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Review

Research Advances of Engineered Exosomes as Drug Delivery Carrier

Lianghui Huang, Enguang Wu, Jiawei Liao, Zongyi Wei, Jin Wang,* and Zhenhua Chen*

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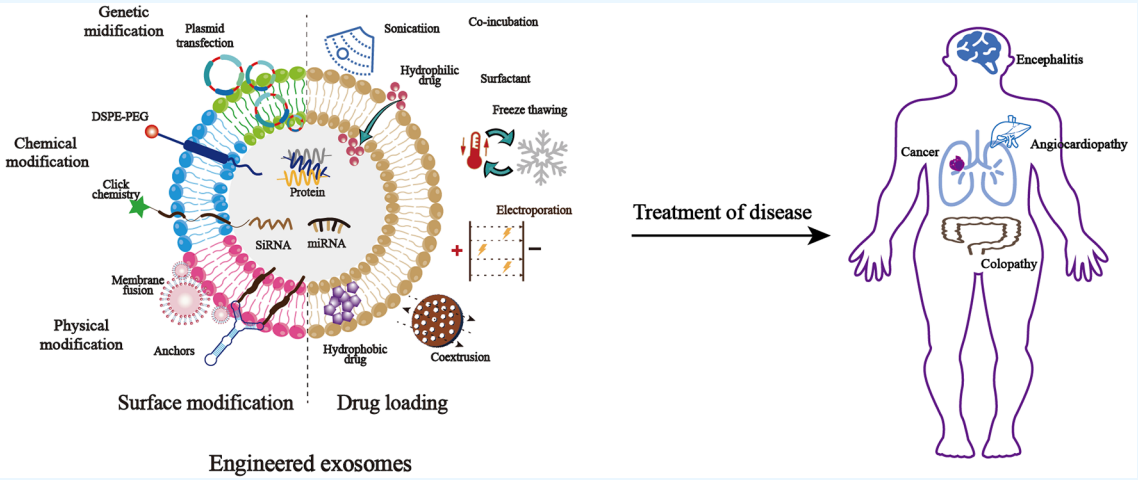

ABSTRACT: Exosomes are nanoscale vesicles secreted by living cells that have similar membrane composition to parental cells and carry a variety of proteins, lipids, and nucleic acids. Therefore, exosomes have certain biological activities and play an important role in intercellular communication. On the basis of its potential as a carrier for drug delivery systems, exosomes have been engineered to compensate for the shortage of natural exosomes through various engineering strategies for improving drug delivery efficiency, enhancing targeting to tissues and organs, and extending the circulating half-life of exosomes. This review focuses on the engineered exosomes loading drugs through different strategies, discussions on exosome surface modification strategies, and summarizes the advantages and disadvantages of different strategies. In addition, this review provides an overview of the recent applications of engineered exosomes in a number of refractory and relapsable diseases. This review has the potential to provide a reference for further research and development of engineered exosomes.

1. INTRODUCTION

The ideal drug delivery system (DDS) is one that the lowest concentrations of therapeutic agents precisely to the lesion without damaging the surrounding healthy tissue.[1] In the past decades, various synthetic nanocarriers have been developed to enhance therapeutic efficacy, such as liposomes,[2] micelles,[3] carbon nanotubes,[4] and dendrimers.[5] Moreover, various functionalized modifications of nanocarriers have been used to improve the key properties of drug delivery.[6] For example, they extended circulation time *in vivo*, stimulated response, and improved drug loading. However, synthetic drug delivery systems still have some limitations. For instance, they have cytotoxicity, immunogenicity, and complex fabrication processes.[7−9] Cell-derived exosomes have extraordinary potential in DDS for its low immunogenicity, high stability, ability to penetrate tissues, and inherent bioactivity.[10]

Exosomes were initially considered as "garbage bags" to dispose of unwanted cellular contents.[11] It contains similar components of parental cells, including nucleic acids, amino acids, lipids, and proteins, which plays an important role in mediating intercellular information transfer and material exchange.[12] Exosomes are commonly characterized by the expression of specific marker proteins, including tetraspanins CD9, CD63, CD81, integrins, major histocompatibility molecules (MHC) class I and II, etc.[13,14] In the Exocrata

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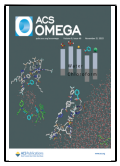

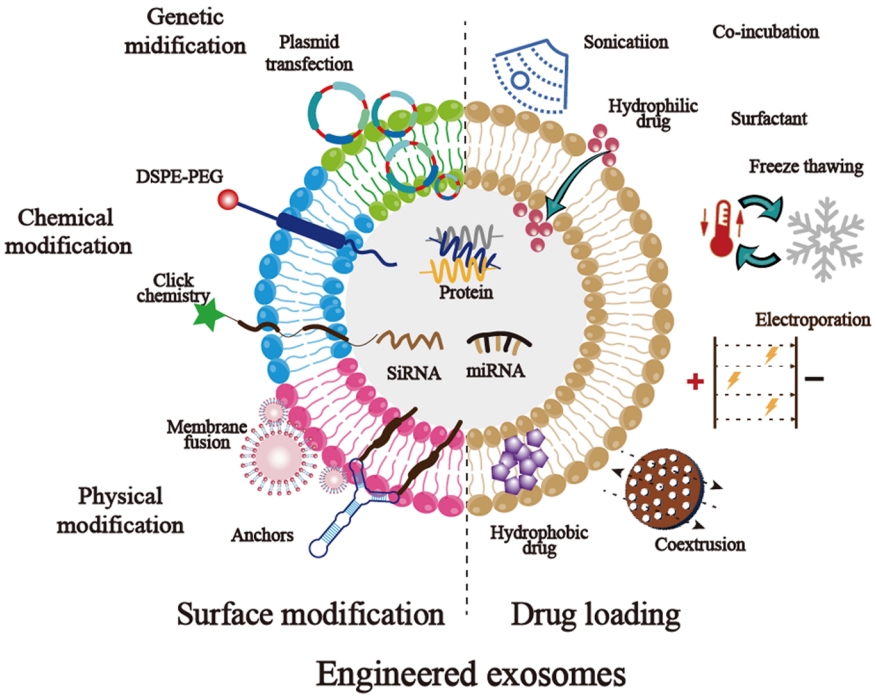

Figure 1. Engineered exosome drug loading and surface modification strategies.

database (http://exocarta.org), 9769 protein species identified in different biological exosomal species have been included so far. As the exosomes are a lipid bilayer nanovacule structure, they offer the possibility of loading hydrophilic and lipophilic drugs.[15] Inspired by the drug loading and functionalized modification of synthetic nanocarriers, natural exosomes were loaded with drugs or exosomes with surface modification by some engineering strategies to obtain engineered exosomes with more desired properties.[16] This review introduces different drug loading strategies for engineered exosomes, including coincubation, sonication, electroporation, freeze−thaw cycles, coextrusion, and surfactant for delivery of various drugs, nucleic acids, peptides, and proteins. Strategies for surface modification of engineered exosomes are summarized, such as chemical modifications, physical modifications, and genetic modifications. In addition, the advantages and disadvantages of different modification strategies are also described. Finally, recent applications of engineered exosomes in diseases such as cancer, cardiovascular disease, brain disease, and ulcerative colitis are reviewed.

2. EXOSOMES OVERVIEW

Exosomes are single-membrane lipid bilayer nanoscale extracellular vesicle (EV), 30−150 nm in diameter, cup holder, or globe in shape.[17,18] Exosomes are produced by the endosomal membrane budding inward, maturing into multivesicular bodies that eventually fuse with the plasma membrane, releasing the exosomes into the extracellular space.[19] It can be secreted by almost any type of living cell and has been found in various body fluids, such as plasma, urine, saliva, semen, tears, amniotic fluid, and breast milk.[20,21] The main components of exosomes include nucleic acids (DNA, mRNA, and miRNA), lipids, and many proteins including extracellular matrix proteins, enzymes, receptors, and transcription factors.[22] Thus, exosomes can carry specific molecular information from parental cells and act as intercellular communication carriers, playing a key role in facilitating the transfer of proteins, functional mRNAs, and miRNAs, especially in long-distance intercellular communication.[23] This secretory signaling and communication mechanism is an efficient, robust, and economical way of exchanging information between cells. Under physiological conditions, exosomes perform important functions in the regulation of homeostasis and various other conditions in the body.[24] Under pathological conditions, the information conveyed by exosomes has been shown to contribute to the diagnosis and treatment of a variety of diseases, such as tumorigenesis and metastasis, inflammation, and cardiovascular disease.[25−27] On the basis of above functions, exosomes have attracted much attention in clinical applications as disease diagnostic markers and therapeutic drug carriers. There are a wide variety of drug carriers, and the relatively mature ones include synthetic drug carriers such as microcapsules, microspheres, and polymer nanoparticles.[28,29] In comparison, exosomes, as a novel cell-derived bionic drug delivery vehicle, have the advantages of low toxicity, low immunogenicity, and high tissue penetration ability, including crossing the blood-brain barrier.[30] The stability of exosomes to drug molecules allows for long-distance transport of various cargos under physiological and pathological conditions.[31] With the continuous efforts of researchers, various types of natural exosomes loaded with cargos or surface modified have shown excellent therapeutic effects for a variety of diseases.[32]

3. ENGINEERED EXOSOMES

The positive benefits of exosomes and their ability to carry cargos make them ideal candidates for new therapeutic targets. However, a few key factors limit the clinical application of natural exosomes, such as rapid systemic circulation clearance and insufficient targeting.[33,34] Engineered exosomes can effectively overcome existing limitations and expand their loading capacity for therapeutic agents. As shown in Figure 1,

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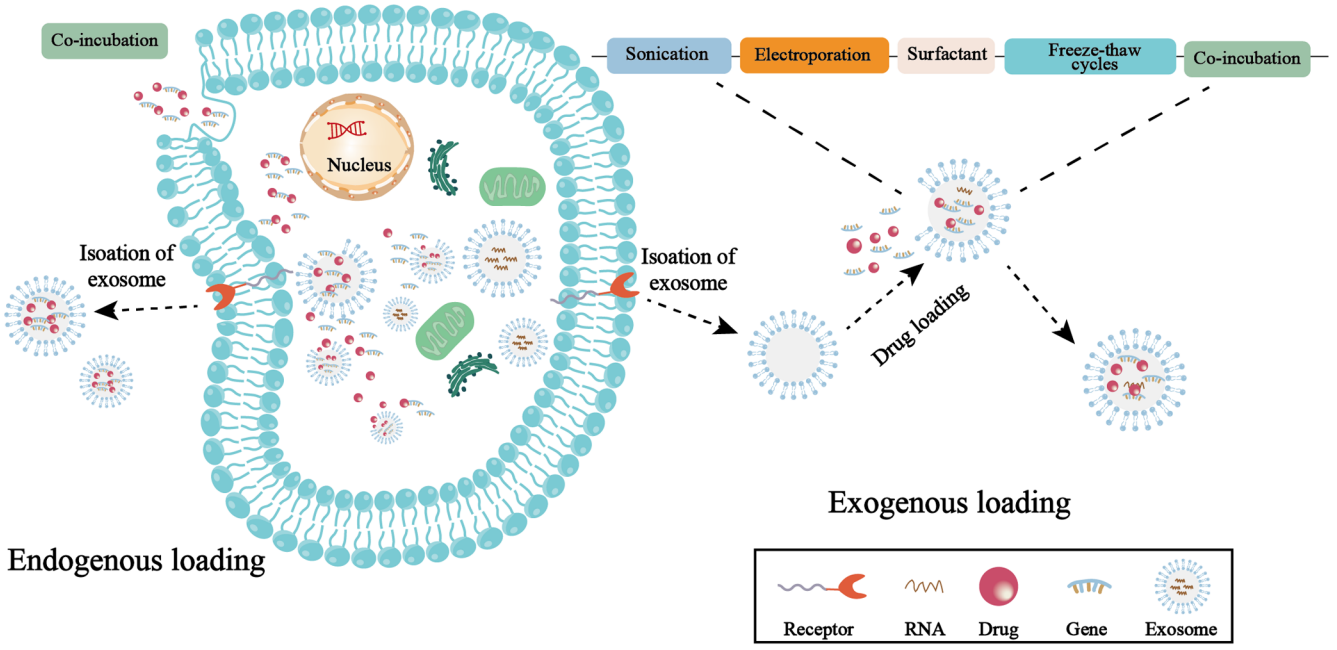

Figure 2. Endogenous loading and exogenous loading of material engineering exosomes.

engineered exosomes are divided into drug loading and surface modification.[35]

3.1. Drug Loading. Low molecular weight therapeutic agents (nucleic acids, peptides, and proteins) or artificial nanoparticles can be loaded into exosomes as a vehicle for combination therapy.[36,37] According to the loading method, it can be divided into endogenous loading and exogenous loading. As shown in Figure 2, the endogenous loading is the carrying of drugs into the parent cells, and the parent cells secrete drug-carrying exosomes. Exogenous loading is the direct loading of drugs into isolated exosomes.[38]

3.1.1. Endogenous Loading. Endogenous loading is the coincubation of cargo with parental cells so that the cargos enter the parental cells and the parental cells load the cargos in the cells into intracellular multivesicular bodies during the production of exosomes, which travel down the luminal vesicles to the extracellular vesicles.[39,40] For instance, Ma et al. fabricated exosomes coated with polydopamine (PDA) through coincubating 293T cells high in expression of ACE2 receptor with PDA nanoparticles, which was used for COVID-19 pneumonia treatment. The results showed that the anti-inflammatory ability of PDA and the competitive binding ability of exosomes exhibited synergistic therapeutic effects, which was contributed to significantly reduced inflammatory cytokine levels and less lung tissue damage by mediating oxidative stress.[41] The endogenous loading method offers simple operation and low equipment requirements. However, the loading efficiency is low, as well as the drug may be toxic to cells during incubation.[39,42]

3.1.2. Exogenous Loading. The fluidity of exosomal lipid bilayers facilitates exogenous loading, which refers to the encapsulation of cargo into isolated and purified exosomes and can be divided into passive and active encapsulation.[43,44] One of the passive encapsulation methods is coincubation of the drug with exosomes at a suitable temperature and pH so that the cargos can be passively diffused through the membrane into the exosomes, which is fast and simple with no effect on exosome integrity.[45] For instance, Hosseini et al. loaded doxorubicin (DOX) into aptamer surface functionalized exosomes (DOX-Apt-Exo) through coincubation for the targeted treatment of colorectal cancer. This study showed that 200 μg exosomes were incubated with 300 μg/mL DOX to obtain the best loading capacity with an encapsulation efficiency close to 13%. Compared with free DOX, DOX-Apt-Exo improved the target homing ability and reduced the cytotoxicity of DOX.[46] Passive encapsulation methods are more suitable for hydrophobic and small molecule cargoes, while for hydrophilic and macromolecular cargoes, stronger stimulation is required to encapsulate them into the exosomes.[47]

Active encapsulation uses mechanical techniques to transiently and reversibly open exosomal membranes to allow cargos to enter the exosomes, with common methods such as sonication, electroporation, freeze−thaw cycles, coextrusion, and surfactant.[39,48,49]

Sonication is the use of ultrasonic energy to reduce the rigidity of membranes.[50] Inspired by liposome preparation, sonication has been applied to load drugs into exosomes.[51] Probe sonication is often used for transient pore formation in exosomal membranes, thus allowing cargos, including nanoparticles, small molecule drugs, nucleic acids, and proteins, to enter the exosomes through simple diffusion. For example, Kanchanapally et al. loaded Honokiol into mesenchymal stem cell (MSC) exosomes by sonication for enhancing antitumor activity against pancreatic cancer cells.[52] However, the large local energy brought by ultrasound may lead to thermal decomposition of some drugs, compromised exosome integrity, and changes in exosome size.[53] Therefore, optimized temperature control and other processing conditions cannot be neglected.

Electroporation, with the help of a conductive buffer, the exosome membrane is stimulated by an external electrical pulse to transiently open small recoverable pores that allow molecules to pass through the membranes.[54] Different potentials are applied according to the source and concentration of exosomes, the parameters can be well controlled in a

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simple way that has become one of the most common methods to load various molecules into exosomes. For instance, Zhang et al. loaded miR-665 into exosomes secreted from osteosarcoma cells by electroporation at 400 V potential and showed proliferation inhibition and apoptosis induction in osteosarcoma cells.[55] In addition, Rodríguez et al. used exosomes extracted from three different cell lines for loading bioactive peptides (insulin) to test the delivery efficiency under different conditions. The results showed that 200 V electroporation achieved the best insulin loading efficiency compared to room temperature incubation, and the maximum exosome uptake was reached at 6 h incubation *in vitro*.[56] The disadvantage of electroporation is that cargos such as siRNA, proteins and DNA tend to aggregate, so addition of alginate disaccharide, citric acid, and ethylenediaminetetraacetic acid (EDTA) to the conductive buffer and optimization of electroporation parameters are helpful to prevent nucleic acid aggregation and improve encapsulation efficiency.[57−59]

The freeze−thaw cycles, in which the exosomes and drug mixture are rapidly frozen at −80 °C and then thawed at room temperature, should be cycled at least three times. The ice crystals formed during the freezing process can temporarily destroy the phospholipid bilayer and then the drug would import into the exosomes.[60] The freeze−thaw cycles drug loading strategy has no significant effect on the structure of exosomes and does not damage the biological properties of exosomes.[61] In general, the freeze−thaw cycles method has low loading efficiency, which is affected by the number of freeze−thaw cycles, temperature, and pH. To improve the loading efficiency, the freeze−thaw cycles method can be improved by combining coincubation, ultrasonic treatment, and surfactant treatment. For example, Ebrahimian et al. combined three methods of incubation, surfactant and freeze−thaw cycles to load the anticancer drug Thymoquinone (Tq) into exosomes secreted by MSCs, and the combination of these three methods has resulted in a 60% loading efficiency of Tq and improved the disadvantages of Tq's instability and poor solubility, while reducing the toxic and side effects of systemic drug delivery.[62]

Co-extrusion is a method in which a mixture of exosomes and cargos together are repeatedly pushed through a polycarbonate membrane with a pore size of 100−400 nm, during which the exosome membranes are broken and mixed with the cargos. It can also be produced by extruding cells or polymer−nanoparticles coating the cell membrane to produce a large number of nanovesicles, which can be used as exosome mimics for drug delivery.[63,64] For instance, Meng et al. mixed M1 macrophages and docosahexaenoic acid (DHA) and extruded them 21 times repeatedly through polycarbonate membrane filters with pore sizes of 1 μm, 0.4 and 0.2 μm using a microextruder to obtain nanoscale vesicles. *In vitro* experiments showed that the M1 macrophage-derived exosome mimics could effectively load DHA, promoted DHA accumulation and the induction of ferroptosis in tumor cells.[65] Extrusion processing has been shown to have high loading efficiency and uniform size distribution, although intense extrusion and excessive shear stress may alter the properties of the exosome membrane, such as the alteration of zeta potential and surface protein structure.[66,67]

Surfactants such as saponins or triton can interact with exosomal membrane cholesterol to create small holes and increase the permeability of the exosome membranes, which promote cargos loading.[68,69] Under the suitable conditions, surfactant strategy has high loading efficiency and hardly damage the exosome integrity. For instance, Jang et al. used incubation, saponin-assisted incubation, extrusion, freeze−thaw cycles, sonication, and electroporation to load astaxanthin (AST) into fetal bovine serum extracellular vesicles (FBS-EV). The results showed that AST was loaded into vesicles by saponin-assisted incubation with the highest loading efficiency.[70] Currently, saponins are the most widely used surfactants in this field. However, saponins have a risk of hemolysis, some drugs require high doses of saponins to assist in loading or have reagent residues due to technical limitations that may limit their application *in vivo*.[71,72]

3.2. Surface Modification. Exosomes can be easily surface modified by bioengineered methods. Besides, surface modified exosomes play an important role in therapeutic applications in the field of nanomedicine, including extended circulation, specific activity targeting, and therapeutic efficacy.[73] Bioengineered methods can be classified as chemical modification, physical modification, and genetic modification.

3.2.1. Chemical Modification. Chemical modification techniques can induce molecules to bind to exosome membranes through covalent and noncovalent interactions without damaging the membrane structure.[74,75] Covalent modifications are used to form stable covalent bonds between the functional groups of the molecule and the exosome. Common covalent modifications include click chemistry and PEGylation. Click chemistry uses covalent interactions between alkynes and azide residues to form stable triazole bonds that can be used to link the targeted portion to the exosome surface in a variety of water-based buffers, including water, alcohols, and dimethyl sulfoxide.[76,77] Neuropilin-1-targeted peptide (RGERPPR, RGE) is a specific targeting peptide for glioma cells. On the basis of the click chemistry principle, Jia et al. affixed RGE peptide with exosomes through the cycloaddition reaction of sulfonyl azide. The experimental results showed that RGE peptide modified drug-loaded exosomes can well cross the blood-brain barrier and effectively concentrate in the tumor area, which got excellent results for targeted imaging and treatment of glioma.[78]

PEGylation is the modification of the exosome surface with branched polyethylene glycol (PEG), the problem of rapid exosome removal can usually be solved by introducing PEG molecules to cover the membrane surface.[79,80] Zhang et al. have been proved that PEGylation of exosome-like nanovesicles can significantly improve the blood circulation cycle and enhance the tumor targeting ability of nanovesicles for inhibiting the proliferation of hepatoma carcinoma cells.[81] The disadvantage of covalent modification is that the induction process of covalent bond may require the involvement of toxic chemicals, so the application of covalent modification methods in therapy needs to be cautious.

Exosome membranes can also be modified using noncovalent modifications such as receptor−ligand binding, electrostatic interaction, and hydrophobic insertion.[75] Electrostatic interaction involves the interaction of the cationic targeting portion with the negatively charged functional groups of the exosome membrane. Due to the lipid bilayer of the exosome membrane, hydrophobic interactions allow direct insertion of the targeting portion into the exosome membrane.[82,83]

3.2.2. Physical Modification. Physical modifications change the surface construction of exosomes through the use of physical forces. Exosomal membranes are able to sponta-

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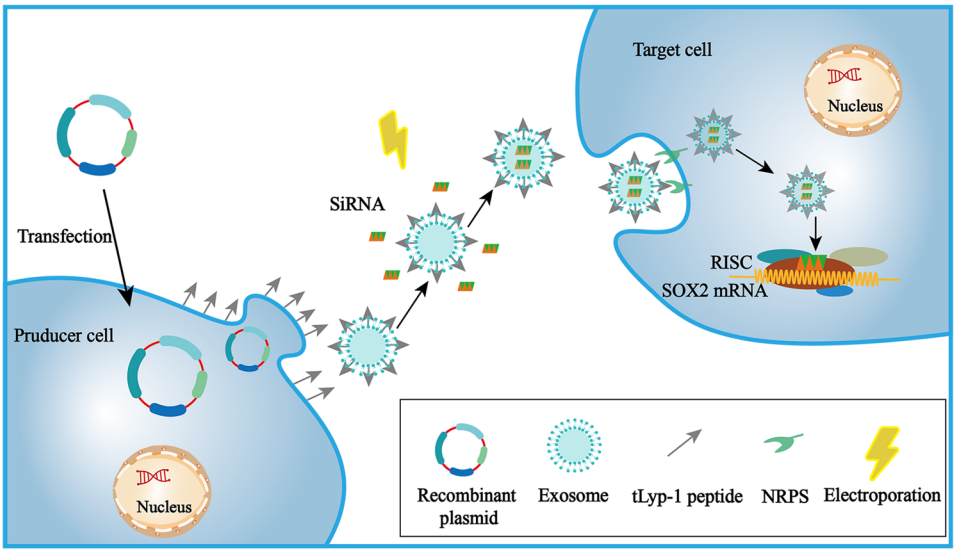

Figure 3. Recombinant plasmid transfected with parental cells and secreted exosomes with targeting portion.

neously fuse with other plasma membranes, and the methods of membrane fusion are usually based on pH difference, freeze−thaw, extrusion, PEG, and natural incubation.[84−86] pH-mediated is membrane bending modulus increases in acidic environments, leading to reorientation of membrane lipid polar groups, which may alter the energy distribution of lipid membrane fusion.[87,88] Freeze−thaw-mediated is the expansion of water in the lipid bilayer during freezing, resulting in changes in the fluidity, curvature, and charge of the lipid head, which are beneficial for interactions between membranes and promote the membrane fusion process during thawing.[89] Extrusion-mediated fusion is achieved using filters or nanopores by applying pressure that causes membrane lipid bilayers to deform and cross and eventually fuse.[90] PEG-mediated fusion involves the use of PEG-modified lipids or liposomes, which facilitate the fusion of two lipid membranes by reducing the interaction energy between the lipid bilayers through PEG.[91] Natural incubation-mediated involves spontaneous membrane fusion by electrostatic or hydrophobic interactions of the membrane itself.[92] Exosome-liposome hybridization methods can be used to optimize the properties of exosome surfaces to reduce immunogenicity, promote colloidal stability, and increase exosome half-life in the blood and target cell uptake.[93] Hybridization increases the size of exosomes and helps to improve drug loading efficiency and facilitate loading of macromolecular cargos. Gomes et al. fused long-circulating and pH-sensitive liposomes containing DOX to breast cancer tumor-derived exosomes for the treatment of breast cancer, with 88.5% DOX encapsulation, lower toxicity, and higher antitumor effect.[94] The disadvantage of membrane fusion is that it changes the integrity and orientation of exosomal membrane proteins, which may weaken the biological function of exosomes. Physical modifications, in addition to membrane fusion, can also involve incorporating lipid components or cell membrane staining dyes with lipophilicity/hydrophobicity into the exosomal membrane as anchors. For example, Kang et al. utilized a fluorescent lipophilic boron-dipyrromethene as a cell anchor and coupled it with a targeted ligand separated by a PEG spacer to create targeted engineered exosomes. Modified exosomes encapsulated with DOX significantly inhibited melanoma growth both *in vivo* and *in vitro*.[95] Antes et al. embedded modified glycero-phospholipid-PEG conjugate into vesicular lipid bilayer membranes as anchors for biotinylated fluorescent molecules or ligand protein couplings to construct an exosome membrane anchoring platform, where any biotinylated molecule can its coupling for extracellular vesicle decoration.[96]

3.2.3. Genetic Modification. With gene modification techniques, it is possible to promote site-specific insertion, deletion, or modification at specific sites in the genome, which indirectly improves exosomal function. This is achieved by plasmid DNA or mRNA transfection of parental cells expressing genes of targeted fractions (such as peptides, receptors, and antibodies) that are fused to exosomal membrane components (such as tetraspanins and lysosome-associated membrane proteins).[97] The genetically modified cells produce exosomes that express targeted parts through the normal process of exosomal biogenesis. As shown in Figure 3, Bai et al. used the engineered tLyp-1-lamp2b plasmid to transfect HEK293T tool cells, and the plasmid-transfected HEK293T cells could secrete tumor-targeting tLyp-1 exosomes, which was used to encapsulated siRNA by electroporation technique. The results showed that targeting siRNA tLyp-1 exosomes are able to knock-down the target genes of cancer cells and reduce the stemness of cancer stem cells.[98] Furthermore, Du et al. transfected donor cells HEK293T with CD47-overexpressing plasmid to obtain CD47 surface functionalized exosomes, and loaded with ferroptosis inducer (Erastin, Er) and photosensitizer (Rose Bengal, RB) into the exosomes by sonication. The results showed that CD47-functionalized exosomes displayed high delivery efficiency to tumors.[99] Compared to chemical modification methods, gene modification acts on exosomes before their biogenesis, which preserves the integrity and functionality of the exosomes.[100] However, this method is complex, costly, has low reproducibility, and insufficient transfection efficiency remains a concern.[101] The mechanisms, advantages and disadvantages of different engineered exosomes strategies are listed in Table 1.

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Table 1. Comparison of Different Strategies for Engineered Exosomes

engineering strategy			mechanisms	advantages	disadvantages	ref.
drug loading	passive-cargo loading	co-incubation	drugs and parental cells/exosomes coincubated for sometimes	simple operation, no additional equipment required, no effects in exosome integrity	low loading efficiency, drug cytotoxicity, more effective for hydrophobic small molecule drugs	102,103
	active-cargo loading	sonication	weakening membrane rigidity with ultrasonic energy	high loading efficiency	heat generation, affecting exosome integrity and size	53,30
		electroporation	reversible pores opened by electrical pulse stimulation of the exosome membrane	high loading efficiency, useful for loading various drugs and macromolecules, ease in control	aggregation of nucleic acids and proteins	57,104
		freeze−thaw cycles	the mixture of drug and exosome is rapidly frozen at −80 °C, thawed at room temperature, and repeatedly freeze−thawed for more than three times	simple operation, no damage to exosome biology	low loading efficiency, aggregation of exosome membranes	61,103
		co-extrusion	drugs and exosomes are repeatedly extruded through porous membranes of 100−400 nm	simple operation, high loading efficiency, uniform size distribution of exosomes	might affect the properties of the membrane	67,105
		surfactant	interaction with cholesterol on the exosome membrane produces pores	high loading efficiency, no effects in exosome integrity	hemolysis risk	71
surface modification	chemical modification	click chemistry	covalent interactions between alkynes and azide residues	simple, effective, and enhanced targeting	possibility of membrane protein alteration	75,106
		PEGylation	modification of exosome surface with branched polyethylene glycol	extending the cycle of exosomes *in vivo*	toxic reagents may be required	79,107
	physical modification	membrane fusion	inducing fusion of exosome membranes with other plasma membranes	optimizing exosome membrane properties, improving loading efficiency	weakening of exosome function	86,108
		anchors	incorporating lipid components or cell membrane staining dyes with lipophilicity/hydrophobicity into the exosomal membrane as anchors	enhanced targeting, traceability	complicated operation, lipids tend to aggregate in aqueous environments	95,109
	genetic modification	transfection	transfection of parental cells with plasmids with target genes	preserved exosome integrity and function	complex, costly and insufficiently efficient transfection	100,101

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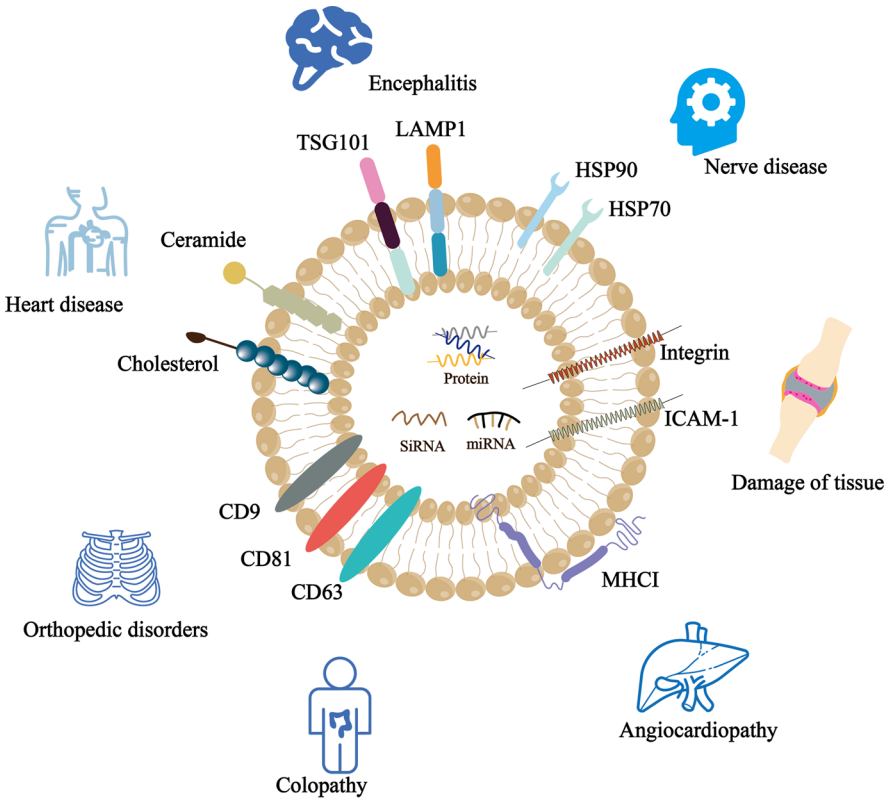

Figure 4. Application of engineered exosomes.

4. APPLICATION OF ENGINEERED EXOSOMES

Engineered exosomes are obtained by modifying natural exosomes with a series of bioengineering techniques.[74] Engineered exosomes as a type of exosomes are tiny vesicles secreted by cells into the extracellular space, involved in the exchange of substances between different cells and play important biological functions in normal physiological and pathological activities. Engineered exosomes with functionalized ligands modified on the membrane surface or nanomaterials encapsulated inside have been applied for disease treatment.[110] Compared with some other delivery systems, engineered exosomes have excellent endogenous and biocompatibility, and can exist stably in body fluids and are not readily cleared by phagocytes, which can provide better protection for drugs.[111,112] Currently, engineered exosomes have been widely applied as targeted drug carriers, such as in cancer, cardiovascular diseases, brain diseases and ulcerative colitis.[113−116] (Figure 4).

4.1. Engineered Exosomes for Cancer Therapy. Cancer, as a disease with high lethality, recurrence, and drug resistance, has always been a challenge to human health problems.[117] Exosomes are nanoscale vesicles secreted by various cells and are capable of participating in a variety of cancer therapeutic processes including antiapoptosis, metastasis, angiogenesis, and immune evasion.[118−120] Due to the biological properties of exosomes, it can provide a stable environment for cancer therapeutic agents and can fuse with the cell plasma membrane to enter cells directly to reduce immune rejection. It is often used to transport antitumor drugs to achieve therapeutic effects.[121] The engineered exosomes obtained through genetic, physical, or chemical modifications have all the biological properties of natural exosomes. In addition, it can improve the shortcomings of natural exosomes such as weak drug loading capacity and poor targeting ability of lesion sites.[122,123] Engineered exosomes are capable of fusing target proteins or gene sequences to the gene sequences of exosomal membrane surface proteins, which produces the corresponding specific proteins to perform their responsive biological functions.[124] For instance, Qiao et al. concluded that tumor exosomes can bind to parental cancer cells and are able to extend the duration of action of DOX in tumor cells, showing excellent therapeutic effects in nude mice. It provides a new direction for targeted therapy of cancer.[125] Zhang et al. developed a kind of neutrophil-derived exosome modified with superparamagnetic iron oxide nanoparticles to induce tumor cell apoptosis. Compared with natural exosomes, DOX loaded exosomes can improve the inhibitory effect on tumor cells and can selectively accumulate in the tumor site to inhibit the growth of tumor cells.[126] Through chemical modification of exosome surface membrane proteins to load different types of ligands, the targeting ability and range of action of exosomes can also be enhanced. For example, Cheng et al. introduced fusion proteins containing CD3 antibody, epidermal growth factor receptor (EGFR) antibody, and platelet-derived growth factor receptor (PDGFR) antibody into cells by cell transfection technique, so that the fusion proteins were expressed on exosome membrane proteins to form exosomes with high affinity for breast cancer cells and T cells, which can specifically cluster T cells around cancer cells and enhance the targeting power of T cells.[127]

4.2. Engineered Exosomes for Cardiovascular Disease Therapy. Cardiovascular disease is a type of disease involving the heart and blood vessels that is high fatality rate and difficult to treat.[128,129] Cardiomyocytes, vascular cells, and fibroblasts

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Table 2. Application of Engineered Exosomes in Disease Therapy

disease model	exosome source	engineering trim	outcomes	ref.
hypertension	fibroblasts	MiR-155−5p	inhibition of angiotensin-converting enzyme gene reduces angiotensin expression and vascular remodeling	149
heart attack	cardiac progenitors	MiR-322	effectively reduced infarct size and fibrosis in mouse models and increased cardiovascular formation	150
neurospinal cord injury	BMSCs	peptide	retains and sustains release, effectively reduces the inflammatory response	151
hepatocellular carcinoma	BMSCs	MiR-199a	transfer of miR199a to hepatocellular carcinoma cells and increased sensitivity of cancer cells to adriamycin	152
colon cancer	THLG-293T	MiR-21/5-Fluorouracil	target lesion sites, down-regulate to reduce tumor proliferation and increase apoptosis	153
osteoarthritis	ADSCs	MiR-140	target chongrocytes, delivery of miR-140, effectively alleviate the symptoms in mouse models	154

release exosomes in the heart with low immunogenicity, and cardiac exosomal miRNAs are able to regulate the expression of sarcomeric genes, antifibrotic activity, and angiogenesis.[130−132] The modification of natural exosomes into engineered exosomes by bioengineering technology can not only optimize its various biological functions, but also compensate for the weak targeting ability and low drug loading efficiency of natural exosomes. It has been shown that genetic modification of engineered exosomes loaded with miR-425 and miR-744 can target the TGF-β pathway to inhibit angiotensin-induced collagen and fibrin synthesis as a way to inhibit myocardial remodeling.[133] Engineered exosomes have shown great potential for application in the treatment of cardiovascular diseases.

Engineered exosomes are widely used for the treatment of cardiovascular diseases, including myocardial infarction, atherosclerosis, and dilated cardiomyopathy. Modifications such as exosome genetic programming or pharmacological intervention can enhance their heart repair function. The enrichment of proangiogenic miRNAs in exosomes secreted by cardiosphere-derived cells (CDCs) improved the palliation of cardiac fibrosis and reduced the apoptosis of cardiomyocytes after myocardial infarction. In addition, engineered exosomal miRNAs were able to greatly inhibit the expression of protein kinase C and enhance the metastasis of M1 and M2 macrophages.[134] For example, Cheng et al. modified mesenchymal stem cell-derived exosomes with hyaluronic acid hydrogel to prepare an injectable Exo-Gel, which was injected into the pericardial cavity of rats with transverse aortic constriction induced heart failure. Exo-Gel therapy reduced LV chamber size and preserved wall thickness. It was confirmed the safety and feasibility of Exo-Gel injection in a pig model.[135]

4.3. Engineered Exosomes for Ulcerative Colitis Therapy. Ulcerative colitis is a chronic nonspecific inflammatory disease at the colonic mucosa that mainly caused by an immune imbalance between immune response and immune tolerance.[136] The current therapies for ulcerative colitis are mainly surgical and pharmacological treatments, while engineered exosomes are a novel strategy for the therapy of ulcerative colitis for its powerful cellular communication and targeting ability in the treatment of ulcerative colitis.[112,137]

Mesenchymal stem cell-derived exosomes (MSC-Exos) have been used as a new strategy for the therapy of ulcerative colitis.[138,139] Compared with traditional treatment methods, MSC-Exos have excellent biocompatibility, active targeting ability, the ability to aggregate at specific lesion sites for effect, and no toxic side effects.[140] However, intravenous therapy for ulcerative colitis may cause some exosomes to remain in the liver and lungs, resulting in poor therapeutic effect. For instance, Deng et al. have used a layer-by-layer (LbL) self-assembly technique to construct an efficient LbL-Exos exosome, which could be released in colitis and alleviate ulcerative colitis by controlling the MAPK/NF-KB signaling pathway.[141]

4.4. Engineering Exosomes for Brain Disease Therapy. Engineered exosomes have excellent efficacy in the therapy of neurodegenerative diseases, brain cancer, and encephalitis due to the advantages of high targeting and efficient drug delivery.[142−144] Engineered exosomes have been shown to be involved in mediating several physiological processes required for normal brain function.[145] Compared with other therapeutic drug delivery systems, engineered exosomes have immunomodulatory effects, strong targeting of lesion sites, and blood-brain penetration.[146] Moreover, the engineered exosomes are modified to reduce their distribution in the liver and spleen, and more effectively target to the brain tissue, which is a promising new approach for the treatment of brain diseases.[147] For instance, Yu et al. have proved that blood-derived exosomes have natural brain-targeting capabilities.[148] Qu et al. loaded dopamine into blood-derived exosomes by coincubation for the treatment of Parkinson's disease (PD). The experimental results showed that the engineered exosomes had better therapeutic efficacy and lower toxicity in a PD mouse model.[144] Therefore, engineered exosomes can be used as a new platform for targeted drug delivery for the treatment of central nervous system diseases. The applications of different sources of engineered exosomes in the therapy of different diseases are listed in Table 2.

5. SUMMARY AND OUTLOOK

To overcome the complex environment in the human body, researchers have been dedicated to exploring various drug delivery carriers. Exosomes, once used as "garbage bags" to dispose of unwanted cell contents, have been proven to have a similar composition to parental cells and play a crucial role in intercellular communication.[155,156] Exosomes with higher biocompatibility, greater tissue penetration and inherent bioactivity are ideal carriers for targeted drug delivery. To overcome the shortcomings of natural exosomes, engineered exosomes loaded with drugs or with surface modifications significantly improve drug loading efficiency, enhanced targeting, as well as extended circulating half-life.[112] Engineered exosomes have shown significant therapeutic effects in various diseases, such as cancer, cardiovascular diseases, ulcerative colitis and brain diseases. However, there are still some factors that limit the practical application of engineered exosomes in clinical applications. For example, the low production of exosomes and the high cost of engineered

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exosomes.[157,158] In order to achieve exosome isolation and purification as well as the large-scale production of engineered exosomes, the limitations by technology and equipment still need to be optimized and solved.[159,160] In addition, different sources of exosomes have different membrane compositions and biological functions. Therefore, the safety and efficacy of different sources of exosomes need to be further evaluated rather than simply acting as empty carriers. For example, tumor cell-derived exosomes are commonly researched for improving the targeting efficiency against cancer cells, but their potential carcinogenic risk must be considered.[161] Despite the challenges, the unique properties of exosomes have attracted extensive attention of many researchers. Exosomes from plant and milk sources have been explored as a relatively economical alternative option.[162,163] In addition, compared to mammals, plants do not carry human pathogens and some plant-derived exosomes carry certain naturally active components. For example, a study found that orally administered ginger-derived exosomes reduced the release of pro-inflammatory factors and promoted intestinal mucosal healing.[7,164] It is believed that as these aforementioned issues continue to be addressed and limitations are broken, engineered exosome-based targeted drug delivery systems may become a safe and efficient novel strategy for the clinical treatment of many diseases in the future.

AUTHOR INFORMATION

Corresponding Authors

Zhenhua Chen − *Jiangxi Province Key Laboratory of Drug Design and Evaluation, School of Pharmacy, Jiangxi Science and Technology Normal University, Nanchang 330013, P. R. China*; orcid.org/0000-0002-6860-5829; Email: zhenhuachen@jxstnu.edu.cn

Jin Wang − *Jiangxi Province Key Laboratory of Drug Design and Evaluation, School of Pharmacy, Jiangxi Science and Technology Normal University, Nanchang 330013, P. R. China*; Email: 1020220803@jxstnu.edu.cn

Authors

Lianghui Huang − *Jiangxi Province Key Laboratory of Drug Design and Evaluation, School of Pharmacy, Jiangxi Science and Technology Normal University, Nanchang 330013, P. R. China*

Enguang Wu − *Jiangxi Province Key Laboratory of Drug Design and Evaluation, School of Pharmacy, Jiangxi Science and Technology Normal University, Nanchang 330013, P. R. China*

Jiawei Liao − *Jiangxi Province Key Laboratory of Drug Design and Evaluation, School of Pharmacy, Jiangxi Science and Technology Normal University, Nanchang 330013, P. R. China*

Zongyi Wei − *Jiangxi Province Key Laboratory of Drug Design and Evaluation, School of Pharmacy, Jiangxi Science and Technology Normal University, Nanchang 330013, P. R. China*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c04479

Author Contributions

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ABBREVIATIONS

DDS, drug delivery system; EV, extracellular vesicle; PDA, polydopamine; EDTA, ethylenediaminetetraacetic acid; DHA, docosahexaenoic acid; AST, astaxanthin; PEG, polyethylene glycol; EGFR, epidermal growth factor receptor; PDGFR, platelet-derived growth factor receptor; LbL, layer-by-layer; PD, Parkinson's disease

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