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Review

Engineering exosomes and exosome-like nanovesicles for improving tissue targeting and retention

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ABSTRACT

Exosomes are natural nano-size particles secreted by human cells, containing numerous bioactive cargos. Serving as crucial mediators of intercellular communication, exosomes are involved in many physiological and pathological processes, such as inflammation, tissue injury, cardiovascular diseases, tumorigenesis and tumor development. Exosomes have exhibited promising results in the diagnosis and treatment of cancer, cardiovascular diseases and others. They are a rapidly growing class of drug delivery vehicles with many advantages over conventional synthetic carriers. Exosomes used in therapeutic applications encounter several challenges, such as the lack of tissue targeting capabilities and short residence time. In this review, we discuss recent advances in exosome engineering to improve tissue targeting and describe the current types of engineered exosome-like nanovesicles, and summarize their preclinical applications in the treatment of diseases. Further, we also highlight the latest engineering strategies developed to extend exosomes retention time in vivo and exosome-like nanovesicles.

1. Introduction

Exosomes represent a class of extracellular vesicles that can be secreted by almost all cells in both normal and pathological state and thus are widely found in tissues, serum and other body fluids [1,2]. Exosomes are smaller than all other extracellular vesicles, with diameters between 40 and 160 nanometers [3]. The biogenesis of exosomes is an intricate, multi-stage process that encompasses the invagination of the plasma membrane, the development of early and late sorting endosome, and eventually the generation of multivesicular bodies (MVBs) containing intraluminal vesicles (ILVs). Upon fusion with the plasma membrane, ILVs contained in MVBs are released to the extracellular space as exosomes [4,5]. Exosomes have the same topological structure as cells and are rich in bioactive substances, such as miRNAs, DNA, proteins, lipids, enzymes and cell metabolites [6,7]. Initially, exosomes were thought to be the "garbage bags" to help the cells remove unwanted substances [8]. Recent discoveries have revealed that exosomes and their cargoes play an important role in cell-to-cell communication. After secretion, exosomes enter the circulation to selectively transport bioactive cargoes from donor cells to near or distant recipient cells. Upon being taken up by recipient cells, the cargoes of exosomes are released into the cells to regulate their phenotypes, such as cell proliferation, apoptosis, migration, invasion, and angiogenesis [9]. By altering cellular signaling, exosomes demonstrated therapeutic value in a variety of diseases, including cancer, tissue repair, cardiovascular disease, respiratory disease and neurodegenerative disease [10–13]. Of note, exosomes are relatively safer than cell-based therapies because they cannot be replicated and their functions cannot be reprogrammed by environmental factors [14]. During the biogenesis of exosomes, a variety of bioactive molecules from donor cells are selectively encapsulated into exosomes, enabling them to inherit many specific biomolecules from parental cells. In addition, the abundant presence and relative stability of exosomes in bodily fluids confers them potential prognostic and diagnostic biomarkers for diseases [15]. Liquid biopsies containing exosomes are suited for longitudinal sampling, enabling the monitoring of prognosis, disease progression and response to treatment, which are superior to circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA) [16]. Exosomes, as natural vehicles to transport substances between cells, exhibit many advantages in drug delivery, including low immunogenicity, endosomal escape, tissue specificity, stability, and excellent biocompatibility. Compared to traditional nanocarriers, exosomes are widely used to deliver various nucleic acids, peptides and small molecule drugs [7,17,18]. Despite be-

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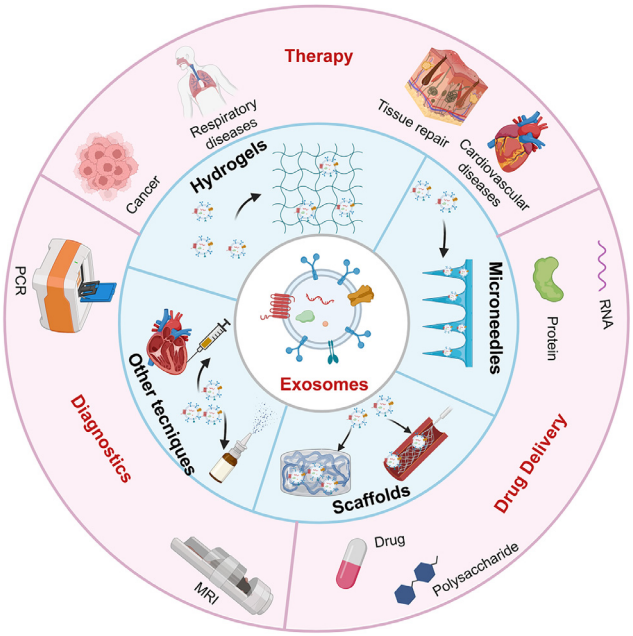

Fig. 1. Application of exosomes. Exosomes showed great potential in the treatment of various diseases, including cancer, respiratory disease, tissue repair and cardiovascular disease, etc. Exosomes are rich in various intracellular molecular signatures of parental cells, including proteins, mRNA, microRNA, therefore making them suitable for disease diagnosis and monitoring. As natural nanocarriers, exosomes can be utilized for cargo delivery, including drugs, polysaccharides, proteins, and RNA.

ing natural nanocarriers, exosomes can be easily modified on their surface with antibodies, fluorescent dyes, and peptides, allowing for the enhancement of cell and tissue specificity or the customization for specific bioactive formulations [19]. In summary, the unique structure, functions and circulation mechanisms of exosomes make them ideal tools for disease diagnosis, treatment, and drug delivery (Fig. 1). At present, some exosomes-based therapies have entered clinical trial phases (Table 1).

Although the biological functions and therapeutic potential of natural exosomes have been extensively validated in a large number of preclinical studies, the non-specific targeting and short retention hindered the therapeutic effect [20]. Moreover, the heterogeneous nature and difficulty in large-scale production restrict the widespread use of exosomes in clinical applications [21]. The present methods for exosomes isolation include differential ultracentrifugation, density gradient ultracentrifugation, size-based isolation techniques, polymer-based precipitation methods, affinity-based capture, and other techniques such as microfluidic techniques [22]. Despite the development of numerous isolation methods, achieving high yield and purity in isolated exosomes remains a significant challenge. As a result, artificial mimics of exosomes have been developed. The biogenesis of exosomes and their functions in various diseases have been described in detail. In this review, we will introduce surface engineering strategies for utilizing exosomes as drug delivery vehicles. Next, a discussion of the currently available types of bionic exosomes, their preparation methods, and advantages and disadvantages will be introduced. Then, based on engineering strategies, we will discuss the applications of exosomes, exosome-like nanovesicles in disease treatment.

2. Surface-functionalized exosomes as drug delivery systems

As natural delivery vectors of intercellular communication, exosomes can efficiently transfer cell-specific informational substances into target cells, either close to or distant from the cells of exosome origin. In contrast to liposomes and other traditional synthetic nanocarriers, exosomes exhibit more favorable biocompatibility, lower immunogenicity, lower toxicity, and greater stability in body fluids, even though they had similar structures and characteristics [23]. Therefore, exosomes have been considered as promising vehicles for drug delivery. Although exosomes from different parental cell lines display tissue targeting capabilities, the insufficient targeting and lack of specificity exhibited by some native exosomes greatly limited their clinical application. Properly modifying the surface of exosomes is an effective strategy for improving their targeting efficiency. Recently, surface engineering methods for exosomes include genetic engineering, chemical modification, and metabolic labeling (Fig. 2).

2.1. Genetic engineering

Genetic engineering is a commonly used indirect method based on modifying exosome-producing cells, which will then display homing peptides or ligands on the surface of exosomes. An expressing vector including the gene sequence of targeted ligands or therapeutic proteins and peptides, fused with exosome membrane proteins, is introduced into parental cells. After transfection with these vectors, recipient cells produce exosomes that have been modified on their surface to express the targeting moieties [24].

LAMP-2 isoform B (LAMP-2B), a membrane protein localized specifically on the exosomes and lysosomal membranes, is one of the most widely used exosome membrane proteins to display peptides, proteins, and antibodies on exosomes surfaces. A previous study has established that therapeutic agents or targeted tags would be exposed on the exosomes surface when fused with LAMP-2B N-terminus, whereas they would be located inside the exosomes when fused with the LAMP-2B C-terminus [25]. Alvarez-Erviti et al. constructed a brain-targeting exosomes by transfecting a plasmid encoding a neuron-specific rabies virus glycoprotein RVG fused to LAMP-2B into dendritic cells [26]. Contrary to unmodified exosomes, injection of these exosomes into the brain delivered loaded siRNA specifically to neurons, microglia, and oligodendrocytes. Through transfection of a vector containing a cardiac muscle targeting peptide (SKTFNTHPQSTP) fused with LAMP-2B into satellite cells, Wang et al. generated cardiac targeting exosomes encapsulating miR-26a (Exo/miR-26a) to treat complications of chronic kidney disease [27]. The collagen-binding domain (CBD) is a polypeptide (TKKTLRT) derived from collagenase with the capacity to bind with collagen-I (Col-I)-based biomaterials. Using a similar genetic engineering approach, Liu et al. engineered a type of exosomes loaded with miR-21 through genetic modification, encapsulated within Col-I scaffolds. This encapsulation was facilitated by the CBD peptide fused to LAMP-2B on the surface of the exosomes [28]. Modified CBD on exosome surfaces anchored them to Col-I scaffolds and promoted miR-21-loaded exosome retention in lesion sites. Peptides are the most commonly used targeting ligands to achieve targeted delivery of exosomes by surface genetic engineering modifications. However, the degradation of peptides by intracellular endosomal proteases during exosomes formation makes it challenging to obtain the desired peptide-functionalized exosomes. In order to increase their stability on exosome surfaces, a glycosylation motif (GNSTM) is added to the N-terminus of peptide–LAMP-2B fusions. To protect LAMP-2B from degradation, Xu et al. fused GNSTM to the N-terminus of the plasmid along with the MSC-targeted peptide E7. Transferring the plasmid into dendritic cells could yield exosomes with the ability to target synovial fluid-derived mesenchymal stem cells (SF-MSCs) targeting. By loading these engineered exosomes with Kartogenin (KGN), a small molecule to induce SF-MSCs differentiation into chondrocytes, they could induce a higher degree of cartilage differentiation [29]. LAMP-2B-based genetic engineering can also present proteins and antibodies on the surface of exosomes. Cavin-2 belongs to the family of Cavin and is involved in caveolae formation [30]. Liao et al. demonstrated that Cavin-2 plays an essential role in EVs uptake by transcriptome sequencing and functional validation. In this study,

Table 1
Clinical applications of exosomes-based therapeutics and delivery nanoplatforms.

Title	Exosome source	Disease	phase	NCT number	status
A Clinical Study of Mesenchymal Stem Cell Exosomes Nebulizer for the Treatment of ARDS	MSCs-derived exosomes	Acute Respiratory Distress Syndrome	Phase I/II	NCT04602104	Unknown
A Clinical Study of Mesenchymal Progenitor Cell Exosomes Nebulizer for the Treatment of Pulmonary Infection	Mesenchymal progenitor cell-derived exosomes	Pulmonary Infection	Phase I/II	NCT04544215	Unknown
The Effect of Stem Cells and Stem Cell Exosomes on Visual Functions in Patients with Retinitis Pigmentosa	Umbilical cord-derived mesenchymal stem cell-derived exosomes	Retinitis Pigmentosa	Phase II/III	NCT05413148	Recruiting
A Tolerance Clinical Study on Aerosol Inhalation of Mesenchymal Stem Cells Exosomes in Healthy Volunteers	MSCs-derived exosomes	Healthy	Phase I	NCT04313647	Completed
Effect of UMSCs Derived Exosomes on Dry Eye in Patients With cGVHD	Umbilical mesenchymal stem cells- derived exosomes	Dry Eye	Phase I/II	NCT04213248	Recruiting
The Use of Exosomes for the Treatment of Acute Respiratory Distress Syndrome or Novel Coronavirus Pneumonia Caused by COVID-19	MSCs-derived exosomes	COVID-19, Novel Coronavirus Pneumonia, Acute Respiratory Distress Syndrome	Phase I/II	NCT04798716	Not yet recruiting
Safety and Efficiency of Method of Exosome Inhalation in COVID-19 Associated Pneumonia	MSCs-derived exosomes	COVID-19, SARS-CoV-2 Pneumonia	Phase II	NCT04602442	Unknown
Study Investigating the Ability of Plant Exosomes to Deliver Curcumin to Normal and Colon Cancer Tissue	Plant-derived exosomes	Colon Cancer	Phase I	NCT01294072	Unknown
Efficacy and Safety of EXOSOME-MSC Therapy to Reduce Hyper-inflammation In Moderate COVID-19 Patients	MSCs-derived exosomes	COVID-19, SARS-CoV-2 Pneumonia	Phase I/II	NCT04491240	Completed
Efficacy and Safety of EXOSOME-MSC Therapy to Reduce Hyper-inflammation In Moderate COVID-19 Patients	MSCs-derived exosomes	SARS-CoV2 Infection	Phase II/III	NCT05216562	Recruiting
Clinical Efficacy of Exosome in Degenerative Meniscal Injury	Synovial fluid-derived mesenchymal stem cells-derived exosomes	Knee, Injury, Meniscus (Lateral) (Medial), Meniscus Tear, Lesion, Degeneration, Laceration	Phase II	NCT05261360	Recruiting
Safety and Tolerability Study of MSC Exosome Ointment	MSCs-derived exosomes	Psoriasis	Phase I	NCT05523011	Completed
COVID-19 Specific T Cell Derived Exosomes (CSTC-Exo)	COVID-19 specific T cell derived exosomes (CSTC-Exo)	Corona Virus Infection, Pneumonia	Phase I	NCT04389385	Unknown
Exosome-based Nanoplatform for Ldlr mRNA Delivery in FH	MSCs-derived exosomes	Familial Hypercholesterolemia	Phase I	NCT05043181	Not yet recruiting
Evaluation of the Safety of CD24-Exosomes in Patients With COVID-19 Infection	T-REx™-293 cells-derived exosomes	SARS-CoV2 Infection	Phase I	NCT04747574	Unknown
A Pilot Clinical Study on Inhalation of Mesenchymal Stem Cells Exosomes Treating Severe Novel Coronavirus Pneumonia	MSCs-derived exosomes	Coronavirus	Phase I	NCT04276987	Completed
Edible Plant Exosome Ability to Prevent Oral Mucositis Associated with Chemoradiation Treatment of Head and Neck Cancer	Plant (grape)-derived exosomes	Head and Neck Cancer, Oral Mucositis	Phase I	NCT01668849	Completed
Intra-articular Injection of MSC-derived Exosomes in Knee Osteoarthritis (ExoOA-1)	MSCs-derived Exosomes	Osteoarthritis, Knee	Phase I	NCT05060107	Unknown
Safety and Efficacy of Injection of Human Placenta Mesenchymal Stem Cells Derived Exosomes for Treatment of Complex Anal Fistula	Placenta-MSCs derived exosomes	Fistula Perianal	Phase I/II	NCT05402748	Recruiting
Safety of Injection of Placental Mesenchymal Stem Cell Derived Exosomes for Treatment of Resistant Perianal Fistula in Crohn's Patients	Placenal MSCs-derived exosomes	Perianal Fistula in Patients with Crohn's Disease	Phase I/II	NCT05499156	Unknown
New Co-transplantation of Mesenchymal Stem Cell Derived Exosomes and Autologous Mitochondria for Patients Candidate for CABG Surgery	MSCs-derived exosomes	Myocardial Infarction, Myocardial Ischemia, Myocardial Stunning	Phase I/II	NCT05669144	Recruiting
Intra-discal Injection of Platelet-rich Plasma (PRP) Enriched with Exosomes in Chronic Low Back Pain	Blood-derived exosomes	Chronic Low Back Pain, Degenerative Disc Disease	Phase I	NCT04849429	Completed
Trial of a Vaccination with Tumor Antigen-loaded Dendritic Cell-derived Exosomes	Tumor antigen-loaded dendritic cell-derived exosomes	Non Small Cell Lung Cancer	Phase II	NCT01159288	Completed
Effect of Microvesicles and Exosomes Therapy on β-cell Mass in Type I Diabetes Mellitus (T1DM)	MSCs-derived exosomes	Diabetes Mellitus Type 1	Phase II/III	NCT02138331	Unknown
MSC-Exos Promote Healing of MHs	MSCs-derived exosomes	Macular Holes	Early Phase 1	NCT03437759	Unknown
Evaluation of Adipose Derived Stem Cells Exo.in Treatment of Periodontitis	Adipose derived stem cells exosomes	Periodontitis	Early Phase 1	NCT04270006	Unknown
The Safety and the Efficacy Evaluation of Allogenic Adipose MSC-Exos in Patients with Alzheimer's Disease	MSCs-derived exosomes	Alzheimer Disease	Phase I/II	NCT04388982	Unknown
MSC EVs in Dystrophic Epidermolysis Bullosa	MSCs-derived exosomes	Dystrophic Epidermolysis Bullosa	Phase I/II	NCT04173650	Not yet recruiting
Extracellular Vesicle Infusion Treatment for COVID-19 Associated ARDS	Bone marrow derived extracellular vesicles	COVID-19, ARDS	Phase II	NCT04493242	Completed

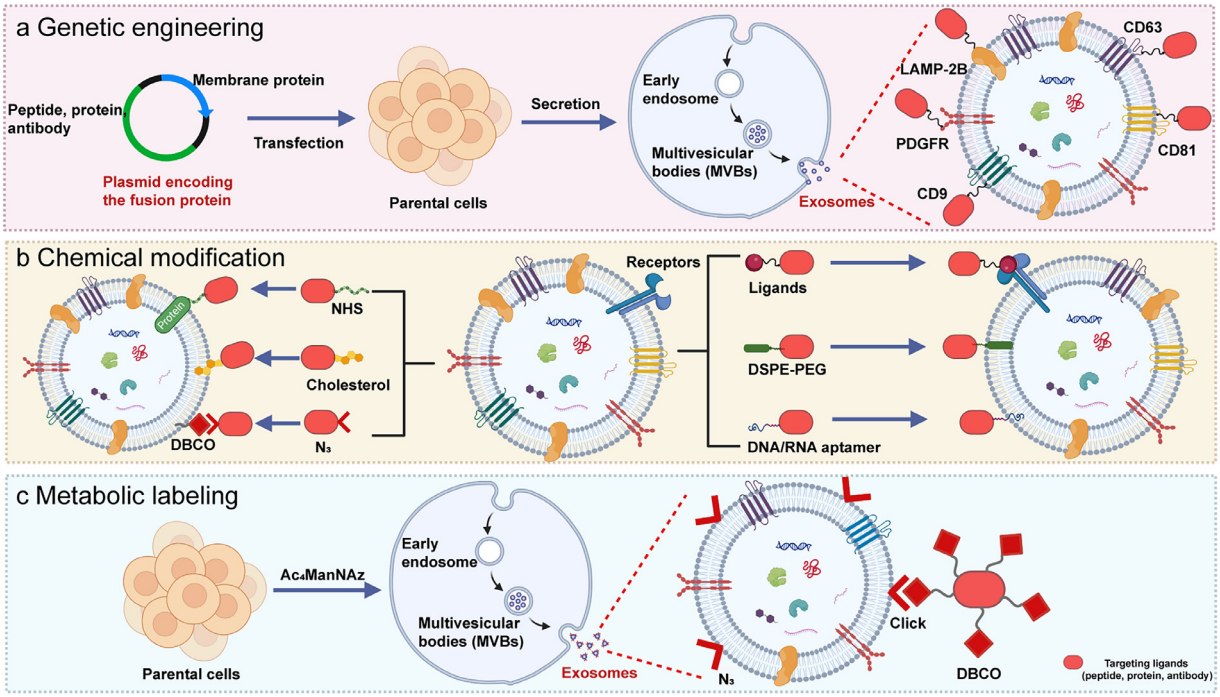

Fig. 2. Strategies for exosomes surface modification. (a) Genetic engineering for modifying exosomes. This strategy involves constructing plasmids with the gene sequence of targeted proteins, peptides or antibody fused with the membrane proteins of exosomes. The target cargos are displayed on the surface of exosomes via transfected plasmids into recipient cells and the biogenesis of exosomes. (b) Chemical engineering for modifying exosomes. Chemical modification involves biological coupling of targeted molecules, such as NHS, ligands, cholesterol, DSPE-PEG to proteins existed in the surface of exosomes. (c) Metabolic labeling for modifying exosomes. The metabolic glycoengineering involves introducing bioorthogonal azide reporters onto the surface glycoconjugates of the parental cells after metabolizing Ac4ManNAz. This is followed by a reaction with a dibenzocyclooctyne-conjugated targeted molecules via click chemistry.

MSCs were transfected with GV492-CAVIN2-LAMP-2B vectors to produce Cavin-2-LAMP-2B positive exosomes. This modification strategy upregulated the uptake of EVs by nucleus pulposus cells (NPCs) under TNF-α treatment [31]. Bellavia's group designed targeted exosomes containing IL3-LAMP-2B to be used for the delivery of imatinib or BCR-ABL siRNA to CML cells overexpressing interleukin-3 receptor (IL3-R). With IL3-LAM-2B expression on the surface of exosomes, IL3-R-mediated endocytosis enabled targeted cellular uptake [32]. Another study fused a HER2-binding antibody to LAMP-2B's N-terminus to modify the surface of exosomes. Experiments conducted in vitro and in vivo demonstrated that the engineered exosomes successfully delivered an inhibitor of miR-21 as well as chemotherapeutics to 5-FU-resistant HCT-116 (HCT-1165FR) cells, effectively inhibiting cancer cell growth [33].

In addition to LAMP-2B, platelet-derived growth factor receptor (PDGFR) and tetraspanins like CD63, CD9, and CD81 existing in exosome membrane have also been reported to be used for membrane display. For example, Ohno et al. used HEK-293 cells as a source of exosomes and transfected these cells with a special pDisplay vector containing the transmembrane domain of PDGFR fused to the GE11 peptide. Since GE11 can selectively bind to EGFR, exosomes modified with the GE11 peptide successfully transported let-7a miRNA to EGFR-expressing heterozygous breast cancer tissues in RAG2$^{-/-}$ mice after intravenous injection [33]. The role of the transmembrane domain (TMD) of platelet-derived growth factor receptor (PDGFR) was crucial in facilitating the presentation of functional monoclonal antibodies on the exosome surface. To prevent steric hindrance, Shi et al. fused a single polypeptide that encodes in-tandem single-chain variable fragments (scFvs) against human CD3 and HER2 receptors to the PDGFR TMD. Using this approach, the authors developed engineered exosomes that displayed both anti-human CD3 and anti-human HER2 antibodies on their surface, facilitating immunotherapy for breast cancer [34]. CD63, CD86 and CD9 are the membrane proteins of exosomes with four transmembrane domains, serving as well-known exosome-specific markers. Inspired by this, Liang et al. developed a plasmid containing a gene fusion of CD63 and Apo-A1 and engineered exosomes produced by plasmid-transfected HEK 293T cells to express Apo-A1, which binds to liver cancer cells with high expression of SR-B1 [35]. Cheng et al. genetically modified HEK-293 cells by co-transfection of constructs expressing αCD3-αEGFR-PDGFR TMD and PD-1-CD9-OX40L fusion proteins to obtain exosomes displaying multiple immunomodulatory proteins on their surface. These exosomes activated T cells to kill EGFR-positive triple-negative breast cancer (TNBC) cells and stimulated an anticancer immunity response [36]. In addition, the surface proteins of exosomes can also be fused with fluorescent proteins and then transfected into recipient cells to produce fluorescently labeled exosomes. Traditional fluorescent lipophilic dyes cannot properly reflect the exact half-life of exosomes in vivo because they could bind to other lipids, resulting in long retention times. Conversely, fluorescent chemical labeling methods are limited to purified exosomes obtained from conditioned media or body fluids, which are ineffective for staining and monitoring exosomes secreted by parental cells in vivo and subsequently tracking their movement between cells. The expression of fluorescent proteins (e.g. GFP, RFP) fused to marker proteins, such as CD63, CD9, CD81, on exosome membranes by genetic engineering allows for monitoring of exosome movement from donor to recipient cells [37]. Through the observation of membrane protein fluorescence, it is possible to directly visualize the transfer of exosomes in both live cultures and in vivo settings. In addition, this labeling strategy circumvents potential false positive signals arising from labeling other lipid entities, as the fluorescent proteins are specifically affixed to exosomes. In another study, fluorescent transgenes technology was used to endogenously label milk-derived exosomes with ZsGreen1 and eGFP [38].

Table 2
Chemically modified exosomes as drug delivery systems.

Classification	Methods	Cargo	Function	Target cells/tissue	Reference
covalent modification	click chemistry	RGE	targeted delivery of curcumin/SPIONs	tumor cells	[39]
		sub-5 nm ultrasmall Prussian blue nanoparticles	reduction of oxidative stress	activated fibroblast-like synoviocytes (FLS)	[40]
		DNA probe	determination of tumor exosomes	tumor cells	[41]
		AlexaFlour®488 (AF488)-azide	intracellular tracking and uptake quantification	tumor cells	[42]
non-covalent binding	receptor-ligand binding	multiple transferrin (Tf) conjugated superparamagnetic magnetite colloidal nanocrystal clusters (SMCNCs)	confering magnetism and targeting properties	tumor cells	[43]
	hydrophobic insertion	siRNAs	gene silencing	neurons	[44]
		cardiac homing peptide (CHP)	improvement the targeting of exosomes	infarcted heart	[48]
		antibody	activation of immune response	tumor cells and DCs	[50]
		polymers	Enhancement of physicochemical properties and biological activity of exosomes	tumor cells	[47]
	electrostatic interaction	cationized pullulan	Augmentation of liver targeting of exosomes	liver	[52]

2.2. Chemical modification

The surface of exosomes can be modified chemically to load functional agents and targeting molecules through covalent and non-covalent methods (Table 2). “Click chemistry” is a copper-catalyzed azide alkyne cycloaddition reaction with the advantages of mild reaction conditions, high yields and fast reaction rates, making it an efficient route to covalently conjugate peptides, proteins and drugs to the surface of exosomes. The mild conjugation reaction has no impact on the biological activity of exosomes and their uptake by target cells. Jia et al. conjugated a neuropilin-1-targeted peptide (RGERPPR, RGE) to the phosphatidylethanolamine on exosomal membrane by a cycloaddition reaction involving sulfonyl azide. The glioma-targeting property of exosomes is conferred by the presence of RGE displayed on their surface [39]. Rheumatoid arthritis (RA) is a persistent systemic inflammatory disease, which currently lacks a specific and effective treatment to alleviate inflammatory conditions. Taking inspiration from the chemotaxis exhibited by neutrophils towards inflammatory regions, Zhang et al. synthesized a neutrophil-derived exosomes covalently conjugated with sub-5 nm ultrasmall PBNPs (uPB) by click chemistry. The exosomes modified with uPB not only retain neutrophil-targeted biological molecules but also exhibit outstanding anti-inflammatory properties [40]. As an early diagnostic tool, the development of reliable, convenient and sensitive methods for exosomes determination has posed challenges in the exosome-based molecular diagnostics. An and co-workers developed an electrochemical aptasensor capable of detecting tumor exosomes in high concentrations, utilizing click chemistry and the DNA hybridization chain reaction (HCR) to achieve signal amplification. This method facilitated the precise and sensitive quantification of exosomes in serum [41]. Unlike conventional copper-mediated click reactions, copper-free click chemistry does not depend on the use of a toxic copper catalyst, making it suitable for biomedical fields. Xu et al. initially modified exosomes surface with NHS-DBCO, and then utilized copper-free click chemistry to label fluorescent molecules on the surface of exosomes for monitoring and measurement of exosomes in vitro and in vivo [42]. However, the influence of the initial linker, for example, NHS-DBCO, on the covalent attachment of exosomal surface protein epitopes could not be neglected.

Non-covalent binding is another important way to directly engineer the surface of exosomes, mainly through receptor-ligand binding, hydrophobic insertion, and electrostatic interactions to immobilize the functionalized agents onto the surface of the exosomes. The principle of ligand-receptor interaction allows the attachment of ligand-bound cargo to exosomes containing the corresponding receptors on their surface. For instance, Qi et al. prepared an engineered exosomes with magnetic and targeting properties by attaching multiple transferrin (Tf) conjugated superparamagnetic magnetite colloidal nanocrystal clusters (SMCNCs) to blood reticulocyte-derived exosomes based on Tf-Tf receptor interaction [43]. Exosome membranes consist of phospholipid bilayers, allowing hydrophobic or lipid molecules to spontaneously insert into their membranes through hydrophobic interactions. It has been demonstrated that the covalent binding of siRNAs to lipids including cholesterol, docosanoic acid, α-tocopheryl succinate, and their PC derivatives promoted the loading of siRNAs onto exosome membrane via hydrophobic interaction. Both in vitro and in vivo experiments validated the efficacy of gene silencing mediated by siRNA-loaded exosomes [44,45]. Exosomes could also be loaded with other types of cargo using the covalent lipid-conjugation strategy, such as SERS nanoprobes, RNA aptamers (PSMA-aptamer, EGFRaptamer, AS1411 aptamer), and folate [46]. *Lathwal* et al. engineered exosomes with different biocompatible polymers on their surfaces, termed exosomal polymer heterodimers (EPHs), based on cholesterol-modified DNA tethers. Two strategies were applied to prepare EPHs. In the first, the amphiphilic Chol-DNA tethers were embedded into the exosome membrane, with DNA strands displayed outside of the exosome surface, resulting in the formation of Exo-ssDNA (single-stranded DNA) structures. The complementary DNA block copolymer (DNA’-Polymer) hybridizes with the DNA tethers on the exosome surface by noncovalent interactions to produce EPHs. In the second strategy, preannealed Chol-DNA and DNA’- initiator strands were used to generate an exosome macroinitiator, and then the atom transfer radical polymerization (ATRP) initiator initiated polymer chains to prepare EPHs. Adjusting and modifying the polymers on the surface of exosomes could enhance their physicochemical properties and bioactivity, promote cellular uptake, and prolong their blood circulation [47]. However, the lipid composition of exosome membranes derived from different cell sources may affect the exact level of cargo loading. In addition, DOPE-NHS (1,2-Dioleoyl-sn-Glycero-3-Phosphoethanolamine-N-hydroxysuccinimide), DSPE-PEG (polyethylene glycol (PEG)-grafted 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine) are commonly used amphiphilic linkers for conjugating targeting peptides or other molecules into the membrane of exosomes. For example, Vandergriff et al. constructed exosomes for the treatment of myocardial infarction by attaching the cardiac targeting peptide CHP to the surface of exosomes through a DOPE-NHS linker. Researchers found that this led to increased retention of exosomes in the heart and reduced fibrosis following ischemia/reperfusion injury [48]. Our lab constructed a novel ROS-responsive exosome-eluting stent (EES) using a DSPE-PEG linker to immobilize exosomes onto the stent. Following 28 days of implantation, stents coated with exosomes facilitated a faster re-endothelialization

process and demonstrated a reduced incidence of in-stent restenosis when compared to both drug-eluting stents and bare-metal stents [49]. Moreover, Fan et al. incubated DSPE-PEG-anti-CD40 and DSPE-PEG-PLGVA-anti-PD-L1 together with donor cells to modify the antibody on the surface of donor cells, and this method obtained dual-targeting drug-loaded exosomes. When the donor cells secrete exosomes, DSPE-PEG-anti-CD40 and DSPE-PEG-PLGVA-anti-PD-L1 could be transferred to the membrane of exosomes [50]. The outer leaflet of the exosome membrane contains negatively charged phosphatidylserine (PS), thus the cationized cargo can be coated onto the surface of the exosomes by electrostatic interaction [51]. A cationized pullulan was successfully modified onto exosome surfaces using electrostatic interaction [52]. The CP05 peptide, identified by Gao et al. through phage display, was demonstrated to bind specifically to the second extravesicular loop of CD63 on the exosomal membrane. The application of the CP05 peptide allowed efficient modification of the surface of exosomes without altering the exosome characteristics and distribution in vivo. In addition, CP05 could be utilized to isolate exosomes from human serum, which provides a straightforward way for surface modification with targeted or therapeutic cargo and facilitates the capture of exosomes [53].

2.3. Metabolic labeling

Metabolic glycoengineering (MGE) is a technique for labeling azido groups on the glycoproteins of living cell surfaces by the metabolic process of unnatural sugars. The combination of MGE and bioorthogonal copper-free click chemistry allows for surface modification of metabolically engineered cells. The MGE strategy is applicable to exosomal glycan labeling and offers characteristics such as small size, good biocompatibility, and flexible modification. By applying the complementary bioorthogonal motif DBCO/N3 to modify the surface of adipose-derived stem cells (ADSCs) based on MGE-mediated click chemistry, You et al. generated activated macrophage-targeting MSC-EXOs. Such surface engineering strategies could be employed to introduce various reactive functionalities to MSC-EXOs without compromising their structural or functional cargo [54]. Zhu et al. designed a dual labeling strategy adapted to visualize the glycosylation of exosomal proteins. This method involves tagging with protein-specific aptamers and employing a metabolic glycan labeling strategy. First, exosomes with azide sialic acid were harvested from parent cells cultured with Ac4ManNAz and then secreted by these cells. This allowed for copper-free bioorthogonal introduction of alkynyl DBCO-Cy5 for metabolic glycan labeling. In addition, a Cy3-labeled PD-L1 aptamer was used for labeling PD-L1 on the surface of exosomes. With this strategy, the glycosylation of exosomal PD-L1 could be characterized in situ by intramolecular fluorescence resonance energy transfer (FRET) between Cy3 and Cy5 incorporated into the same exosomal protein [55].

3. Types of engineered exosome-like nanovesicles

There is an increasing interest in developing drug delivery systems and therapeutics that mimic exosomes for potential replacement of exosomes. The integration of the inherent advantages of exosomes with the enhanced functionalities of synthetic nanomaterials is achievable through the development of bioinspired exosome-like nanoparticles. These hybrid structures have the potential to establish a positive feedback loop between exosomes and synthetic nanomaterials. There are currently three main types of exosome-like nanoparticles: cell-derived nanovesicles (CDNs), hybrid exosome-like nanovesicles, and exosome-like liposomes.

3.1. Cell-derived nanovesicles

CDNs represent promising bioinspired nanovesicles with similar structure and size to exosomes, offering a scalable and efficient alternative to exosome production [56]. CDNs are self-assembled exosomes-like structure, which are prepared though subjecting cells to a physical manipulation. At present, multiple approaches are employed for CDNs biofabrication, including extrusion, filtration, microfluidic technology, and sonication. Compared to the processing of exosome collection, CDNs offer advantages of higher production yield (~200-fold higher) and shorter processing times [56]. Formed by self-assembly using membrane fragments from precursor cells, CDNs have a high degree of similarity to exosomes, with long circulation times, low clearance rates and cytotoxicity compared to synthetic nanoparticles. Moreover, they partially retained membrane proteins, lipids, and mRNA molecules originating from their parent cells and preserved the biological characteristics of their parental cells. Quite a few studies have investigated the difference between CDNs and naturally secreted exosomes. Jo et al. fabricated nanovesicles from living embryonic stem cells (ES cells) using microfluidic techniques and found that these mimetic nanovesicles were similar to exosomes in shape. In addition, they contained the mRNAs, cytoplasm, and membrane proteins of the original cells and could deliver their contents to acceptor cells by fusion with the cytoplasm membrane in a manner similar to exosomes [21]. Goh et al. reported that CDNs generated by spin cups via a cell shearing approach have scores similar to exosomes in almost all aspects, including physical attributes (zeta potential, hydrodynamic diameter), protein markers, lipidomic profiles, the mechanisms of cellular uptake as well as in vivo biodistribution [57]. More recently, similar results were confirmed by Wen et al. Not only that, they also found that the membrane proteins of CDNs have ~71% similarity to EVs and their small RNAs have ~ 65% similarity [58]. Given the advantage of CDNs, they were considered to be potential substitutes for exosomes. Cells including mesenchymal stem cells (MSCs), dendritic cells, T cells, and tumor cells were used to produce CDNs [59]. Our research group previously prepared CDNs from MSCs by squeezing the cells through a set of extrusion filters sequentially. We found that the production of CDNs was over 20-fold higher than that of EVs. Moreover, CDNs treatment reduced scar sizes and preserved cardiac functions by promoting angiogenesis and proliferation of cardiomyocytes after ischemia reperfusion injury, functions similar to natural EVs [60]. One mechanism by which CDNs may promote cardiac repair is through the microRNAs they contain. Wang et al. demonstrated that microRNAs in CDNs were associated with cardiac repair, fibrosis repression, and angiogenesis [28]. Hong et al. demonstrated that programmed cell death protein 1 (PD-1) and TGF-β receptors are present on the surface of T-cell-derived nanovesicles (TCNVs). These nanovesicles are prepared through the serial extrusion of cytotoxic T cells, endowing them with the capability to impede T cell exhaustion and exhibit anti-tumor activity [59]. The specific proteins on the membrane surface are thought to provide CDNs with their natural targeting abilities. Jang et al. developed monocytes or macrophages-derived CDNs by serial extrusion and the chemotherapeutics were loaded during plasma membrane self-assembly. They found that doxorubicin-loaded CDNs performed the targeted delivery of encapsulated chemotherapeutics to malignant tumors and demonstrated comparable in vivo antitumor activity to doxorubicin-loaded exosomes. By removing the plasma membrane proteins, the CDNs lost their therapeutic effects both in vitro and in vivo [61]. While CDNs hold promise as alternatives to exosomes, the preparation of CDNs is a random process, and determining their final composition may be difficult, which will affect their manufacturing reproducibility and therapeutic efficacy.

3.2. Hybrid exosome-like nanovesicles

Hybrid exosome-like nanovesicles are generated by the hybridization of exosomes and other synthetic or biological systems via different membrane fusion methods, thereby overcoming the disadvantages of two independent systems and combining both advantages. The fusing of exosomes with liposomes is the most common form of hybrid exosome-like nanovesicles. Apart from having a high capacity for

drug loading, excellent stability, and easy surface modification, hybrid nanovesicles exhibit remarkable biocompatibility and minimal immunogenicity. A freeze-thaw method was used to produce hybrid exosomes derived from Raw 264.7 cells and liposomes. In vitro cellular uptake studies confirmed that the uptake rate of hybrid exosomes was higher than that of untreated exosomes [62]. In another experiment, Cheng et al. employed the freeze-thaw method to fuse gene-engineered exosomes with drug-loaded thermosensitive liposomes, resulting in the formation of hybrid nanovesicles. These hybrid nanovesicles blocked the CD47 signal, thereby inhibiting macrophage phagocytosis. They specifically targeted homologous tumors in mice and effectively transported photothermal agents loaded in hybrid nanovesicles to the tumor sites [63]. Although the freeze-thaw method may realize high fusion efficiency, the biological activity of the drug in the vesicle and the structure of the exosome membrane may be damaged by high-frequency freeze-thaw cycles. Lin et al. generated hybrid nanovesicles through the incubation of HEK-293T exosomes with liposomes expressing CRISPR/Cas at 37 °C for 12 h, maintaining the integrity of the lipid bilayer and preventing any leakage of vesicle contents. They revealed that this hybrid vesicle could effectively encapsulate large plasmids compared with exosomes and liposomes alone. Moreover, CRISPR/Cas9 cleavage systems loaded in hybrid vehicles were successfully delivered into MSCs and regulated the target gene expression regulate the target gene expression [64]. The membrane extrusion method was also employed to prepare hybrid exosome-like nanovesicles. In this approach, exosomes and liposomes were initially mixed in different ratios, then sonicated and vortexed the above mixture, and finally the mixture was extruded through a pore size of 400 nm, 200 nm, or 100 nm to produce hybrid nanovesicles [65,66]. To enhance drug delivery to lung fibrotic tissue, Sun et al. hybridized clodronate-loaded liposomes (CLD) and fibroblast-derived exosomes by extrusion, which enhanced pulmonary anti-fibrotic agents nintedanib delivery [67]. Hu et al. produced hybrid nanoparticles based on the fusion of $CXCR4^{+}$ exosomes with liposomes containing antagomir-188 by physical extrusion. These hybrid nanoparticles were shown to specifically deliver antagomir-188 in bone marrow, thereby inhibiting adipogenesis and promoting osteogenesis of bone marrow mesenchymal stem cells in aged mice [68]. Although hybrid nanoparticles possess the low immunogenicity of exosomes, the toxicity of their liposomal fraction may not be ignored.

Additionally, we have constructed platelet membrane and stem cell exosome hybrids to increase exosome binding and accumulation in injured tissues. These exosome-like nanovesicles were mainly synthesized in three steps. Initially, MSCs-derived exosomes were isolated and extracted. Subsequently, platelet membrane components were extracted by freeze-thawing. Finally, the exosomes and platelet membranes were fused using an extrusion method. Importantly, the process did not affect the integrity of exosomes and platelet membrane function. By hybridizing with platelet membranes, exosomes were able to target the injured heart and reduce macrophage uptake. Thus, they enhance therapeutic potential in mice with myocardial infarction [69]. Using the same procedure, other researchers have successfully prepared engineering extracellular vesicles hybrid with platelet membrane to target delivery of functional microRNAs to damaged sites in myocardial ischemia reperfusion (MI/R) injury [70].

3.3. Exosome-like liposomes

Liposomes are entirely synthetic, enclosed lipid vesicles that possess a similar size and structure to exosomes. The effectiveness of liposomes as drug delivery systems has been well-established over the past decades, with a variety of products based on liposomes available, such as Doxil® and AmBisome® [71,72]. Liposomes with their well-characterized structure, versatility in size, ease of generation, and scalability offer considerable advantages in drug delivery. In addition, liposomes can also be modified to improve their structural stability and targeting ability. Nevertheless, similar to many other types of nanoparticles, liposomes are susceptible to immune system recognition and display restricted internalization by target cells [73].

Exosomes and liposomes both have advantages and disadvantages, so positive feedback between these fields would be beneficial. Based on the advantages of exosomes in terms of specific lipid components that facilitate cellular internalization or targeted accumulation, researchers have designed more efficient and biocompatible liposome delivery vehicles. These liposomes provide the advantages of endogenous exosomes (e.g., effective drug delivery, and limited immunogenicity). Haque et al. demonstrated that exosome-mimicking liposomes present remarkable membrane fusion properties and stability, very similar to natural exosomes [74]. Lu et al. compared the intracellular delivery of siRNA function between exosome-mimicking liposomes and conventional liposomes. The results indicated that exosome-mimicking liposomes demonstrated significantly enhanced cellular uptake and silencing efficiency in comparison to PC–Chol liposomes (> three-fold). Consequently, exosome-mimicking liposomes' distinctive lipid composition improves their intracellular delivery efficiency. However, the drug delivery effect is still not satisfactory when compared with cationic Lipo 2000 and DOTAP liposomes [75].

4. Design of exosomes and artificial nanovesicles for disease-specific therapeutics

Extensive studies have broadened our understanding of the biological, physical, and chemical properties of exosomes, but the clinical translation of exosome-based therapeutics for specific diseases is still highly restricted. The main reason is that exosomes, as natural nanovesicles, are deficient in some features for therapeutic applications [76,77]. For example, the exosomes lack the specific cell-targeting capability, which highly hampers the delivery efficiency for various disease therapy. In addition, sometimes it is necessary to integrate new drugs into exosomes to provide or enhance the therapeutic effect. To this end, various biotechnological and chemical techniques have been utilized to design and fabricate engineered exosomes and artificial nanovesicles for the treatment of specific diseases, such as cancer, cardiovascular diseases, and respiratory diseases. These highly specialized exosomes, designed using multiple exosome engineering techniques, are referred to as designer exosomes. Designer exosomes offer attractive novel features to achieve an improved disease pharmacokinetic profile for targeted therapeutic applications [77,78]. In this chapter, we introduce the disease-specificized engineered exosomes and artificial nanovesicles.

4.1. Cancer

Cancer remains one of the leading causes of global human death because of late diagnosis, poor prognosis, drug resistance, and metastasis. The efficacy of commonly used clinical therapies, including surgery, chemotherapy, and radiotherapy, is limited, and novel therapeutics are required for cancer treatment [79]. Exosomes and artificial nanovesicles are considered promising nanoplatforms for targeted cancer therapy due to their capability for cargo delivery, mediating cell-cell communication, and inducing specific immune responses [80]. For example, our group found that the cancer cell-derived exosomes targeted their parent cells in vitro and returned to the original tumor tissue in vivo, suggesting the application of exosomes for drug delivery [81]. Hao and Wang designed an engineered exosome-based nanoplatform for the delivery of ferroptosis inducer (Er) and photosensitizer (RB) for chemo-photodynamic therapy [82]. CD47 was introduced to the surface of the exosomes to avoid the phagocytosis by the mononuclear phagocyte system and increase the accumulation in the tumor. The developed drug-loaded exosomes induced potent ferroptosis under 532 nm laser irradiation.

In addition to drug delivery, efforts have been made using engineered exosomes and artificial nanovesicles for enhanced immunotherapy. For instance, Xie and coworkers modified CD47 antibodies (aCD47) and SIRPα antibodies (aSIRPα) on the surface of M1 macrophages-derived exosomes [83]. The developed exosome nanobioconjugates could specifically release aSIRPα and aCD47 and block SIRPα on macrophages and CD47, to abolish the "don't eat me" signaling pathway and enhance phagocytosis. In addition, M1 exosomes induced the transition of M2 macrophages to the antitumoral M1 phenotype. The synergism of M1 exosomes and antibodies induced potent antitumor effects. Our group also developed antibody-engineered exosomes for CAR-T cancer immunotherapy. By stimulating DCs with tumor antigens, we found that the derived exosomes can provide MHC-antigen complexes and co-stimulatory molecules for CD86. We then engineered two antibodies, anti-CD3 and anti-EGFR, on tDC-Exo surface (Exo-OVA-aCD3/aEGFR). The developed Exo-OVA-aCD3/aEGFR could induce potent activation of T cells and enhance the interaction between T cells and cancer cells. In vivo results demonstrated the excellent anticancer therapeutic effect of the Exo-OVA-aCD3/aEGFR in combination with anti-PD-L1 antibodies [84]. Most recently, we modified anti-PD-L1 antibodies (aPD-L1) on the surface of Exo-OVA (Exo-OVA-aPD-L1). The developed Exo-OVA-aPD-L1 could efficiently activate T cells and inhibit the immune escape of cancer cells, leading to enhanced anti-cancer T cell immunotherapy [85]. The typical immunosuppressive tumor microenvironment (TME) highly limited the efficacy of tumor immunotherapy. To overcome the obstacle, our group developed an exosomal vaccine to regulate the macrophage phenotype for enhancing cancer immunotherapy. We used LPS to stimulate primary macrophages (M0) to get the polarized M1-type macrophages. After that, we incubated the M1 macrophages with the model antigen ovalbumin (OVA) and harvested the exosomes (M1OVA-Exos). The M1OVA-Exos could potently polarize macrophages to the M1 phenotype and induce immune responses. Animal experiments demonstrated that the M1OVA-Exos led to significant inhibition of tumor growth and metastasis. Our exosomal vaccine presents a novel idea to enhance cancer immunotherapy [86]. CD47 is anti−phagocytic molecules overexpressed on certain kinds of tumor cells that, when interacts with signal regulatory protein α (SIRPα) on macrophages, activates the "don't eat me" signal that impedes macrophage phagocytosis of tumor cells [87,88]. Thus, blocking the binding of CD47 to SIRPα on macrophage can improve macrophage phagocytosis of tumor cells. Liang and coworkers produced a hybrid nanovehicles (SPI@hEL) by fusing M1 macrophages-derived extracellular vesicles (M1 EVs) with liposomes, followed by further modification with the RS17 peptide to produce SPI@hEL-RS17 nanoparticles. Intravenously injected SPI@hEL-RS17 displayed superior tumor targeting ability and improved drug accumulation at the tumor site, attributed to the natural targeting capability of M1 EVs and the specific affinity between RS17 peptide and CD47 on tumor cells. In addition, RS17 peptide bind to CD47 on tumor cells, blocking CD47-SIRPα signaling and promoting macrophage phagocytosis, thus reshaping the immunosuppressive tumor microenvironment [89]. Liu and colleagues generated a hybrid therapeutic nanovesicles (hGLV) with surface overexpression of CD47 by fusing gene-engineered exosomes with drug-loaded thermosensitive liposomes. The designed nanovesicles demonstrated prolonged blood circulation and preferential accumulation at the tumor site. In addition, hGLV enhanced macrophage-mediated phagocytosis of tumor cells by inhibiting CD47 signaling. The loading the photothermal agent into hGLV could completely eliminating tumors through a combination of photothermal therapy and immunotherapy [63].

Cancer patients who receive chemotherapy suffer severe toxicity to various organs, such as hepatotoxicity and cardiotoxicity, and often induce death. Thus, alleviating chemotherapy-induced toxicity would be an effective way to prolong the lifespan of cancer survivors [90,91]. Exosome utilization holds great promise to overcome the toxicity-based limitation of cancer chemotherapy. In previous work, we found that cardiac stem cells (CSCs)-derived exosomes protected the heart against doxorubicin (DOX) induced dilated cardiomyopathy with significantly improved heart function via decreasing apoptosis and fibrosis [92]. Most recently, our group designed decoy exosomes for the reduction of chemotherapy-induced toxicity [93]. The MSC exosomes were functionalized with tetrahedral DNA nanostructures (Exo-TDN) that are abundant in GC base pairs in the sequences. In this design, the exosomes functioned as the repairing carriers, and the TDNs were used for DOX delivery. The CLSM results indicated that the Exo-TDN avoided DOX from entering the nucleus and thus protected the cells from DOX toxicity. In addition, the Exo-TDN could efficiently induce macrophages to transform from M0 or M1 phenotypes to M2, confirming their anti-inflammatory activities. In an MCF-7 tumor mice model, Exo-TDN treatment significantly reduced liver injury caused by DOX via suppression of hepatocellular apoptosis without affecting the antitumor efficiency of DOX. Thereafter, we modified the Exo-TDN with a cardiomyopathic peptide (Exo-TDN-PCM) to broaden their appliance. Biodistribution results revealed the enhanced accumulation of Exo-TDN-PCM in the heart. Furthermore, Exo-TDN-PCM induced potent alleviation of the DOX-caused cardiotoxicity through the reduction of cell apoptosis and inflammation in myocardial tissue.

4.2. Cardiovascular diseases

Cardiovascular diseases (CVDs), including peripheral vascular disease, coronary artery disease, cerebrovascular disease, ischemic heart disease, and heart failure, remain the predominant cause of global mortality, resulting in a severe challenge to the worldwide health and economy [94]. Researchers have proposed stem cell-based therapy and regenerative medicine to treat CVDs. However, the disadvantages of poor engraftment and survival, tumorigenic potential, and immune rejection limit the clinical translation of cell therapy [95]. Numerous studies have revealed that EVs are involved in many cardiovascular physiological or pathological pathways, such as angiogenic regulation, blood pressure maintenance, cardiac hypertrophy, fibrosis, and apoptosis. In addition, stem cell-derived EVs preserve the favorable therapeutic effect and arise as an appealing alternative in CVD treatment [96]. Furthermore, various approaches for engineering EVs with improved therapeutic properties has been proposed [97]. Recently, extruded MSC nanovesicles were found to show similar therapeutic effects to natural EVs in cardiac repair [60]. Considering their relatively high yield (more than 20-fold), extruded nanovesicles could be an alternative choice in regenerative medicine applications.

To enhance the targeting capability, targeting moieties could be added to EV surfaces to allow specific accumulation in interested tissues or cells. For instance, Shen and Yang introduced an ischemic myocardium-targeting peptide (IMTP) onto the exosome surfaces via fusion with LAMP-2B. IMTP-exosomes indicated efficient cellular uptake in vitro and improved accumulation in ischemic myocardium in vivo. The IMTP-exosome treatment significantly inhibited inflammation and cell apoptosis, enhanced angiogenesis, and improved cardiac function. In addition, the phospholipid component enables the direct modification of the EV surface via lipid-based techniques, such as self-insertion [98]. Antes and coworkers modified EVs from cardiosphere-derived cells (CDC-EVs) with DMPE-PEG-streptavidin, in which DMPE is an anchor and PEG-streptavidin is for coupling with biotinylated IMTP. The developed CDC-EVs indicated enhanced accumulation in the injured myocardium in a rat I/R model [99]. In addition, our group developed heart-targeting exosomes via conjugation of exosomes from human CDCs (hCDC-XOs) with a cardiac homing peptide (CHP). E*x vivo* imaging indicated that the CHP labeling enhanced the retention of exosomes to the infarcted heart. The injection of CHPXOs caused an improvement in cardiac function and a reduction in fibrosis in an ischemia/reperfusion injury model after 21 days. The immunohistochemistry analysis indi-

cated that the CHP-XOs treatment significantly enhanced cardiomyocyte proliferation and angiogenesis [48]. Cardiac-targeting EVs represent a potential method for treating the myocardial infarction.

The targeting capability of EVs could also be enhanced through the fusion of EVs with lipid-based artificial or natural biomaterials. For example, Vader et al. fused cardiac progenitor cell (CPC) derived-EVs (CPC EVs) with liposomes, and these hybrid nanoparticles significantly enhanced wound healing compared with blank liposomes [100]. In another work, we hybridized MSC-derived exosomes (MSC-XOs) with platelet membranes (P-XOs) to improve their cardiac targeting ability. The cellular uptake of developed P-XOs, which was mediated by micropinocytosis, by endothelial cells and cardiomyocytes was significantly enhanced. In vivo imaging results indicated that the P-XOs could target and accumulate in the injured heart. In addition, compared with unmodified exosomes, P-XOs demonstrated enhanced improvement of cardiac function by promoting vessel formation and inhibiting cell apoptosis in the infarct border zone. Our study provides a universal technique to improve the targeted accumulation of exosomes in injured organs.

4.3. Respiratory diseases

As a leading causes of global morbidity and mortality, respiratory diseases have become a heavy burden. Although several drugs, including small molecules, mRNA, proteins, and stem cells, have been developed for treating respiratory diseases, their therapeutic effects are limited due to antigenic drift, compromised host immune response, or drug resistance [101]. The exceptional viral complexity, high transmissibility, and rebounding possibility drive the need for effective antiviral therapeutics to cure present respiratory diseases and avoid future outbreaks. Exosomes offer an alternative therapeutic approach for respiratory disease treatment via using the host machinery and molecular components to inhibit viral replication.

Lee and coworkers carried out a pioneer study investigating the therapeutic potential of MSC-EVs in the treatment of acute lung injury (ALI). The authors found that the therapeutic effects of EVs isolated from human bone marrow-derived MSC (BM-MSC EVs) were comparable with the MSCs in treating the *E. coli* endotoxin-induced ALI. Intratracheal administration of BM-MSC EVs significantly decreased pulmonary edema, lung protein permeability, and inflammation in mice ALI model. The KGF siRNA treatment of BM-MSC decreased the therapeutic effects of BM-MSC EVs, indicating the important role of KGF protein in EV-mediated treatment of ALI [102]. Qu et al. also reported the protective effects of BM-MSC EVs on lipopolysaccharide (LPS)-induced ALI in mice. The authors further indicated that the therapeutic effects and immunomodulatory activities of BM-MSC EVs in ALI were partly mediated via the contained Ang-1 mRNAs [103]. In another study, Krasnodembskaya and colleagues demonstrated that MSCs induced reduced proinflammatory cytokines, increased CD206 expression, and improved phagocytic capacity in macrophages in the inflammatory environment of acute respiratory distress syndrome (ARDS). MSC EV-pretreated alveolar macrophages offered protection against LPS-induced lung injury in mice model. Further investigation revealed that macrophage polarization is triggered by the transfer of functional mitochondria mediated by MSC EVs in ARDS [104]. Moreover, Khatri et al. showed that porcine BM-MSCs-derived EVs could efficiently suppress replication of the influenza virus, and significantly mitigated influenza virus-caused acute lung injury (ALI) in a pig model [105]. In addition to the animal models, other groups also investigate the effects of MSC EVs on *ex vivo* perfused human lungs against transplantation rejection or *E. coli*-induced pneumonia [106,107]. These researches demonstrated that EVs protect against respiratory diseases.

Currently, the world is still suffering from the ongoing pandemic, called coronavirus disease of 2019 (COVID-19), caused by novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The present pandemic drives scientists to explore the therapeutic potential of EVs in combating COVID-19. For example, Moon and coworkers reported that MSC EVs rescued the pathogenic effect of SARS-CoV-2 virus and suppressed the proinflammatory activities via inhibition of viral replication [108]. More excitingly, a BM-MSC exosome-based agent, ExoFloTM, has been examined in a non-randomized open-label cohort study in treating severe COVID-19. The ExoFlo^{TM} treatment significantly improved the recovery of the patients via restoration of oxygen storage capacity, downregulation of cytokine storm, and enhancement of immunity [109]. Exosome-based drug delivery for COVID-19 treatment is also under heated investigation. For example, Gould et al. reported a method for fabricating mRNA-encapsulated exosomes for localized mRNA delivery. Loading of mRNAs encoding SARS-CoV-2 Spike and Nucleocapsid proteins induced immune responses against these two proteins for months. This work demonstrated that exosomes could be used as nanoplatforms for functional mRNA delivery [110]. Yet, the pulmonic bioavailability and stability limited the development of COVID-19 therapies based on exosomes. To solve the problem, our group developed lung-derived exosomes (Lung-Exos) as inhalable drug carriers of mRNA and protein. The Lung-Exos were further formulated as a lyophilized dry powder that showed excellent stability at room temperature. The inhalation of Lung-Exos dry powder resulted in delivery to the lungs of the mouse and African green monkey (AGM). In addition, Lung-Exos loaded with mRNA encoding the SARS-CoV-2 Spike protein demonstrated stronger immune responses than the liposome counterpart [111]. Furthermore, we systematically compared the biodistribution of the developed Lung-Exos with commercial HEK293T-derived exosomes (HEK-Exos) and synthetic LNPs (Lipo). The Lung-Exos demonstrated superior delivery and accumulation of mRNA and protein in the bronchioles and parenchyma after jet nebulization administration, suggesting that the inhalable Lung-Exo could deliver molecular drugs with improved pulmonary bioavailability and therapeutic efficacy [112].

Alternatively, exosomes could be designed as “nanodecoys” for specifical binding with the virus to prevent viral infection of host cells. Angiotensin-converting enzyme 2 (ACE2) receptors on host cells assist the cellular uptake of SARS-CoV-2 via binding to the spike (S) protein of the virus. Thus, Inal proposed that engineering MSC EVs with angiotensin-converting enzyme 2 (ACE2) expression would competitively suppress infection of SARS-CoV-2 to ACE2-expressing alveolar type II cells [113]. Our group successfully developed human lung spheroid cells (LSCs)-derived ACE2 nanodecoys for the protection of the host lung cells from SARS-CoV-2 infection (Fig. 3a) [114]. As shown in Fig. 3b, LSCs and derived nanodecoys showed higher ACE2 expression levels than HEK293 cells and corresponding nanodecoys. The TEM images indicated the typical spherical morphology of the prepared nanodecoys (Fig. 3c). ACE2 was confirmed to be located at the nanodecoy surface via flow cytometry analysis. LSC-nanodecoys showed efficient capture and binding with spike S1, while HEK293-nanodecoys did not (Fig. 3d). Fig. 3e revealed that the LSC-nanodecoys could competitively bind spike S1 and be internalized by macrophages, suggesting the clearance of nanodecoys and neutralized virus. In addition, the LSC-nanodecoys potently bound designed SARS-CoV-2 viral mimics and blocked their entry into host lung cells (Fig. 3f). Following the inhalation of LSC nanodecoys, we studied their biodistribution and found that they were retained for 72 h in the lungs. More importantly, Fig. 3g and 3h indicated that the inhaled LSC-nanodecoys markedly reduced SARS-CoV-2 mimics and promoted their clearance from the lung. Furthermore, we evaluated the therapeutic efficacy of LSC-nanodecoys in a macaque model. Inhalation treatment of LSC-nanodecoys led to significantly rapid clearance of SARS-CoV-2 virus, reduced polymorphonuclear cells and neutrophils, and decreased lung fibrosis (Fig. 3i-l). Our study confirmed that the LSC-nanodecoys could be used as potential therapeutic agents to treat COVID-19. Most recently, we designed an inhalable COVID-19 vaccine based on exosomal virus-like-particles (VLPs). The SARS-CoV-2 receptor-binding domain (RBD) was conjugated to the surface of the LSC-derived exosomes (LSC-Exo) todevelop RBD-Exo VLP (RBD-Exo) that simulates the SARS-CoV-2 morphology. RBD-Exo demonstrated enhanced distribution and retention

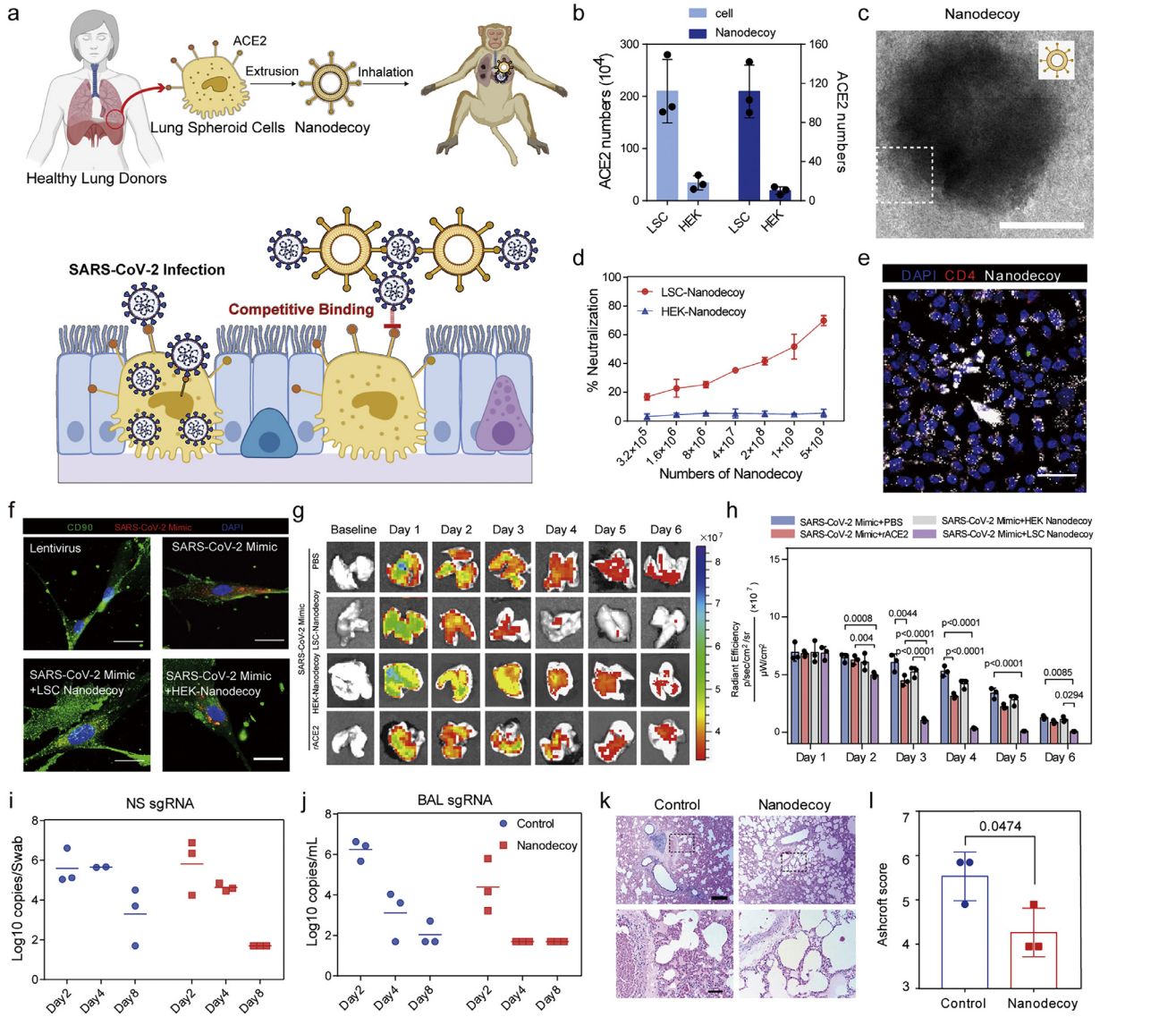

Fig. 3. Nanodecoys for the neutralization of SARS-CoV-2 and mitigation of COVID-19 lung injury. (a) Schematic illustration of the LSC-nanodecoys for treating SARS-CoV-2 infection. (b) Measurement of ACE2 numbers on LSC and HEK293 cells, and corresponding nanodecoys. (c) TEM image of LSC-nanodecoys. (d) Neutralization of spike S1 by LSC- and HEK293-nanodecoys. (e) Confocal image indicating internalization of nanodecoys in macrophages. (f) Immunocytochemistry showing entry blockage of SARS-CoV-2 mimics in host cells by LSC-nanodecoys. (g) Ex vivo IVIS imaging of SARS-CoV-2 mimics in mice lung tissues and (h) corresponding quantified fluorescence intensities after various treatments. Viral sgRNA copies per swab in nasal swabs (NSs) (i) and bronchoalveolar lavage (BAL) (j) at various time points after inhalation. (k) H&E images of lung tissues of cynomolgus macaques after SARS-CoV-2 infection. (l) Quantification of lung fibrosis in infected cynomolgus macaques via Ashcroft scoring [109].

in the mucus-lined respiratory airway and lung parenchyma compared to commonly used liposomes. In vivo experiments showed that RBD-Exo inhalation induced robust humoral and cellular immune responses in mice against SARS-CoV-2 mimics and in hamsters infected with live SARS-CoV-2. Also, RBD-Exo vaccination led to high levels of RBD-specific IgA and CD4+ and CD8+ T cells in the lungs, which is beneficial for the protection of the lungs from viral infection. In addition, the lyophilized RBD-Exo VLPs indicated excellent physical and antigenic stability at room temperature, which is superior to currently approved vaccines and could promote vaccine accessibility [115]. Huang and coworkers also developed CAR-T cell-derived nanovesicles that express single-chain fragment variables (scFv) CR3022 and B38 (CR3022/B38 NVs) to target SARS-CoV-2. The developed CR3022/B38 NVs could efficiently neutralize Spike-pseudotyped virus and enhance the antiviral ability via inhibition of viral replication and reduction of adverse drug reactions [116].

5. Biomaterials engineered exosomes and exosome-like nanovesicles for enhanced retention

As discussed above, exosomes and artificial nanovesicles have demonstrated their potential applications in gene regulation, tissue regeneration, drug delivery, and disease therapy. Nevertheless, the clinical translation of exosomes and artificial nanovesicles is hindered by their administration route, fast clearance from circulation, and rapid accumulation in non-targeted organs [117,118]. In this regard, various types of biomaterial-based platforms and devices, including hydrogels, scaffolds, and microneedles, have been developed to engineer exosomes or exosome-like nanovesicles for prolonged localized release. This significantly enhances therapeutic efficacy while minimizing side effects. [119–121]. This chapter summarizes examples of various kinds of biomaterials for engineering exosomes or exosome-like nanovesicles to improve retention (Table 3).

Table 3
Biomaterial-based platforms for enhancing the retention of exosomes or exosome-like nanovesicles.

Biomaterials	Cargo	Site of action	Function	Reference
collagen-based hydrogel	iCM-EVs	heart	reduction of myocardial injury	[119]
chitosan/silk hydrogel	GMSCs-derived exosomes	Skin	promotion of skin wound healing and neuronal ingrowth	[124]
decellularized extracellular matrix (ECM) hydrogels	MSC-derived exosomes	heart	promotion of cardiac repair	[125]
exosome/β-TCP combined scaffolds	hiPS-MSC-Exos	bone	therapy of calvarial bone defects	[136]
silk fibroin (SF)-based 3D scaffolds	hAMSCs-derived exosomes	bone	promotion of bone regeneration	[137]
ROS-responsive exosome-eluting stents (EES)	MSC-derived exosomes	heart	enhancement of vascular healing in ischemia-reperfusion injury	[138]
hyaluronic acid (HA)-based core-shell microneedle	Fe-MSC-NVs	skin	promotion of wound healing	[145]
GelMA/PEGDA microneedles	HUVECs-exos	skin	promotion of diabetic wound healing	[146]
polymeric matrix of microneedles	MSC-derived exosomes	skin	Promotion of hair regrowth.	[148]
bacterial cellulose membrane	HUCMSCs-Exos	subcutaneous tissue and muscle	Suppression of epidural fibrosis and peridural adhesions	[152]
minimally invasive exosome spray	MSC-derived exosomes	heart	Promotion of heart repair	[155]

5.1. Hydrogels

A hydrogel is a 3D crosslinked polymer network that can absorb a substantial amount of water or biological fluid. In general, hydrogels can be prepared from hydrophilic natural polymers and derivatives. For instance, these may include chitosan, alginate, dextran, collagen and polylysine, along with synthetic polymers, including poly (ethylene glycol) (PEG), poly(N-vinyl pyrrolidone) (PNVP), and poly(hydroxy butyrate) (PHB). The hydrophilic property of hydrogels offers excellent biocompatibility and capability of carrying drugs, including cells, proteins, nucleic acids, nanoparticles, and recently, exosomes and artificial nanovesicles [122,123].

Liu and coworkers demonstrated that the induced-pluripotent-stem-cell-derived CMs (iCMs) could secrete functionally active EVs in similar yield but with greater therapeutic potential compared to their parent induced pluripotent stem (iPS) cells. The next-generation miRNA sequencing revealed the abundance of cardiac-specific miRNAs in iCM-EVs. To further study the therapeutic effects on heart injury and recovery, the authors developed an engineered collagen-based hydrogel patch with excellent capability of encapsulating iCM-EVs [119]. After direct application to rat myocardium in a myocardial infarction model, the sustained release of iCM-EVs from the hydrogel patch led to reduced injury and enhanced recovery by increasing arrhythmia burden, decreasing infarct size and pathological hypertrophy, and remarkably preventing hypoxia-induced apoptosis. In another work, Shi et al. showed a chitosan/silk hydrogel sponge laden with gingival mesenchymal stem cells (GMSCs)-derived exosomes [124]. The authors first isolated and purified the GMSCs-derived exosomes via the qEV size exclusion column. After that, the chitosan/silk hydrogel sponge was prepared according to the freeze-drying approach and subsequently loaded with GMSCs-derived exosomes. Application of the hydrogel-exosomes platform in a streptozotocin-induced rat diabetic model resulted in significantly promoted skin wound healing with accelerated re-epithelialization and deposition, remodeled extracellular matrix, increased angiogenesis, and facilitated neuronal ingrowth. Our group also reported a technique for minimally invasive delivery of biocompatible decellularized extracellular matrix (ECM) hydrogels containing therapeutic reagents via intrapericardial (iPC) injection for cardiac repair [125]. In this approach, the pericardial cavity serves as a natural mold in which the injectable hydrogels uniformly assemble a cardiac patch. The iPC injection of induced pluripotent stem cell-derived cardiac progenitor cells (iPS-CPCs) and MSC-derived exosomes, used as model therapeutic agents, led to promoted cardiac repair with sustained release and prolonged retention of therapeutics, and mitigated immune responses throughout the heart, suggesting that the iPC injection is a safe, effective, and clinically feasible method for heart delivery of exosomes or other drugs. Lv and coworkers generated an injectable multifunctional composite hydrogel with cationic KLD2R peptide and MSC-derived nanovesicles (MSC-NVs) loaded with transforming growth factor β receptor inhibitor (TGF-βRi) to prevent postoperative adhesion (PA) formation [126]. Briefly, MSC-NVs were initially prepared by serial extrusion of MSCs through a series of pore-sized membrane filters, after which drug-encapsulated MSC-NVs were obtained by incubating TGF-βRi with MSC-NVs. Then, the composite hydrogel was simply prepared by adding the purified drug@NVs to the KLD2R solution without undergoing any chemical reactions. Injection of the multifunctional composite hydrogel into the surgical areas of the mouse could effectively prevent PA by attenuating local inflammation and fibrosis.

In addition, stimuli-responsive hydrogels have also been developed for the controlled release of exosomes and EVs in response to specific factors, such as biomolecules, pH, temperature, light, etc. [118]. For instance, Ferreira and coworkers designed a light-responsive hydrogel through hyaluronic acid (HA) cross-linking by a photocleavable linker in conjugation with EVs derived from human umbilical cord blood mononuclear cells (hUCBMNCs) [127]. The EVs release, which is dependent on irradiation time and number, can be achieved via light-triggered hydrogel disassembly. The EVs delivery efficacy of this platform was tested in a diabetic wound healing model for 10 days with daily laser irradiation of 1 min, resulting in improved wound healing and skin tissue formation. In addition, Wang and colleagues reported a pH-responsive hydrogel based on antibacterial polypeptides for the release of adipose-derived mesenchymal stem cell exosome (AMSCs-exo) [128]. The multifunctional hydrogel facilitated skin regeneration and wound healing through the release of pH-responsive exosomes and exhibited excellent antibacterial properties.

5.2. Scaffolds

The use of scaffolds is widespread in tissue engineering and regenerative medicine because they are porous biomaterials with constructive properties [129,130]. The ideal scaffolds should be able to accelerate cell proliferation and tissue regeneration to replace the damaged tissues for functional restoration. Typically, a scaffold provides the proper location for loading of cells, growth factors, or drugs and controlled release of these cargoes to modulate the cellular microenvironment and promote tissue regeneration [131]. Up to now, various natural and synthetic biomaterials, such as silk fibroin, chitosan, PLGA, and PVA, have been employed for scaffold formation in tissue engineering [132].

MSC-secreted exosomes (MSC-exos) have been shown to have the potential to promote cellular self-repair, restore the cellular microenvironment, and boost tissue healing [133,134]. Considering the advantages of higher therapeutic effects, simple preparation and storage, and excellent biocompatibility, the combination of scaffolds with exosomes is a promising concept in the field of tissue engineering [135].

For instance, Wang et al. combined tricalcium phosphate (β-TCP) and exosomes derived from human-induced pluripotent stem cell-derived mesenchymal stem cells (hiPS-MSC-Exos) for the therapy of calvarial bone defects [136]. The designed exosome/β-TCP combined scaffolds contributed to increased proliferation, migration, and osteogenic differentiation of human bone marrow-derived mesenchymal stem cells (hBMSCs). Gene expression profiling, bioinformatics analyses, and further functional studies confirmed that the exosome/β-TCP combination scaffolds-induced osteogenic responses of hBMSCs were due to the involvement of PI3K/Akt signaling pathway. The study provided evidence for the application of exosome and scaffold combinations in tissue regeneration and the underlying mechanisms. Also, Lee and coworkers developed silk fibroin (SF)-based 3D scaffolds coated with human adipose-derived mesenchymal stem cells (hAMSCs)-derived exosomes (Exo-SF-BMSC scaffolds) for bone regeneration. Cell experiments demonstrated that the Exo-SF-BMSC scaffolds promoted the proliferation and osteogenic differentiation of hBMSCs. Additionally, the implantation of Exo-SF-BMSC scaffolds led to significant bone regeneration and reinforcing abilities in a rat model of calvarial defects [137]. Of note, scaffolds usually raise inflammatory issues due to foreign-body reactions, requiring specific modifications to improve their biosafety. For example, bare-metal stents (BMS) and drug-eluting stents (DES) suffer from the risks of neointimal hyperplasia and late restenosis despite their widespread use in vascular diseases. To prevent the limitation, our group recently developed biocompatible and reactive oxygen species (ROS)-responsive exosome-eluting stents (EES) for enhanced vascular healing in ischaemia-reperfusion injury (Fig. 4) [49]. We chose exosomes secreted from MSCs (MSC-XOs) as therapeutic cargoes because of their reported potential in treating ischaemic injuries and superior biocompatibility as natural products. As shown in Fig. 4a, MSC-XOs were coated on the DSPE-modified stents with an inserted ROS linker, endowing the EES with ROS-responsive property. SEM images indicated the nanoscale roughness of the EES compared to the BMS with a smooth surface, confirming the MSC-XOs coating (Fig. 4b). In vitro H_2O_2 incubation increased the MSC-XOs release from the EES, demonstrating the ROS-responsive feature of the EES (Fig. 4c). In addition, adhesion of activated platelets and monocytes was significantly decreased after incubation with EES compared to that of BMS, revealing excellent safety (Fig. 4d). More importantly, the EES incubation enhanced the endothelial tube formation and proliferation of HUVECs (Fig. 4e) but inhibited the migration ability of vascular smooth muscle cells (SMCs) (Fig. 4f). As shown in Fig. 4g, the EES treatment resulted in a preserved healthy muscle morphology in the unilateral hindlimb ischaemia model. In addition, CD31-positive capillaries and Ki67-positive endothelial cells were significantly increased in the EES group, indicating the pro-angiogenesis effect of the EES treatment (Fig. 4h).

Exosome-coated scaffolds have also been used to modulate the immune system to achieve certain functions. For example, Zhang and colleagues proposed a construct of U-MSC exosomes and collagen-based scaffolds (CS/Exos) for endometrium regeneration. The local CS/Exos transplantation significantly promoted the endometrium regeneration, collagen remodeling, estrogen receptor α and progesterone receptor expression, leading to the restoration of fertility in a rat model of endometrial damage. More importantly, the authors investigated the underlying mechanism for tissue regeneration. Results from in vitro and in vivo experiments showed that the CS/Exos increased M2 macrophage polarization and decreased inflammation. Specifically, RNA-seq analyses revealed that the macrophage polarization was mainly due to the involvement of the miRNAs enriched in exosomes [138]. Luo et al. designed multifunctional scaffolds by immobilizing MSC exosomes on PEI-modified electrospun fibers (Exo-PEF) for tissue repair. In vitro and in vivo investigations indicated that the release of exosomes from Exo-PEF scaffolds was due to direct contact with cells, and released exosomes were predominantly taken up by macrophages in wounds. Implantation of Exo-PEF scaffolds activated local immunomodulatory responses as evidenced by increased M2 macrophage phenotype and T_{reg} cells in skin wound models. In addition, Exo-PEF treatment also induced lymph node-remote adaptive immune responses. The authors further investigated the therapeutic potential of Exo-PEF in the healing of large skin wounds. The results indicated that the Exo-PEF significantly promoted wound closure and reepithelialization, collagen deposition, and blood vessel formation [139]. In addition, Roura and coworkers developed novel cardiac decellularized scaffolds for local delivery of EVs from porcine cardiac adipose tissue-derived MSC (cATMSC-EV) for cardiac repair. cATMSC-EV was proven to decrease the secretion of pro-inflammatory cytokines and accelerate angiogenesis. The administration of cATMSC-EV-loaded scaffolds contributes to vascular density increment and macrophage and T cell reduction in a myocardial infarction (MI) model [140]. Most recently, the same group reported the long-term study, revealing the local and systemic anti-inflammatory activities of the cATMSC-EV-loaded scaffolds, which is beneficial for cardiac healing in MI [141].

5.3. Microneedles

Microneedles are medical devices consisting of an array of micron-scaled needles on a small patch [142]. Typically, the microneedles are designed to be small enough to penetrate the skin but have to be large enough for efficient transdermal delivery of almost any drug, such as small molecules and large particles [143]. As an emerging platform for skin-targeted drug delivery, microneedles possess many advantages, such as minimal invasiveness, improved transdermal efficacy, superior compliance, and convenient operation [144]. Traditional applications of exosomes or EVs in treating skin-associated diseases often require multiple administrations because of their poor transdermal permeability and short-term retention. Accordingly, researchers have recently developed different kinds of innovative microneedles for the delivery of therapeutic exosomes and EVs in skin diseases.

Sun and colleagues proposed a hyaluronic acid (HA)-based core-shell microneedle patch, which encapsulates ferrum-mesenchymal stem cell-derived artificial nanovesicles (Fe-MSC-NVs) and PDA nanoparticles (PDA NPs) in its tips to initiate wound healing. The progressive release of PDA NPs could inhibit inflammation induced by ROS, and the Fe-MSC-NVs could promote the migration, proliferation, and tube formation of human umbilical vein endothelial cells (HUVEC). The in vivo experiments revealed the outstanding diabetic wound healing capability of the developed microneedle patch [145]. In another study, Chen and coworkers designed microneedle patches made of methacrylate gelatin (GelMA) to control the release of HUVECs-derived exosomes (HUVECs-exos) and pro-angiogenic drug tazarotene for diabetic wound healing [146]. Most recently, Sun and Zhao developed novel microneedle patches with the spatio-temporal variation capability for MSC-exos and Ag nanoparticles (AgNPs) delivery. The release of MSC-exos could remarkably inhibit inflammation and promote angiogenesis, leading to accelerated wound healing. In addition, the AgNPs could suppress bacterial infection to further promote wound repair [147].

Researchers have also investigated the application of the microneedle technique to other diseases. For instance, Gu *et al.* reported a microneedle-based delivery platform for transdermal codelivery of exosomes derived from MSCs and a drug UK5099 for hair regrowth. Keratin was used to prepare the microneedles on an HA-based patch base, endowing the microneedles with features of high mechanical strength, continuous payload release ability, excellent biocompatibility, and enhanced treatment compliance. The direct and efficient transportation of MSC-derived exosomes and UK5099 through the microneedle device significantly induced pigmentation and hair regrowth in less than 6 days. Promoted activation of hair cycle and improved hair condition were also observed, confirming the great therapeutic potential of the developed microneedle platform in hair loss therapy [148]. Wang and coworkers also proposed a soluble microneedle patch decorated with hair nanoparticles for the delivery of human amniotic mesenchymal stem cells (hAMSCs)-derived exosomes to promote hair regrowth [149]. In

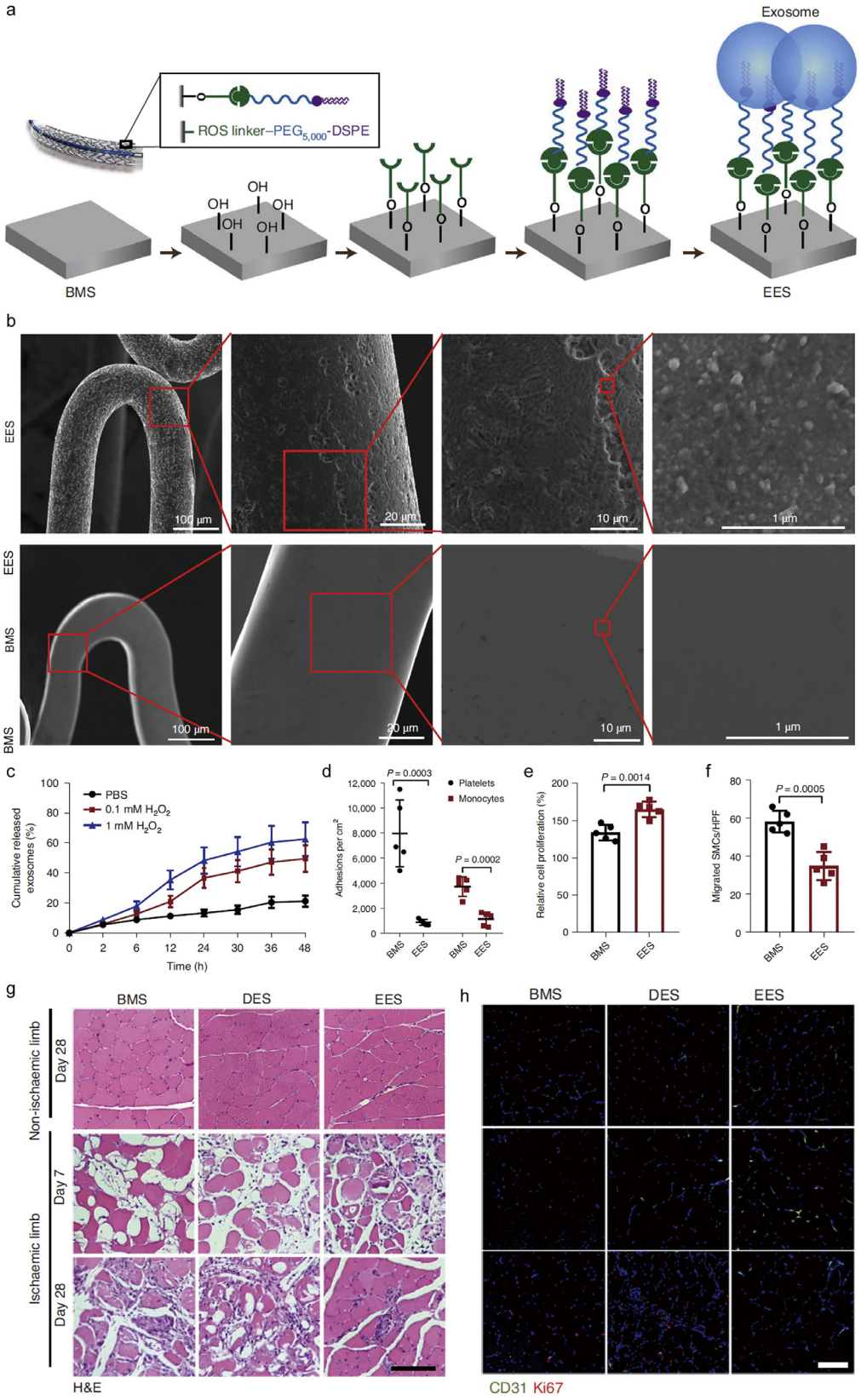

Fig. 4. ROS-responsive exosome-releasing stents for enhanced vascular healing after ischaemic injury. (a) Schematic illustration of the fabrication process of the EES. (b) SEM images of EES and BMS. (c) MSC-XOs release profiles from EES in PBS and H_2O_2 of different concentrations (0.1 mM and 1 mM). (d) Quantification of adherent platelets and monocytes. (e) CCK-8 assays of HUVECs and (f) quantified migrated SMCs incubated with BMS or EES. (g) H&E staining and (h) immunofluorescent images of non-ischaemic leg and ischaemic leg with various treatments [132].

addition, Mao and colleagues developed a gelatin methacryloyl (GelMA) and PVA-based detachable microneedle patch for the transdermal delivery of nitric oxide nanomotor-modified exosomes (EXO/MBA) derived from tendon stem cells for Achilles tendinopathy (AT) treatment. The sustained release of EXO/MBA from the microneedle led to significantly inhibited inflammation, promoted tendon cell proliferation, enhanced Col1a expression, and reduced extracellular matrix degradation, demonstrating its potential in enthesopathy healing [150]. Xin and Liu also designed a 3D microneedle patch for the effective delivery of MSCs-derived exosomes for spinal cord injury (SCI) therapy. The 3D culture of exosomes in the microneedle patch resulted in significantly decreased inflammation and glial scarring in the SCI model, indicating its potential in SCI treatment [151].

5.4. Other techniques

In addition to the approaches discussed above, scientists have developed other techniques for the delivery of exosomes and artificial nanovesicles [118]. For example, Mao and coworkers developed a specific bacterial cellulose (BC) membrane that contains human umbilical cord mesenchymal stem cells (HUCMSCs)-derived exosomes (HUCMSCs-Exos). The membrane was able to significantly suppress epidural fibrosis and peridural adhesions in a laminectomy model [152]. Sun et al. reported a strategy to immobilize MSC-EVs on titanium surfaces via biotin-streptavidin interaction to improve the bio functions of titanium implants [153]. Recently, our group used a needle-free injector to deliver 3D human dermal fibroblast spheroids-derived exosomes (3D HDF-XOs), which expresses increased tissue inhibitor of metalloproteinases-1 (TIMP 1) and differential miRNAs than 2D HDF-XOs, for cutaneous aging treatment. The jet injector for needle-free injection offers an easy but efficient technique for exosome transdermal delivery [154]. In another study, we fabricated a minimally invasive exosome spray (EXOS) for heart repair. The EXOS was sprayed into the rat heart using a high-precision tweezer or needle tip which inserts into a small incision under the guidance of a mini-endoscope. The EXOS significantly rescued the cardiac function, decreased fibrosis, and facilitated angiomyogenesis in an acute myocardial infarction model [155]. Furthermore, we developed several inhalable exosomes or EVs-based vaccines or drug carriers for disease treatment [111,112,115].

6. Conclusion and perspectives

Advances in bionanotechnology have brought striking influence on human health and the next-generation of personalized nanomedicine. The rapidly increasing research on the components, biogenesis, and physiological and pathological functions of exosomes is leading to expanded possibilities in the biomedical fields of nanomedicine, drug delivery, and disease therapeutics. Extensive studies have revealed the superior potential of exosomes in overcoming the limitations of synthetic nanomaterials-based strategies due to their superior physical and chemical properties, such as biocompatibility, targeting capability, and functionality [156]. Despite the vast academic success, the shortcomings of natural exosomes still significantly hamper the clinical and industrial translation of exosome-derived products.

The large-scale production and manufacturing of exosomes remains a dominant challenge for further academic research and clinical translation. The abundance of exosomes via present preparation techniques could not fully meet the demand for various applications. Large-scale production of natural exosomes could profit from the achievements in the manufacture of classical biologics, such as protein production and cell therapy. Accordingly, multilayered culture flasks, bioreactors, and hollow fiber cartridges are suggested for scalable cell culture. For example, stainless steel bioreactors lead to up to 20,000 L scaled cell culture, and platform-rocker wave bags and disposable bioreactors up to 500 L and 2000 L, respectively [157]. Meanwhile, scientists developed various top-down strategies to generate exosome-like artificial nanovesicles. For instance, Jang et al. first proposed extrusion-based approach for the large-scale production of cell-derived nanovesicles that is comparable to natural exosomes with regards to morphology, size, proteins, and drug loading efficacy [61]. Cells could be converted into artificial nanovesicles with maintained biological biomarkers by sequentially extruding them through polycarbonate membrane filters with decreasing pore size. More excitingly, the yield of these nanovesicles is significantly higher (> 100-fold) than that of natural exosomes. Also, the development of other techniques, such as microfluidic device-based method, filtration-based method, and sonication-based method, expanded the choices for large-scale production of exosome-like artificial nanovesicles. Due to the intrinsic heterogeneity of the EVs, size inconstancy, and batch-to-batch variability suffered during their production, the quality control of the harvested exosomes and artificial nanovesicles is another concern for biomedical applications. Although some strategies, including differential ultracentrifugation, size exclusion chromatography, affinity chromatography, and tangential flow filtration, have already been assessed, there is no consensus on a standard technology for large-scale purification of natural or artificial exosomes. It is therefore essential to develop beneficial purification techniques that are suitable for the scalable isolation of functional exosomes.

Another challenge of exosome translations is the inadequate therapeutic effect of natural exosomes. Exosomes themselves contain biomolecules, such as proteins, nucleic acids, and lipids, and could therefore participate in and regulate biological processes as therapeutics. However, the natural drugs in exosomes display diversity according to the cellular origin, and the loading capacity is relatively low compared to synthetic nanocarriers. Novel techniques are required to improve drug loading efficiency and promote drug release. In addition, the efficacy of exosome-mediated targeted drug delivery is not satisfactory though natural exosomes have been reported to possess homing capability and targeting property. To date, various approaches, including genetic engineering, chemical modification, and metabolic labeling, have been suggested to enhance the targeting capability and change the biodistribution of exosome-based nanoplatforms. Despite the gratifying achievements, there are still issues that need to be reconsidered in the development of engineered exosomes. For example, the heterogeneity of parent and target cells results in difficulties in choosing optimal exosome sources and corresponding modifications. Also, modifications of the exosome surface may have a negative impact on the properties of exosomes, including stability, biocompatibility, and production simplicity. Most importantly, the ultimate translation of exosomes into the clinic requires careful and profound investigation of their therapeutic effect and safety in patients, a situation that is completely different from the widely tested cells and small animals in academic research.

In addition, the biostability of exosomes and systematic immunogenicity restrict the therapeutic outcome of exosomes. Realizing the maximum therapeutic effect of exosomes in the aimed tissues is another problem concerning scientists. In this respect, various biomaterials have been combined with exosome technology to enhance the retention and long-term release of exosomes and artificial nanovesicles in diseased tissues. However, the detailed architecture design, mechanical properties, and biocompatibility of the material-based exosome delivery systems need to be studied before their translation. Besides, the exosome loading and release profiles in clinically relevant systems are necessary, as the in vivo environment is much more dynamic and complicated than cell culture conditions.

Exosomes and artificial nanovesicles are considered next generation nanomedicine for human health improvement. At present, natural and artificial nanovesicles are not yet prepared for clinical translation due to their primary problems, including large-scale production, limited therapeutic effects, and concerns about bioavailability. Breakthroughs in biology, chemistry, nanotechnology, and pharmacology will certainly

provide positive influences on exosome technology and speed up their development. We look forward to the clinical translation of exosomes and artificial nanovesicles in the future.

Declaration of competing interest

The authors declare that they have no conflicts of interest in this work.

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