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Progress of research on engineered extracellular vesicles from different sources for disease treatment

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Summary. Extracellular vesicles (EVs) are lipid bilayer nanoparticles that encapsulate proteins, lipids, nucleic acids, and small molecules and display low immunogenicity, high stability, and cross-species transmission. By applying engineering technologies, the surface of EVs can be modified and loaded with cargo with therapeutic properties. Thus, engineered EVs can play important roles in preventing and treating various diseases. However, many challenges and uncertainties are faced in the clinical translation of EVs. In this review, we comprehensively analyzed the types of EVs derived from animal cells, plant cells, and microorganisms and summarized their biological properties and potential for engineering modifications. Furthermore, we also explored their therapeutic potential and discussed recent advancements in relevant clinical trials, aiming to provide scientific guidance for future research on the engineering of EVs and precision treatment of clinical diseases.

Key words: Extracellular vesicles, Cargos, Engineering modification, Disease treatment, Clinical trial

Introduction

Extracellular vesicles (EVs) are nanoparticles released by natural membranes that cannot replicate and contain bioactive proteins, lipids, and nucleic acids, which can potentially be transferred to recipient cells to exert different functions (Zhao et al., 2024b). Natural EVs have gained interest as modern drug vehicles for targeted therapy due to their higher biocompatibility, lower immunogenicity, and better capacity for biofilm fusion compared with those of traditional artificial drugs (Chen et al., 2024a). However, the application of natural EVs in the treatment of diseases is constrained by their short half-lives, limited targeting ability and low yield. To address this, EVs can be loaded with drugs and artificially modified to target certain recipient cells, thereby broadening their prospects for clinical utility (Chen et al., 2024a). The main strategies for EV engineering include genetic engineering (GE), EV exogenous membrane modification, and exogenous loading cargos. A previous study revealed the effectiveness of engineered animal EVs (AEVs), plant-derived EVs (PEVs), and bacteria-derived EVs (BEVs) in the treatment of diseases (Jones et al., 2022). Dendritic cell-derived EVs have been used to encapsulate melanoma tumor cell epitope peptides and, once introduced into the organism, target and activate $CD8^+$ T cells to kill melanoma cells (Escudier et al., 2005). In another study, anti-inflammatory methotrexate (MTX) was mixed with grapefruit-derived PEVs to develop MTX-PEVs, which improved colitis in mice (Wang et al., 2014). BEVs can be categorized into two types: cytoplasmic membrane vesicles (CMVs) released by Gram-positive bacteria and outer membrane vesicles (OMVs) released by Gram-negative bacteria (Jnana et al., 2023). In another study, siRNA targeting kinesin spindle protein (KSP) was loaded into *E. coli* OMVs, which were injected into ovarian tumor cells, resulting in increased apoptosis (Gujrati et al., 2014). These studies show that engineered EVs originating from animals, plants, or bacteria have promising clinical applications (Herrmann et al., 2021). Engineered EVs represent a significant advancement in the field; however, their application is limited by the lack of evaluations of their clinical safety (Liu et al., 2023c). Thus, in this review, we systematically summarized the biogenesis of natural EVs and their cargos, highlighted the strategies for engineering EVs and their applications in treating various diseases, and discussed future challenges and research directions in the field of engineered EVs.

Biological properties of EVs

EVs are found in biological organisms such as animals, parasites (parasites EVs, PAEVs), plants,

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bacteria, fungi (fungi EVs, FEVs), and mycoplasma (mycoplasma EVs, MPEVs) (Zeng et al., 2024a). According to size and occurrence, EVs are divided into exosomes, microvesicles and apoptotic bodies. However, there is no strict consensus on upper and lower size cut-offs. Following the suggestions of the International Society for Extracellular Vesicles, the generic term "EVs" will be used in this review, which are classified by the prefix of biological origin (Welsh et al., 2024) (Table 1). In the following section, the biological properties of EVs are briefly introduced, including the EV biogenesis and cargo.

Biogenesis of EVs

Increasing evidence has shown that there are significant differences in the biogenesis of EVs among various species (Fang et al., 2022). Notably, it has been widely accepted that the biogenesis of AEVs, PAEVs, and FEVs primarily occurs via the endosomal mechanism and membrane budding (Jiang et al., 2024) (Fig. 1a). Briefly, they commence with the invagination of the plasma membrane, resulting in the formation of an early endosome (EE). The Golgi can also contribute to the formation of EE. After maturation, the EE undergoes further invagination, leading to the formation of intraluminal vesicles (ILVs). These ILVs subsequently cluster to create multivesicular bodies (MVBs). The maturation of MVBs facilitates their fusion with the plasma membrane, mediated by molecules such as phosphatidylinositol, culminating in the extracellular release of ILVs as AEVs (Han et al., 2022). Moreover, AEVs, PAEVs, and FEVs are formed through direct outward budding and fission of the plasma membrane (Ma et al., 2025).

Alternatively, in addition to the endosomal pathway described above, PEVs can be produced through the vacuole regulatory pathway and the exocyst-positive organelle (EXPO) pathway (Fang et al., 2022) (Fig. 1b). The vacuole can release antibacterial proteins outside of *Arabidopsis* cells by fusing with the plasma membrane during infection by *P. syringae* (Rutter and Innes, 2017). Small vacuoles (SVs) containing ILVs were mainly matured from MVB fusion, while central vacuoles are derived from the subsequent fusion of SVs, suggesting potential connections between the vacuolar and endosomal pathways (Cui et al., 2019b). The EXPO pathway represents an unconventional secretion in plants characterized by a double-membrane EXPO that fuses with the plasma membrane and releases EVs into the cell wall (Wang et al., 2010).

MPEVs bud from the surface of the mycoplasma cells (Gaurivaud et al., 2018), where treatment of mycoplasma culture with ciprofloxacin elicits a notable increase in the level of MPEVs (Medvedeva et al., 2014). However, research concerning the mechanisms involved in the biogenesis of MPEVs remains limited (Gaurivaud and Tardy, 2022). The biogenesis processes of OMVs can be classified into two main forms: outer membrane budding and cell autolysis (Toyofuku et al., 2023). OMVs are primarily generated in the peptidoglycan (PG) layer sites, which interact less with the outer membrane. The formation of OMVs is triggered by the accumulation of lipids, PG fragments, or misfolded proteins in the periplasmic space, which exert pressure against the outer membrane. Consequently, OMVs do not contain cytoplasmic contents but are rich in periplasmic proteins. Furthermore, some outer membrane components, such as lipopolysaccharide (LPS), PG, and other molecules, can enhance the bending process (Gan et al., 2023). Moreover, the PG layer of bacteria can be degraded by autolysin, phage invasion, and endolysin expression, resulting in the inner membrane protruding into the peripheral space or the membrane fragments generated by explosive cleavage of bacteria to form OMVs, thereby producing OMVs containing cytoplasmic contents. Bacteria can also autolyze and release OMVs when treated with sucrose fatty acid esters or exposed to environmental stress, such as cold shock, starvation, and hypoxia (Ofir and Sorek, 2017) (Fig. 1c). In contrast, CMVs are released directly from the bacterial cell membrane (Fig. 1d), which can be

Table 1. Biological properties of EVs.

		Size (nm)	Surface marker	Biogenesis	Reference
AEVs	Exosomes	30-150	CD9, CD63, CD81	Endosomal mechanism	Krylova and Feng, 2023
	Microvesicles	150-1000		Membrane budding	Xiong et al., 2024
	Apoptotic bodies	1000-5000		Programmed cell death	
PAEVs		30-500	HSP70, HSP83, PRL-1	Endosomal mechanism	Wowk et al., 2017; Da Silva Lira Filho et al., 2024
PEVs		30-500	PEN1, TET8	Endosomal mechanism, Vacuole, EXPO	Rutter and Innes, 2017
BEVs	OMVs	20-1000	LPS, OMPA, OMPF	Outer membrane budding, cell autolysis	Liu et al., 2022; Xie et al., 2024a
	CMVs	10-400	Lipoprotein	Membrane budding	Kopparapu et al., 2021
FEVs		20-100	SUR7, EVP1	Endosomal mechanism	Dawson et al., 2020
MPEVs		70-220	Lipoprotein	Membrane budding	Chernov et al., 2014; Gaurivaud and Tardy, 2022

HSP, heat Shock Proteins; TET9, tetraspanin 9; PEN1, penetration1; TET8, tetraspanin 8.

induced by endolysins, antibiotics, the environmental conditions surrounding the bacteria, spontaneous cell lysis within bacterial populations, cell wall-modifying enzymes, and PG-damaging enzymes (Toyofuku et al., 2017; Liu et al., 2024a).

Cargos carried by EVs

EVs encapsulate nucleic acids, proteins, and lipids, but predominantly nucleic acids (mainly sRNA and lncRNA), which are involved in various biological processes, including cellular communication, the regulation of physiological and pathological processes, intrinsic defense mechanisms, and immune system regulation (Chen et al., 2024c). AEVs and PAEVs can encapsulate viruses and, thus, could potentially influence disease progression by mediating viral spread. AEVs from liver cancer cells can encapsulate the hepatitis B virus (Wu et al., 2023), while PAEVs released by *Leishmania* encapsulate *Leishmania protozoa* RNA virus 1, which triggers severe cutaneous leishmaniasis in mice (Atayde et al., 2019). Furthermore, emerging research has suggested that AEVs encapsulate and transport malfunctioning organelles when the lysosome is compromised. AEVs entrap and remove damaged mitochondria to sustain cellular homeostasis and prevent the buildup of harmful metabolites, and reactive oxygen species (ROS) (Liu et al., 2023b). Moreover, the number and quality of mitochondria within sperm cells directly influence male fertility, which, remarkably, can be modulated through mechanisms based on AEVs (Liang et al., 2023). Notably, tRNA can exist as tRNA-derived small RNA (tsRNA) and tRNA-derived fragments (tfRNA) in PAEVs and FEVs, and some may directly inhibit protein synthesis, and miRNA in host cells (Lambertz et al., 2015; Peres Da Silva et al., 2015;

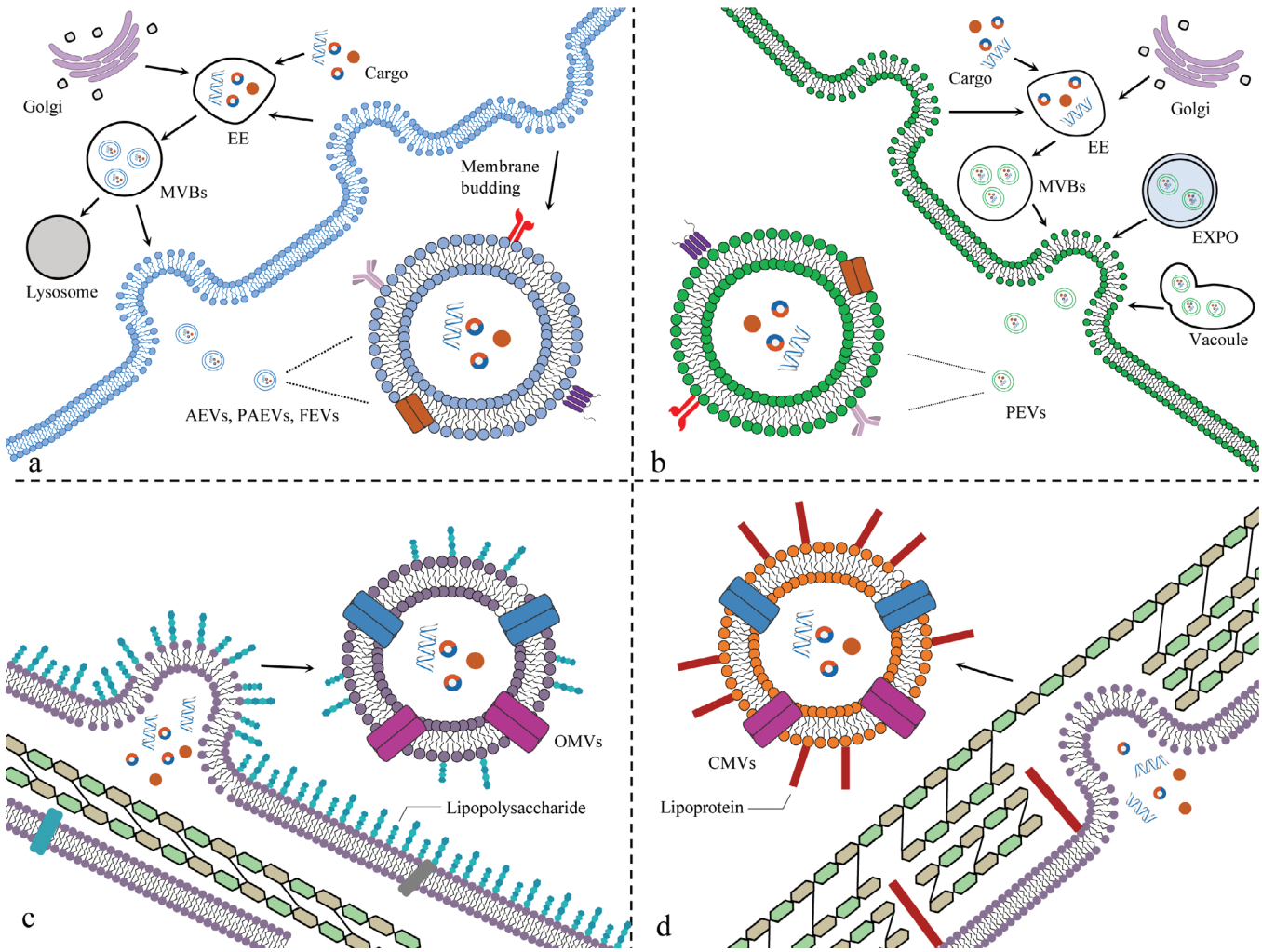

Fig. 1. Biogenesis of EVs. AEVs, PAEVs, and FEVs are mainly generated by MVBs fusion with the plasma membrane and membrane budding. Plant cells also produce and uptake vesicles, but they use a unique organelle called EXPO and a vacuole to produce PEVs. OMVs and CMVs are produced through membrane budding and bacterial autolysis.

Rayner et al., 2017; Wang et al., 2019). Moreover, PEVs and FEVs contain few proteins and are associated with cell wall remodeling (Iravani and Varma, 2019; De Palma et al., 2020; Medina-Castellanos et al., 2023; Valdez et al., 2023). PEVs transport small secondary metabolites (such as flavonoids, saponins, polysaccharides, and alkaloids) (Zhao et al., 2024a). Capsaicin and gingerol in ginger-derived PEVs, naringin and naringenin in grapefruit PEVs, and sulforaphane in broccoli PEVs display strong anti-inflammatory properties (Kim et al., 2022b). Additionally, prions are secreted as unique proteins in FEVs with full infectious ability (Kabani and Melki, 2015; Kabani et al., 2020). However, the most abundant molecules present in FEVs are carbohydrates and glucuronoxylomannan (GXM), recognized as a primary virulence factor (Marina et al., 2020). Interestingly, OMVs contain bacteriophage-associated genes that are involved in gene transfer. This interaction can be mediated not only by bacteria and bacteriophages (Yaron et al., 2000) but also through the direct binding of bacteriophages to OMVs by receptor proteins (Stephan et al., 2020). However, the mechanism involved in the encapsulation of bacteriophages by OMVs is still unclear. In addition, OMVs released by intestinal microbiota can carry a variety of glycoside hydrolases, which are responsible for the degradation of polysaccharides in ingested foods (Sartorio et al., 2023). Few studies have reported the contents of MPEVs, but MPEVs released by *A. laidlawii PG8* (Chernov et al., 2014) and *M. gallisepticum* (Wang et al., 2024) have been found to contain proteins associated with mycoplasma virulence (Gaurivaud et al., 2018). Antibiotics can be packaged into MPEVs by culturing mycoplasma in a medium with antibiotic supplementation. MPEVs mediate the export of a quinolone-resistance-determining region (QRDR) sequence to another mycoplasma, which suggests MPEVs might spread mutant genes within mycoplasma communities via horizontal gene transfer (Medvedeva et al., 2014). In general, EVs transport various cargos, and in-depth analysis of these cargos will support the selection and optimization of cargos in engineered EVs in the realms of targeted drug delivery and personalized medicine.

The method of engineered EVs

Engineered EVs can be loaded with specific drugs and their modified surfaces, enabling targeting capabilities that promote targeted cellular uptake. EV engineering methods mainly involve GE, EV exogenous membrane modification, and exogenous loading cargos (Zhang et al., 2023a). The exogenous loading of cargos is used to load drugs directly into EVs, using methods such as incubation, transfection, electroporation, extrusion, freezing and thawing, surfactants, dialysis, and ultrasound. Since exogenous loading cargos have been extensively described in previous studies, this section is mainly focused on the GE of EVs and EV exogenous membrane modification.

Genetic Engineering of EVs

GE can be used to modify the surface of EVs and for specific drug loading by acting on parent cells. Surface modification primarily involves transfecting a modified peptide plasmid or viral vector into a parent cell. This process allows the modified peptide to fuse with proteins on the membrane of EVs, assembling the modified peptide on the EV membrane. Common proteins used in this process include Lamp2b, Syntenin1, CD6, CD63, CD81, GPI, and Nef^{mut} (Ferrantelli et al., 2019; Salunkhe et al., 2020). EV producer cells are genetically engineered to co-express a glycosylation domain within the EV scaffold protein CD63, enabling engineered EVs to display specific glycan ligands and achieve high targeting specificity (Zheng et al., 2022b). Additionally, EVs have been modified by linking Lamp2b to a chondrocyte affinity peptide (CAP) for chondrocyte targeting (Liang et al., 2022). Despite the rapid and specific nature of this method, the modifying peptides in EVs are prone to degradation by intracellular proteases. To enhance peptide stability, the stability of EV-modifying peptides can be increased by attaching a glycosylation sequence to the peptides before fusing them to Lamp2b (Hung and Leonard, 2015).

Specific drug loading typically involves loading substances into cells and then relying on cargo-sorting processes to sort specific goods into EVs, which does not damage the integrity of the EV and has higher loading efficiency compared with exogenous loading (Chen et al., 2024a). Thus, understanding this complex sorting mechanism is critical for the use of this technology (Fig. 2). The sorting of miRNA into EVs may depend on EV-motifs such as CGGGAG (Rädler et al., 2023), CAUGUG, CCUCGC, and UGUGU (Garcia-Martin et al., 2022). For example, EVs naturally carry anti-miR-6359 by adding CGGGAGC to the 3'-end of the anti-miR-6359 sequence (Xie et al., 2024d). Accordingly, the enrichment of specific sequence motifs within EV RNA may involve RNA-binding proteins (RBPs), including SYNCRIP (Hobor et al., 2018), AGO2 (Shirazi et al., 2021), YBX1 (Li et al., 2022a), HUR (Mi et al., 2022), VAP-A (Barman et al., 2022), FMR1 (Wozniak et al., 2020), LC3 (Leidal et al., 2020), ALIX FUS, and SYNCRIP (Statello et al., 2018; Rädler et al., 2023). Interestingly, LC3 participates in the loading of chromatin DNA (chDNA) into EVs by interacting with FOXM1 during autophagy (Zhang et al., 2024c). Additionally, EVs show a preference for mRNA fragments with regulatory elements in the 3′-UTR, especially those with a 25-nt "Zip code" sequence featuring a core GTGCC motif and a single miR-1289 binding site, and the MS2 binding site (MS2bs), a specific stem-loop structure in viral RNA affiliating to the bacteriophage coat protein MS2 (Bolukbasi et al., 2012; Si et al., 2023). The interaction of protein and amino acid sequences determines whether a protein is encapsulated in EVs. The NDFIP1 molecule binds the WW motif of NEDD4, a ubiquitin ligase, through the L

domain, resulting in NEDD4 ubiquitination and sorting into EVs, which can be used to link the WW motif to target proteins and enable them to be sorted into EVs (Sterzenbach et al., 2017; Cheng et al., 2023). The lysosome-associated membrane protein 2, isoform A (LAMP2A), and the molecular chaperone heat shock 70 (HSC70) were considered regulating proteins with the KFERQ motif for loading into EVs (Ferreira et al., 2022). The coatomer proteins, COPA and COPB2, bind with an 18-amino-acid-signal sequence (PVRASRNKRP TFLKIKKP) to direct protein secretion into EVs (Gurriaran-Rodriguez et al., 2024). In addition, human renal cells treated with an N-terminal glycosylation inhibitor showed a significant decrease in glycosylated antimicrobial proteins within EVs, indicating that N-terminal glycosylation is crucial for the efficient packaging of proteins into EVs (Naito et al., 2021). Moreover, tetraspanins on EVs (including CD63, CD81, TSPAN14, and CD9) are efficient loading scaffolds (Silva et al., 2021). Similarly, FK506-binding protein (FKBP) and FKBP12-rapamycin-binding protein (FRB) are efficient for EVs loading. Specifically, CD81 is coupled with FKBP, and the target cargos are engineered to have the FRB domain, which are sorted into EVs following the catalysis of rapamycin (Ilahibaks et al., 2023). Besides, other EV-sorting proteins, including VSVG (Do et al., 2019), MARCKS, MARKCSL1, BASP1 (Dooley et al., 2021), LMP1 (Nkosi et al., 2020), BASP-1, PTGFRN (Dooley et al., 2021), TSPAN2, TSPAN3 (Zheng et al., 2023a), and ARRDC1 (Wang et al., 2018) have been identified.

EVs can be engineered into effective drug carriers by incorporating therapeutic proteins or nucleic acids and by modifying their surfaces for the treatment of conditions such as cancer, infections, and various diseases. At present, many studies have developed efficient RNA loading systems based on tetraspanins. For example, the CD9-HUR fusion protein effectively enriched EVs with miR-155 (Li et al., 2019). Similarly, most surface modifications are based on tetraspanins. We expect future research to focus on sorting mechanisms and to invent more efficient ways to improve the GE of EVs.

EV exogenous membrane modification

In addition to GE, EVs can be directly designed using techniques such as non-covalent chemical

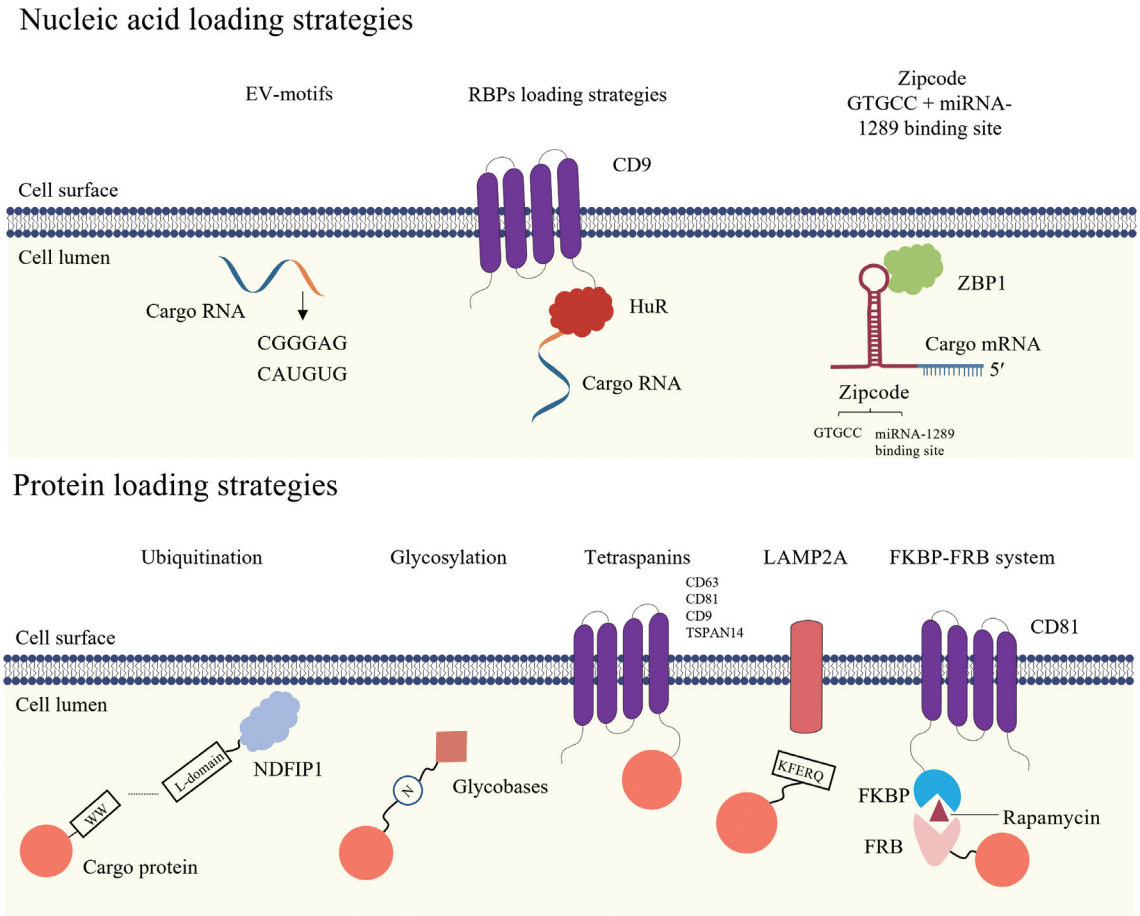

Fig. 2. The mechanisms of sorting cargo into EVs (Plotted based on references (Erana-Perez et al., 2024)).

modification (NCM), covalent chemical modification (CM), hybridized membrane modification (HM), and external magnetic fields (EMF) (Fig. 3).

NCM refers to EVs being connected via weak interactions such as electrostatic interactions, aptamer binding, and hydrophobic interactions. Electrostatic interactions are often utilized to generate positive charges on the surface of EVs, which can then bind to negatively charged cell membranes, resulting in a targeting effect. For example, HeLa cell-derived EVs, which are charged due to cationic lipids, readily enter cells through charge affinity (Nakase and Futaki, 2015). Aptamer binding involves ligands such as short oligonucleotides or peptides with a specific three-dimensional structure. These ligands can link to specific receptors to mimic the functions of antibodies (Wang et al., 2023). Previously, the E3 aptamer has been linked with EVs, which enables selective targeting of prostate cancer cells (Han et al., 2021). Nonetheless, this method of assembly tends to disrupt the lipid bilayer structure of EVs, which can affect their stability. EV membranes contain a high number of amphiphilic substances, such as PG, cholesterol, and glycolipids, allowing hydrophobic compounds to be loaded onto the surface. Polyethylene glycol (PEG) is a hydrophobic compound that is coupled to epidermal growth factor receptor (EGFR) to generate EGFR-PEG. When these complexes were incubated with EVs, the EGFR-PEG lipids were transferred to the EV membranes, endowing them with enhanced cell orientation and cycling time (Kooijmans et al., 2016).

CM is mainly achieved through click chemistry, peptide covalent binding, and protein ligases, and couples the modification groups to the surface of EVs to improve their targeting ability; however, the safety of these chemical groups still needs to be evaluated and the efficiency of the chemical reaction is difficult to control (Johnson et al., 2023). Click chemistry is a relatively simple and interference-resistant chemical reaction. Specifically, generating triazole rings through the catalysis of azide-alkynyl groups by copper ions enables the molecules to be covalently attached to the surface of EVs, thereby directly modifying the EV membrane (Xie et al., 2024c). This modification preserves the size and receptor cell internalization of EVs, successfully enhancing the targeting ability of EVs without impacting their functions and properties (Smyth et al., 2014). However, the copper used in click chemistry has cytotoxic effects. cRGD-EVs have been developed using copper-free click chemistry to modify mesenchymal stromal cells (MSCs)- derived EVs, which exhibited an enhanced affinity for integrin $\alpha_v\beta_3$ of cerebral endothelial cells post-ischemia (Tian et al., 2018). Nevertheless, click chemistry is time-consuming and the reaction conditions are hard to control; therefore, researchers developed an alternative method known as peptide covalent binding. For example, EVs linked with rabies viral glycoprotein (RVG) via a dioleoyl-phosphatidylethanolamine N-hydroxysuccinimide (DOPE-NHS) linker have been developed. These

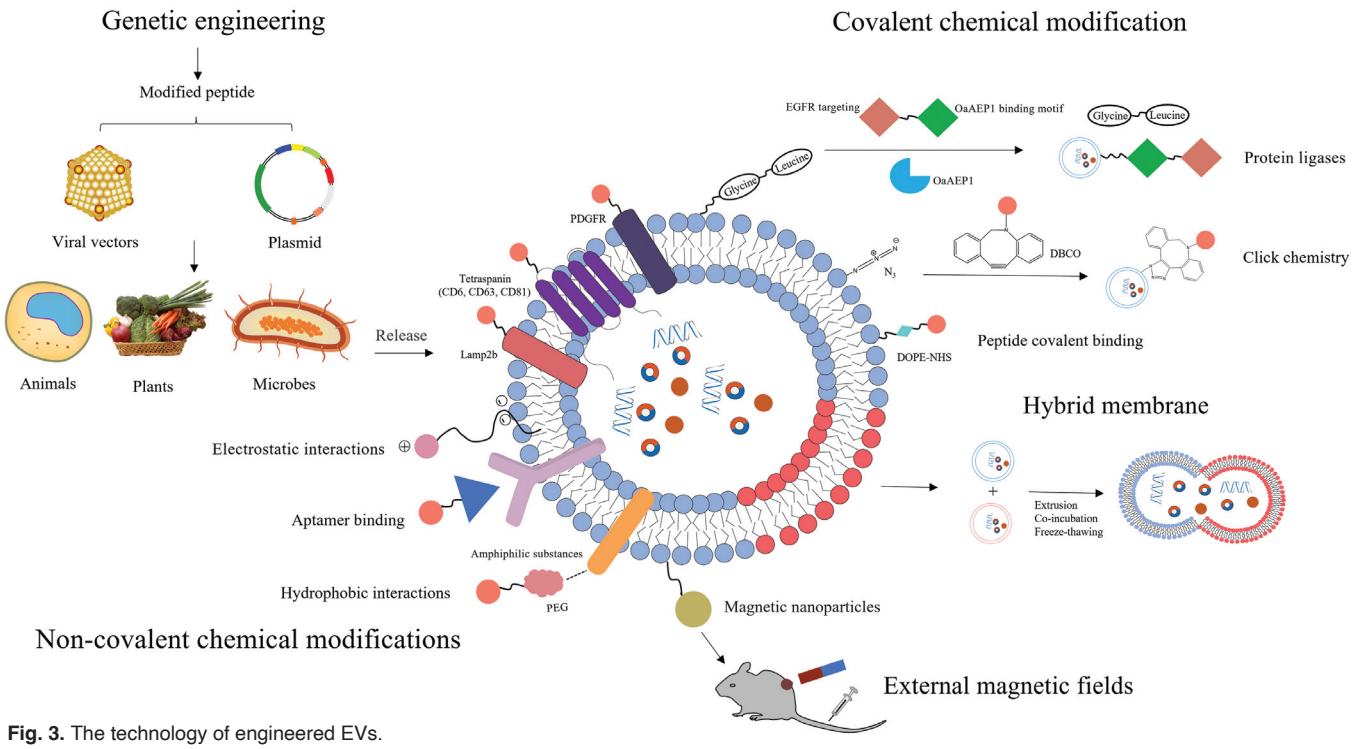

Fig. 3. The technology of engineered EVs.

modified EVs precisely target the hippocampus in mice (Cui et al., 2019a). Alternatively, surface modification of EVs can be achieved through protein ligases, which can bind proteins with a specific sequence of amino acids and target proteins to EVs. The protein ligases SORTASE A and OaAEP1 have been used to modify EVs by attaching EGFR-targeting peptides. This approach enabled EVs to specifically bind to lung cancer cells (Pham et al., 2021).

HM modification involves the fusion of EVs with other lipid molecular layers (e.g., liposomes) through repeated freeze-thawing, extrusion, and co-incubation. Interestingly, AEVs and PEVs can be fused due to the similar biophysical properties of their bilayer lipid membranes (Lu et al., 2024). This optimizes the properties of EV surfaces, reducing their immunogenicity, improving their half-life in the bloodstream, and aiding their transfer to target cells (Liang et al., 2021). In addition, HM modification increases the size of EVs and improves their ability to encapsulate a large cargo or drugs, thus addressing the disadvantage of natural EVs being unable to encapsulate large drugs. Furthermore, a hybrid delivery system that combines clodronate-loaded liposome and fibroblast-derived EVs with non-specific phagocytosis inhibition and fibroblast homing properties has been designed for the treatment of pulmonary fibrosis (Sun et al., 2021). However, this method destroys proteins on the membrane of EVs and makes it difficult to separate EVs from lipid molecular layers after binding (Choi et al., 2021).

EMF enhances EV modification by combining them with magnetic nanoparticles, in addition to chemical modification (Mishra et al., 2021). In 2016, Qi et al. coupled superparamagnetic nanoparticles-transferrin (M-Tf) with EVs isolated from mouse blood to produce M-Tf-EVs, and under the effect of EMF, the M-Tf-EVs could accurately target diseased cells (Qi et al., 2016). Superparamagnetic iron oxide nanoparticles (SPIONs) are used in another research; combinations of SPIONs and EVs loaded with BAY55-9837, a peptide used for the treatment of type 2 diabetes mellitus (T2DM), induced insulin secretion in diabetic mice using EMF, and these BAY-SPIONs-EVs had a longer half-life in plasma, 27-fold greater than that of BAY55-9837 alone (Zhuang et al., 2019).

In summary, each of these methods has advantages and disadvantages. GE provides products with high specificity and stability, but they are constrained by limited peptide expression, costliness, and long development times. CM and NCM modifications are simple and fast, but involve chemical reactions and produce by-products, and it is difficult to guarantee the yield. Additionally, it has a certain degree of toxicity. HM modification alters the structure of EVs, and EMF requires magnetism, which is not very common. These engineering strategies extend the targeting efficiency and therapeutic capabilities of EVs. Moreover, modifications can now be achieved by combining multiple technologies. SPIONs can be packaged into EVs, which bind to neuropilin-1-targeted peptides via click chemistry. These dual-modified EVs can successfully cross the blood-brain barrier (BBB) to specifically target gliomas and effectively divert chemotherapeutic agents (Jia et al., 2018). Although the targeting abilities of EVs when using EMF or click chemistry alone and in combination have not been compared, this study provides a reference for the development of combined modification strategies.

Application of engineered EVs in disease therapy

To date, studies on engineered EVs have not progressed to the clinical translation stage for human disease treatment. Nonetheless, the potential of engineered EVs in disease treatment warrants exploration and is a promising therapeutic avenue for healthcare (Lai et al., 2022). In the following sections, we detail the application of engineered EVs in tumors and infectious diseases. Table 2 shows the novel research on engineered EVs in other diseases.

Application of engineered EVs in tumor therapy

Tumors are one of the most challenging and major problems in the world. Precise drug delivery and drug resistance pose significant challenges. Currently, the main treatment methods for tumors are surgery, chemotherapy, radiotherapy, and immunotherapy (Desai et al., 2024). A study showed that engineered EVs can combined with radiotherapy to develop novel augmented radiotherapy. Au–OMVs, with two specific nanoparticles: gold nanoparticles (AuNPs) and OMVs from *E. coli*. have been synthesized. When administered in conjunction with radiotherapy, Au-OMVs produce radiosensitizing and immunomodulatory effects, successfully suppressing tumor growth in both subcutaneous glioblastoma-bearing and in situ (brain) tumor-bearing mice (Chen et al., 2021).

Chemotherapy is highly toxic to normal cells and presents multidrug resistance, and chemotherapy drugs cannot efficiently cross the BBB, blood-eye barrier (BEB), and blood-testis barrier (BTB), hindering the treatment of tumors of the brain, eyes, and testicles. Therefore, there is an urgent need to achieve precisely targeted drug delivery and combat drug resistance. To solve this challenge, engineered EVs are loaded with chemotherapy drugs. For example, attenuated Salmonella-derived OMVs containing fluorouracil, which resulted in fluorouracil inhibiting DNA synthesis in the tumor cells by inhibiting thymidylate synthetase, considerably inhibiting melanoma tumor growth (Chen et al., 2020). EVs loaded with chemotherapy drugs are modified to further improve toxicity to normal cells. Complex, DOX- and cRGD-modified fruit-derived EVs were assembled for manufacture. cRGD has a high affinity for integrin αvβ3 overexpressed in glioblastoma cells, which was modified to the fruit-EV's surface, enabling the complex to highly efficiently cross the BBB

and deeply penetrate glioblastoma tissues, thereby significantly extending the survival time of glioblastoma mice (Chen et al., 2023).

Immunotherapy is a treatment approach that leverages the immune system to recognize and attack tumor cells. This is primarily achieved through two key strategies: immune checkpoint inhibitors (ICIs) and cell therapy represented by CAR-T therapy (Brandenburg et al., 2024). ICIs are inhibitory signals that block immune checkpoint molecules, thereby enhancing the immune system's ability to recognize and attack tumor cells. Multiple immune checkpoint pathways have been identified, such as cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed death-ligand 1 (PD-1) (Li et al., 2018). A study reported that EVs derived from M1 macrophages were loaded with anti-

Table 2. Application of engineered EVs in disease treatment.

Disease	EV source	Drug	Method	Model	Function	Reference
Tissue injury	LO2 cells	Cytochrome C	Electroporation	Hepatic ischemia/ reperfusion injury mouse	Ameliorating the damage to ischemic liver regions	Liu et al., 2024b
	MSCs	Angiogenin-1	Co-incubation, GE	Myocardial infarction mouse	Inducing angiogenesis and vascular maturation	Hu et al., 2022
	ADSCs	Natural	HM	HUVECs	Promoting angiogenesis	Ma et al., 2023
	M2-macrophage	miR-99a-5p	HM	Ldlr–/– mouse	Attenuating HOXA1; inhibiting foam cell formation	Xie et al., 2024b
	Microglia cells	NR2B9c	CM	TBI mouse	Mitigating the lesion volume	Haroon et al., 2024
Visceral disease	Macrophage cells	Resveratrol	Co-incubation, NCM	Sepsis mouse	Modulating the NF-B signaling pathway to mitigate disease	Zheng et al., 2023b
		vMIP-sII	GE, HM	VM mouse	Regulating the immune micro-environment to achieve cardiac repair	Pei et al., 2024
	Fibroblast	IL-4	Transfection, GM	Lung injury mouse	Reducing IL-6 and IL-1 secretion; mitigating lung injury	Salazar-Puerta et al., 2023
	MSCs	miR-223, miR-21	CM	Myocardial injury mouse	Inhibiting cardiomyocyte apoptosis; reducing inflammation	Fan et al., 2022
		Natural	HM	MI/R mouse	Switching M1 macrophage polarization towards M2 for cardiac repair	Li et al., 2022b
	HEK293 cells	NOX4 siRNA	Co-incubation, CM	Cardiac hypertrophy mouse	Reducing fibrosis and cardiomyocyte cross-sectional area	Kang et al., 2023
	Blood	miR-34a-RNPs	CM, Electroporation	MI mouse	Editing miR-34a genome; ameliorating MI injury and cardiac function	Mun et al., 2024
	Endothelium	miR-125b-5p	Co-incubation, GE	ALI mouse	Inhibiting cell apoptosis; protecting the intercellular junction	Gu et al., 2024
	WJ-MSCs	miR-let-7a-5p	Electroporation	ALI mouse	Downregulating SMAD2/3 phospho-rylation; Mitigating the fibrotic phenotype	Chen et al., 2024b
	Panax	miR-182-5p	Electroporation,	ALI mouse	Regulating NOX4/Drp-1 to improve ALI	Ma et al., 2024
Neurological diseases	Macrophages	TGF-β	GM	Spinal cord injury mouse	Restoring nerve function; promoting angiogenesis	Peng et al., 2023
	M2 microglia	SDF-1	NCM	Ischemic brain stroke mouse	Recruiting NSCs; promoting neuronal differentiation	Ruan et al., 2023
	BMSCs	Netrin-1	Transfection, GE	Spinal cord injury mouse	Attenuating inflammation and accelerating axonal growth	Lu et al., 2023b
		lncRNA KLF3-AS1	Transfection	MCAO mouse	Promoting Sirt1 deubiquitination eased I/R injury	Xie et al., 2023
	HEK293T cells	RVG, sgRNA, dCas9-DNMT3A	Transfection	PD mouse	Decreasing α-syn elevation; improving motor ability	Kong et al., 2024
	MSCs	BDNF	Transfection	Ischemic brain mouse	Upregulating related genes; activating BDNF/TrkB signaling	Zhou et al., 2023
		CB2 receptor agonist AM1241	NCM	AD mouse	Suppressing Aβ-induced neuronal apoptosis	Zhu et al., 2023
		FTO-siRNA	Ultrasound	PD mouse	Suppressing α-Syn expression and dopaminergic neuronal death	Geng et al., 2023
		Natural	EMF	MCAO-induced rats	Promoting the anti-inflammatory response and angiogenesis	Kim et al., 2020
	HEK293 cells	Sox10	Transfection	Demyelinating mouse	Enhancing MBP expression; reducing myelin damage	He et al., 2024
		circ-DYM	Transfection, CM	MDD mouse	Binding to TAF1 reduces target gene expression and neuroinflammation	Yu et al., 2022
	Tomafran	Crocins	GE	Neuroblastoma cells	Reducing oxidative stress and inflammation	Etxebeste-Mitxeltorena et al., 2024
	E. coli	Pioglitazone	Electroporation	Ischemic stroke mouse	Downregulating the activation of NLRP3 inflammasomes and reducing reperfusion injury	Pan et al., 2023

Table 2. (Continued).

Bone and Osteoarticulation	RBCs	anti-miR-214	Electroporation	OP mouse	Inhibiting miR-214 and osteoclast proliferation	Xu et al., 2023
	Blood platelet	Alendronate	Co-incubation, NCM	OP rats	Promoting osteoblast proliferation; reducing symptoms	Zheng et al., 2022a
	Expi293F cells	MMP13-siRNA	NCM	OA rats	Reducing MMP13; increasing COL2A1 and proteoglycan	Zhang et al., 2024a
	BMSCs	lncRNA NRON	Transfection	OVX mouse	Reversing bone loss; increasing bone mass	Yang et al., 2022
		RNF146	Transfection, NCM	OP mouse	Facilitating osteogenesis	Gui et al., 2024
	iPSCs	Shn3-siRNA	Electroporation, NCM	OP mouse	Silencing Shn3-gene-enhanced osteogenic differentiation	Cui et al., 2022
	E. coli	SOST siRNA	Electroporation, GE	OP mouse	Regulating the WNT signaling pathway to induce osteogenic differentiation of BMSCs	Liu et al., 2023a
		BMP-2	GE	OVX mouse	Targeting BMSCs and induce osteogenic differentiation of BMSCs	Liu et al., 2024a.b
Skin diseases	hASCs	NF-κB siRNA	Transfection, GE	Skin lesions mouse	Inhibiting NF-κB expression; accelerating diseased skin repair	Lu et al., 2023a
	PMSCs	miR-146a	Transfection, GE	Diabetic wound mouse	Driving wound healing with less inflammation, collagen deposition	Li et al., 2023
Eye Diseases	T cells	VEGF	CM	Choroidal neovascularization mouse	Inhibiting neovascularization in the eye area	Tian et al., 2021
	MSCs	miR-5068; miR-10228	Electroporation	DR mouse	Inhibiting the HIF-1α/EZH2/PGC-1α pathway; enhancing retinal repair	Sun et al., 2024a
Beauty	Dermal fibroblast	COL1A1 mRNA	Electroporation	Mouse	Producing continuous collagen to reduce wrinkle formation	You et al., 2023
	ADSCs	lncRNA H19	Transfection	UVB-irradiated mouse	Reversing epidermal thickening and collagen degradation	Gao et al., 2023
	Leo	Acetyl hexapeptide-8	Ultrasound, CM	Mouse and zebrafish	Optimizing skin penetration to intensify beauty effects	Hou et al., 2024
Viral diseases	HEK293T cells	spCas9 RNP	GE	HSV-infected mouse	Restraining viral replication; clearing HSV1 virus in neural tissues	Wan et al., 2024
Metabolic disorders	HEK293T cells	LDLR mRNA	Transfection	FH mouse	Restoring LDLR expression; attenuating liver steatosis	Yang et al., 2023
	M2-macrophage	RIPK3-siRNA	Transfection	AIH mouse	Suppressing RIPK3 expression; mitigating hepatitis	Zhang et al., 2024b
Autoimmunity	MSCs	PD-L1	GE	Psoriasis mouse	Reshaping the inflammatory ecosystem	Xu et al., 2022
	HEK293 cells	CD40, MMF	GE	SLE mouse	Blockading of CD40L; restraining production of antibodies from B cells	Fang et al., 2023
Autism	MSCs	miR-137	Co-incubation	ASD mouse	Targeting the TLR4/NF-κB pathway; ameliorating autism-like behaviors	Qin et al., 2024
Chronic illness	HEK293T cells	miR-511-3p	GE	miR-511-3p $^{-/-}$asthma mouse	Binding and activating C3; reversing increased airway inflammation	Tu et al., 2024
	Macrophage cells	PD-L1 and Gal-9	GE	NOD mouse	Declining the proportion of $CD4^+$ and $CD8^+$ T cells infiltrating the pancreas notably	Yang et al., 2024
	Milk	Insulin	Ultrasound	T1D rats	More excellent and sustained hypoglycemic effect	Wu et al., 2022
Oral diseases	M2-macrophages	Ckip-1-shRNA	Transfection	Cementoblasts	Boosting mitochondrial biogenesis through targeting Ckip-1	Huang et al., 2024
ART	EndMSCs	CRISPR/dCas9	HM	Mouse	Inducing miR-30d gene expression; improving blastocyst adhesion, invasion, and angiogenesis	Yaghoobi et al., 2023
Fertility disease	ADSCs	TSG6	GE	IUA mouse	Reducing endometrium fibrosis in IUA mice	Sun et al., 2024b

ADSCs: adipose-derived mesenchymal stem cells; AIH: autoimmune hepatitis; ALI: acute lung injury; ART: assisted reproductive techniques; ASD: autism spectrum disorder; Aβ: amyloid β; BDNF: brain-derived neurotrophic factor; BM: Brain metastasis; BMSCs: bone marrow mesenchymal stem cells; C3: complement C3; CDPs: cartilage-degrading proteases; Ckip-1: casein kinase 2 interacting protein-1; CL4: coupled cytokine-like 4; COL1A1: extracellular-matrix α1 type-I collagen; DC: dendritic cells; DHODH: dihydroorotate dehydrogenase; DR: diabetic retinopathy; ECs: endothelial cells; EndMSCs: endometrial mesenchymal stem cells; FH: familial hypercholesterolemia; GBM: glioblastoma; GPX4: glutathione peroxidase 4; hASCs: adipose mesenchymal stem cells; HBV: hepatitis B virus; HUVECs: human umbilical vein endothelial cells; IFN-γ: interferon-γ; IUA: intrauterine adhesion; Ldlr–/–: lacking low-density lipoprotein receptors; LDLR: low-density lipoprotein receptor; MCAO: middle-cerebral-artery occlusion; MDD: major depressive disorder; MI/R: myocardial ischemia reperfusion; MMP: myelin basic protein; MSR-1: Magnetospirillum gryphiswaldense; NOD: non-obese diabetic; NOX4: NADPH Oxidase 4; NPNs: nano-pathogenoids; NLRP3: nucleotide oligomerization-like receptor protein 3; NSCs: neural stem cells; OA: osteoarthritis; OC: ovarian cancer; OP: osteoporosis; OS: osteosarcoma; OSCC: oral squamous cell carcinoma; OVX: ovariectomized; PCG-1α: proliferator-activated receptor gamma coactivator-1α; PD: Parkinson's disease; PMSCs: placental mesenchymal stem cells; PPM1D: p53-induced protein phosphatase 1; RBCs: red blood cells; ReN: ReNcell VM; RGC: receptor gamma coactivator; RHCs: retinoblastoma human cells; RNPs: ribonucleoprotein complexes; SDF-1: stem cell recruiting factor; SLE: systemic lupus erythematosus; T1D: type I diabetic; TAMs: tumor-associated macrophages; TBI: traumatic brain injury; TNBC: Triple-negative breast cancer; TRAIL: tumor necrosis factor related apoptosis-inducing ligand; VEFG: vascular endothelial growth factor;VM: viral myocarditis; VMP-II: viral macrophage inflammatory protein-II;VPA: valproic acid; VSV-G: vesicular stomatitis virus-glycoprotein; WJ-MSCs: Wharton's jelly-mesenchymal stem cells;α-syn; α-synuclein.

PD-1 and anti-CTLA-4, potentiating immunotherapeutic efficacy (Lin et al., 2024). Moreover, the combination of RNAi technology and engineered EVs has also been used in immunotherapy. EVs released from ReNcell VM (viral myocarditis) cells were loaded with PD-L1-siRNA and combined with c(RGDyK), reversing PD-L1 expression on tumor cells and increasing $CD8^+$ cytotoxic T-cell activity, halting tumor growth (Tian et al., 2022). CAR-T therapy is manufactured using lentiviral or retroviral vector transduction. However, the production of lentiviral or retroviral vectors is a time-consuming and expensive process. Engineered EVs can be produced at a lower cost and in a shorter time than viral vectors and can be used as vectors for CAR expression and targeted delivery of CAR constructs to T cells (Spokeviciute et al., 2024). Engineered EVs deliver CAR mRNAs by MS2-MS2bs, and express anti-CD3/CD28 single-chain variable fragments (scFvs) on their surface, which activate T cells by engaging with their membrane CD3/CD28 and facilitate delivery of CAR mRNA into T cells with cancer cells killing capacity (Si et al., 2023). Moreover, engineered EVs can directly carry CAR activation T cells. For example, EVs expressing CD19 antigen were co-incubated with anti-CD19 CAR-T cells with anti-tumor efficacy (Zhang et al., 2023b).

Application of engineered EVs in infectious disease therapy

Infectious diseases induce inflammation and damage organs and systems and are usually caused by the infection of bacteria, fungi, and viruses. As described previously, EVs can be loaded with antimicrobials. For instance, *Kaempferia parviflora* EVs were co-incubated with clarithromycin (CLA) to produce CLA-EVs, which were localized to the cytoplasm and perinuclear region of gastric cells, inhibiting gastric cell inflammation mediated by *H. pylori* infection (Nemidkanam et al., 2024). EVs equipped with antibiotics can also be combined with EMF across BBB for sonodynamic treatment of bacterial meningitis (Shi et al., 2023). Besides, mRNA can be directly encapsulated and delivered to recipient cells to express inhibitory proteins. In Shrivastava's research, they developed mRNA-loaded EVs, expressing ZFP-DNMT3A (recombinant fusion protein of zinc finger protein and DNA methyl-transferase 3A) causing the HIV-1 virus promoter to be methylated, significantly reducing expression levels (Shrivastava et al., 2021). Research has shown that engineered EVs carry overexpressed proteins for infectious disease therapy. Blocking the interaction between angiotensin-converting enzyme 2 (ACE2) on the cell membrane and S proteins is an important target for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) drugs. ACE2-EVs were designed by CD9 scaffolds as a decoy receptor for the S protein of SARS-CoV-2 with superior antiviral efficacy (Kim et al., 2022a). CRISPR/Cas9 can precisely identify and cleave specific sequences in the viral genome by designing guide RNAs (gRNAs). EVs offer a promising delivery for CRISPR/Cas9 ribonucleoproteins (RNPs). A previous study has shown that RNPs-EVs suppress Hepatitis B virus (HBV) antigens and load. RNPs were localized into EVs by GE technology, and vesicular stomatitis virus glycoprotein (VSV-G) was fused to EV membranes to enhance the nuclear translocation of Cas9 in recipient cells. The gRNA guides the Cas9 protein to precisely cleave the covalently closed circular DNA (cccDNA) of HBV to suppress viral antigens and load (Zeng et al., 2024b). Moreover, hybrid EVs, formed by combining liposomes and OMVs, were loaded with CRISPR/Cas9 plasmids. These plasmids replicated within multidrug-resistant *Acinetobacter baumannii* and drug-resistant *Pseudomonas aeruginosa* and expressed ribonucleic proteins, leading to DNA cleavage and the introduction of irreparable chromosomal lesions (Jia et al., 2024).

Advances in clinical trials of engineered EVs

As the number of studies on the role of EVs in disease pathways has increased, engineered EVs have been increasingly developed for disease treatment. Although the Food and Drug Administration (FDA) has not approved any engineered EV products for clinical use, the number of ongoing engineered EV-based clinical trials has rapidly increased in recent years (Lai et al., 2022). Some studies on engineered modified EVs for drug delivery are in the clinical trials stages; for

Table 3. Clinical trials of engineered EVs.

Disease	EVs source	Drug	Phases	Recruitment	Number
Colon cancer	Grapes	Curcumin	I	Recruiting	NCT01294072
Liver cancer	HEK293 cells	STAT6 ASO	I	End	NCT05375604
Solid tumors	HEK293 cells	STING agonists	I	End	NCT04592484
Lung cancer	Dendritic cells	Tumor antigens	I	End	NCT01159288
Irritable bowel syndrome	Ginger	Curcumin	Unknown	End	NCT04879810
SARS-CoV-2	HEK293 cells	CD24	I/II	End	NCT04747574; NCT04969172
Familial hypercholesterolemia	MSCs	LDLR mRNA	I	Not yet recruited	NCT05043181
Cerebrovascular disease	MSCs	miR-124	I	End	NCT03384433

STAT6 ASO: STAT6 anti-sense oligonucleotide.

example, modified MSC-secreted EVs can unambiguously target the oncogene Kras in pancreatic cancer and improve the overall survival rate of mice with pancreatic ductal adenocarcinoma (Kamerkar et al., 2017). This study has entered the phase I clinical trial stage (NCT03608631). The engineered-EV clinical trial studies are shown in Table 3 (clinicaltrials.gov).

Engineered EVs are capable of treating specific diseases, but their clinical translation must be achieved through industrial production. However, there is an urgent need to establish a systematic and standardized evaluation system for engineered EVs. The properties of EVs are dependent on their culture conditions, and engineered EVs from different mediums have distinct characteristics. For human disease applications, the cultivation method used should avoid non-human biomolecules, including bovine serum albumin (Song et al., 2022). Furthermore, the deleterious effects of serum on EVs cannot be overcome by removing identified components of serum, therefore, serum-free media is more suitable for culturing EVs (Bost et al., 2022). Producing large quantities of engineered EVs remains a significant challenge, but bioreactors such as the shaker bag and hollow fiber systems can be used to cultivate cells at scale for EV collection. Brief hyperthermia at 40°C can boost production by 5.4 times, with enhanced EV properties remaining above 90% (Keysberg et al., 2024). It is crucial to detect and assess cell culture parameters, such as eliminating contaminants and the number of cell passages and cell densities (Patel et al., 2017). Additionally, storing EVs whilst maintaining their stability is a major challenge following the production of EVs in large quantities. Commonly, -80°C is used as the storage temperature, but it can impair functionality (Paganini et al., 2019). Adding protective agents like alginate or DMSO before freezing is a prevalent method to enhance stability (Görgens et al., 2022). Additionally, freeze-drying is frequently used for preservation (Charoenviriyakul et al., 2018). Engineered EVs risk immune rejection and adverse reactions. Therefore, they necessitate rigorous safety assessments, which involve monitoring parental cell selection, EV toxicity, and immunogenicity testing. Due to the lack of uniform safety standards for EVs, there is a need to refine testing methods like nanoparticle-tracking analysis, flow cytometry, and sequencing or discover new ones and establish a recognized evaluation system for safety and efficacy.

Conclusions and Future Perspectives

EVs play a crucial role in intercellular communication, due to their low immunogenicity and high stability, and have great potential in clinical applications as they facilitate cross-species information exchange and regulation. EVs can be loaded with drugs and surface modified. GE mainly involves transfecting mother cells with vectors and viruses to enable the released EVs to carry targeted components. Simultaneously, drugs are sorted into EVs through cargo sorting processes, including EV-motifs, RBPs, mRNA fragments with regulatory elements in the 3′-UTR, interaction of protein and amino acid sequences, N-terminal glycosylation, and EV sorting protein. Chemical modification, covalent chemical modification, hybridization membrane modification, and external magnetic fields can enable EVs to be modified with targeted components and enhance their targeting capacity, thereby promoting their uptake and absorption by target cells. In comparison with traditional drugs, engineered EVs can more precisely attack diseased cells while reducing the risk of damage to normal cells. Consequently, engineered EVs have tremendous potential as drug carriers for various clinical treatments. However, engineered modifications may change the composition of EV and trigger biotoxicity, leading to activation of the immune system. At the same time, the selection of parental cells, culture conditions, and the detection of EV toxicity have hindered the large-scale generation and clinical application of EVs. In order to solve these problems, recognized and well-established safety standards are required to promote their application in clinical medicine. Research on safety modifications and more accurate targeted EVs is still in its nascent stages, necessitating systematic evaluation to facilitate their application in the clinic. Currently, clinical trials focus on natural EVs more than engineered EVs. This indicates significant potential for advancements in the therapeutic applications of engineered EVs, which hold promise for treating a wide array of diseases in the future.

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References

Atayde V.D., Da Silva Lira Filho A., Chaparro V., Zimmermann A., Martel C., Jaramillo M. and Olivier M. (2019). Exploitation of the *Leishmania* exosomal pathway by *Leishmania* RNA virus 1. Nat. Microbiol. 4, 714-723.

Barman B., Sung B.H., Krystofiak E., Ping J., Ramirez M., Millis B., Allen R., Prasad N., Chetyrkin S., Calcutt M.W., Vickers K., Patton J.G., Liu Q. and Weaver A.M. (2022). VAP-A and its binding partner CERT drive biogenesis of RNA-containing extracellular vesicles at ER membrane contact sites. Dev. Cell. 57, 974-994.e8.

Bolukbasi M.F., Mizrak A., Ozdener G.B., Madlener S., Ströbel T., Erkan E.P., Fan J., Breakefield X.O. and Saydam O. (2012). miR-1289 and "Zipcode"-like sequence enrich mRNAs in microvesicles. Mol. Ther. Nucleic Acids 1, e10.

Bost J.P., Saher O., Hagey D., Mamand D.R., Liang X., Zheng W., Corso G., Gustafsson O., Görgens A., Smith C.E., Zain R., El Andaloussi S. and Gupta D. (2022). Growth media conditions influence the secretion route and release levels of engineered

extracellular vesicles. Adv. Healthc. Mater. 11, e2101658.
Brandenburg A., Heine A. and Brossart P. (2024). Next-generation cancer vaccines and emerging immunotherapy combinations. Trends Cancer 10, 749-769.
Charoenviriyakul C., Takahashi Y., Nishikawa M. and Takakura Y. (2018). Preservation of exosomes at room temperature using lyophilization. Int. J. Pharm. 553, 1-7.
Chen Q., Bai H., Wu W., Huang G., Li Y., Wu M., Tang G. and Ping Y. (2020). Bioengineering bacterial vesicle-coated polymeric nanomedicine for enhanced cancer immunotherapy and metastasis prevention. Nano Lett. 20, 11-21.
Chen M., Liu T., Chen Y. and Chen M. (2021). Combining augmented radiotherapy and immunotherapy through a nano-gold and bacterial outer-membrane vesicle complex for the treatment of glioblastoma. Nanomaterials 11, 1661.
Chen J.P., Pan J.H., Liu S.J., Zhang Y.N., Sha S., Guo H., Wang X., Hao X., Zhou H., Tao S., Wang Y. and Fan J. (2023). Fruit-derived extracellular-vesicle-engineered structural droplet drugs for enhanced glioblastoma chemotherapy. Adv. Mater. 35, e2304187.
Chen M., Chen Z., Cai M., Chen H., Chen Z. and Zhao S. (2024a). Engineered extracellular vesicles: A new approach for targeted therapy of tumors and overcoming drug resistance. Cancer Commun. 44, 205-225.
Chen S., Chen Y., Li P., Cheng T., Chu Y., Shen Y., Chen H., Tsai W., Huang C., Sieber M., Yeh Y., Liu H., Chiang C., Chang C., Lee A.S., Tseng Y., Lee L., Liao H., Yip H. and Huang C.F. (2024b). Engineered extracellular vesicles carrying let-7a-5p for alleviating inflammation in acute lung injury. J. Biomed. Sci. 31, 30.
Chen Y., Luh F., Ho Y. and Yen Y. (2024c). Exosomes: A review of biologic function, diagnostic and targeted therapy applications, and clinical trials. J. Biomed. Sci. 31, 67.
Cheng Y., Lu X., Li F., Chen Z., Zhang Y., Han Q., Zeng Q., Wu T., Li Z., Lu S., Williams C. and Xia W. (2023). NDFIP1 limits cellular TAZ accumulation via exosomal sorting to inhibit NSCLC proliferation. Protein Cell 14, 123-136.
Chernov V.M., Mouzykantov A.A., Baranova N.B., Medvedeva E.S., Grygorieva T.Y., Trushin M.V., Vishnyakov I.E., Sabantsev A.V., Borchsenius S.N. and Chernova O.A. (2014). Extracellular membrane vesicles secreted by mycoplasma *Acholeplasma laidlawii PG8* are enriched in virulence proteins. J. Proteomics 110, 117-128.
Choi H., Choi Y., Yim H., Mirzaaghasi A., Yoo J. and Choi C. (2021). Biodistribution of exosomes and engineering strategies for targeted delivery of therapeutic exosomes. Tissue Eng. Regen. Med. 18, 499-511.
Cui G., Guo H., Li H., Zhai Y., Gong Z., Wu J., Liu J.S., Dong Y., Hou S. and Liu J.R. (2019a). RVG-modified exosomes derived from mesenchymal stem cells rescue memory deficits by regulating inflammatory responses in a mouse model of Alzheimer's disease. Immun. Ageing 16, 10.
Cui Y., Cao W., He Y., Zhao Q., Wakazaki M., Zhuang X., Gao J., Zeng Y., Gao C., Ding Y., Wong H.Y., Wong W.S., Lam H.K., Wang P., Ueda T., Rojas-Pierce M., Toyooka K., Kang B. and Jiang L. (2019b). A whole-cell electron tomography model of vacuole biogenesis in Arabidopsis root cells. Nat. Plants 5, 95-105.
Cui Y., Guo Y., Kong L., Shi J., Liu P., Li R., Geng Y., Gao W., Zhang Z. and Fu D. (2022). A bone-targeted engineered exosome platform delivering siRNA to treat osteoporosis. Bioact. Mater. 10, 207-221.
Da Silva Lira Filho A., Lafleur A., Marcet-Palacios M. and Olivier M. (2024). Identification of potential novel proteomic markers of *Leishmania* spp.-derived exosomes. Front. Cell. Infect. Microbiol. 14, 1354636.
Dawson C.S., Garcia-Ceron D., Rajapaksha H., Faou P., Bleackley M.R. and Anderson M.A. (2020). Protein markers for Candida albicans EVs include claudin-like Sur7 family proteins. J. Extracell. Vesicles 9, 1750810.
De Palma M., Ambrosone A., Leone A., Del Gaudio P., Ruocco M., Turiák L., Bokka R., Fiume I., Tucci M. and Pocsfalvi G. (2020). Plant roots release small extracellular vesicles with antifungal activity. Plants 9, 1777.
Desai I., Thakur S. and Pagariya P. (2024). Current advances in immunotherapy for cancer. Oral Oncol. Rep. 12, 100652.
Do M.A., Levy D., Brown A., Marriott G. and Lu B. (2019). Targeted delivery of lysosomal enzymes to the endocytic compartment in human cells using engineered extracellular vesicles. Sci. Rep. 9, 17274.
Dooley K., Mcconnell R.E., Xu K., Lewis N.D., Haupt S., Youniss M.R., Martin S., Sia C.L., Mccoy C., Moniz R.J., Burenkova O., Sanchez-Salazar J., Jang S.C., Choi B., Harrison R.A., Houde D., Burzyn D., Leng C., Kirwin K., Ross N.L., Finn J.D., Gaidukov L., Economides K.D., Estes S., Thornton J.E., Kulman J.D., Sathyanarayanan S. and Williams D.E. (2021). A versatile platform for generating engineered extracellular vesicles with defined therapeutic properties. Mol. Ther. 29, 1729-1743.
Erana-Perez Z., Igartua M., Santos-Vizcaino E. and Hernandez R.M. (2024). Genetically engineered loaded extracellular vesicles for drug delivery. Trends Pharmacol. Sci. 45, 350-365.
Escudier B., Dorval T., Chaput N., André F., Caby M., Novault S., Flament C., Leboulaire C., Borg C., Amigorena S., Boccaccio C., Bonnerot C., Dhellin O., Movassagh M., Piperno S., Robert C., Serra V., Valente N., Le Pecq J., Spatz A., Lantz O., Tursz T., Angevin E. and Zitvogel L. (2005). Vaccination of metastatic melanoma patients with autologous dendritic cell (DC) derived-exosomes: Results of thefirst phase I clinical trial. J. Transl. Med. 3, 10.
Etxebeste-Mitxeltorena M., Niza E., Fajardo C.M., Gil .C, Gómez-Gómez L., Martinez A. and Ahrazem O. (2024). Neuroprotective properties of exosomes and chitosan nanoparticles of Tomafran, a bioengineered tomato enriched in crocins. Nat. Prod. Bioprospect. 14, 9.
Fan M., Li H., Shen D., Wang Z., Liu H., Zhu D., Wang Z., Li L., Popowski K.D., Ou C., Zhang K., Zhang J., Cheng K. and Li Z. (2022). Decoy exosomes offer protection against chemotherapy-induced toxicity. Adv. Sci. 9, e2203505.
Fang Y., Wang Z.W., Liu X.L. and Tyler B.M. (2022). Biogenesis and biological functions of extracellular vesicles in cellular and organismal communication with microbes. Front. Microbiol. 13, 817844.
Fang T., Li B., Li M., Zhang Y., Jing Z., Li Y., Xue T., Zhang Z., Fang W., Lin Z., Meng F., Li L., Yang Y., Zhang X., Liang X., Chen S., Chen J. and Zhang X. (2023). Engineered cell membrane vesicles expressing CD40 alleviate system lupus nephritis by intervening B cell activation. Small Methods 7, e2200925.
Ferrantelli F., Arenaccio C., Manfredi F., Olivetta E., Chiozzini C., Leone P., Percario Z., Ascione A., Flego M., Bonito P.D., Accardi L. and Federico M. (2019). The intracellular delivery of anti-HPV16 E7 scFvs through engineered extracellular vesicles inhibits the proliferation of HPV-infected cells. Int. J. Nanomed. 14, 8755-8768.
Ferreira J.V., Da Rosa Soares A., Ramalho J., Máximo Carvalho C.,

Cardoso M.H., Pintado P., Carvalho A.S., Beck H.C., Matthiesen R., Zuzarte M., Girão H., van Niel G. and Pereira P. (2022). LAMP2A regulates the loading of proteins into exosomes. Sci. Adv. 8, eabm1140.

Gan Y., Zhao G., Wang Z., Zhang X., Wu M. and Lu M. (2023). Bacterial membrane vesicles: Physiological roles, infection immunology, and applications. Adv. Sci. 10, e2301357.

Gao W., Zhang Y., Yuan L., Huang F. and Wang Y. (2023). Long non-coding RNA H19-overexpressing exosomes ameliorate UVB-induced photoaging by upregulating SIRT1 via sponging miR-138. Photochem. Photobiol. 99, 1456-1467.

Garcia-Martin R., Wang G., Brandão B.B., Zanotto T.M., Shah S., Kumar Patel S., Schilling B. and Kahn C.R. (2022). MicroRNA sequence codes for small extracellular vesicle release and cellular retention. Nature 601, 446-451.

Gaurivaud P. and Tardy F. (2022). The *Mycoplasma spp*. 'Releasome': A new concept for a long-known phenomenon. Front. Microbiol. 13, 853440.

Gaurivaud P., Ganter S., Villard A., Manso-Silvan L., Chevret D., Boulé C., Monnet V. and Tardy F. (2018). Mycoplasmas are no exception to extracellular vesicles release: Revisiting old concepts. PLoS One 13, e0208160.

Geng Y., Long X., Zhang Y., Wang Y., You G., Guo W., Zhuang G., Zhang Y., Cheng X., Yuan Z. and Zan J. (2023). FTO-targeted siRNA delivery by MSC-derived exosomes synergistically alleviates dopaminergic neuronal death in Parkinson's disease via m6A-dependent regulation of ATM mRNA. J. Transl. Med. 21, 652.

Görgens A., Corso G., Hagey D.W., Wiklander R.J., Gustafsson M.O., Felldin U., Lee Y., Bostancioglu R.B., Sork H., Liang X., Zheng W., Mohammad D.K., van de Wakker S.I., Vader P., Zickler A.M., Mamand D.R., Ma L., Holme M.N., Stevens M.M., Wiklander O.P.B. and El Andaloussi S. (2022). Identification of storage conditions stabilizing extracellular vesicles preparations. J. Extracell. Vesicles 11, e12238.

Gu Z., Sun M., Liu J., Huang Q., Wang Y., Liao J., Shu T., Tao M., Mao G., Pei Z., Meng W., Zhang X., Wei Y., Zhang S., Li S., Xiao K., Lu Y. and Xu Q. (2024). Endothelium-derived engineered extracellular vesicles protect the pulmonary endothelial barrier in acute lung injury. Adv. Sci. 11, e2306156.

Gui L., Ye Q., Yu L., Dou G., Zhou Y., Liu Y., Zhang Y., Yang X., Jin F., Liu S., Jin Y. and Ren L. (2024). Bone-targeting peptide and RNF146 modified apoptotic extracellular vesicles alleviate osteoporosis. Int. J. Nanomed. 19, 471-488.

Gujrati V., Kim S., Kim S.H., Min J.J., Choy H.E., Kim S.C. and Jon S. (2014). Bioengineered bacterial outer membrane vesicles as cell-specific drug-delivery vehicles for cancer therapy. ACS Nano 8, 1525-1537.

Gurriaran-Rodriguez U., Datzkiw D., Radusky L.G., Esper M., Javandoost E., Xiao F., Ming H., Fisher S., Marina A., De Repentigny Y., Kothary R., Azkargorta M., Elortza F., Rojas A.L., Serrano L., Hierro A. and Rudnicki M.A. (2024). Identification of the Wnt signal peptide that directs secretion on extracellular vesicles. Sci. Adv. 10, eado5914.

Han Q., Xie Q.R., Li F., Cheng Y., Wu T., Zhang Y., Lu X., Wong A.S.T., Sha J. and Xia W. (2021). Targeted inhibition of SIRT6 via engineered exosomes impairs tumorigenesis and metastasis in prostate cancer. Theranostics 11, 6526-6541.

Han Q., Li W., Hu K., Gao J., Zhai W., Yang J. and Zhang S. (2022). Exosome biogenesis: Machinery, regulation, and therapeutic implications in cancer. Mol. Cancer 21, 207.

Haroon K., Zheng H., Wu S., Liu Z., Tang Y., Yang G., Liu Y. and Zhang Z. (2024). Engineered exosomes mediated targeted delivery of neuroprotective peptide NR2B9c for the treatment of traumatic brain injury. Int. J. Pharm. 649, 123656.

He J., Wang Y., Zhao Z., He J., Gao M., Wang J., Wang L., Zhang Y. and Li X. (2024). Exosome-specific loading Sox10 for the treatment of Cuprizone-induced demyelinating model. Biomed. Pharmacother. 171, 116128.

Herrmann I.K., Wood M.J.A. and Fuhrmann G. (2021). Extracellular vesicles as a next-generation drug delivery platform. Nat. Nanotechnol. 16, 748-759.

Hobor F., Dallmann A., Ball N.J., Cicchini C., Battistelli C., Ogrodowicz R.W., Christodoulou E., Martin S.R., Castello A., Tripodi M., Taylor I.A. and Ramos A. (2018). A cryptic RNA-binding domain mediates Syncrip recognition and exosomal partitioning of miRNA targets. Nat. Commun. 9, 831.

Hou J., Wei W., Geng Z., Zhang Z., Yang H., Zhang X., Li L. and Gao Q. (2024). Developing plant exosomes as an advanced delivery system for cosmetic peptide. ACS Appl. Bio Mater. 5, 3050-3060.

Hu X., Ning X., Zhao Q., Zhang Z., Zhang C., Xie M., Huang W., Cai Y., Xiang Q. and Ou C. (2022). Islet-1 mesenchymal stem cells-derived exosome-incorporated angiogenin-1 hydrogel for enhanced acute myocardial infarction therapy. ACS Appl. Mater. Interfaces 14, 36289-36303.

Hung M.E. and Leonard J.N. (2015). Stabilization of exosome-targeting peptides via engineered glycosylation. J. Biol. Chem. 290, 8166-8172.

Huang X., Deng Y., Xiao J., Wang H., Yang Q. and Cao Z. (2024). Genetically engineered M2-like macrophage-derived exosomes for *P. gingivalis*-suppressed cementum regeneration: From mechanism to therapy. Bioact. Mater. 32, 473-487.

Ilahibaks N.F., Ardisasmita A.I., Xie S., Gunnarsson A., Brealey J., Vader P., de Jong O.G., de Jager S., Dekker N., Peacock B., Schiffelers R.M., Sluijter J.P.G. and Lei Z. (2023). TOP-EVs: Technology of protein delivery through extracellular vesicles is a versatile platform for intracellular protein delivery. J. Control Release 355, 579-592.

Iravani S. and Varma R.S. (2019). Plant-derived edible nanoparticles and miRNAs: Emerging frontier for therapeutics and targeted drug-delivery. ACS Sustain. Chem. Eng. 7, 8055-8069.

Jia G., Han Y., An Y., Ding Y., He C., Wang X. and Tang Q. (2018). NRP-1 targeted and cargo-loaded exosomes facilitate simultaneous imaging and therapy of glioma *in vitro* and *in vivo*. Biomaterials 178, 302-316.

Jia X., Yuan B., Wang W., Wang K., Ling D., Wei M., Hu Y., Guo W., Chen Z., Du L. and Jin Y. (2024). Gene editing tool-loaded bio-mimetic cationic vesicles with highly efficient bacterial internalization for *in vivo* eradication of pathogens. J. Nanobiotechnology 22, 787.

Jiang B., Lai Y., Xiao W., Zhong T., Liu F., Gong J. and Huang J. (2024). Microbial extracellular vesicles contribute to antimicrobial resistance. PLoS Pathog. 20, e1012143.

Jnana A., Sadiya S., Satyamoorthy K. and Murali T.S. (2023). Extracellular vesicles in bacterial and fungal diseases - pathogenesis to diagnostic biomarkers. Virulence 14, 2180934.

Johnson V., Vasu S., Kumar U.S. and Kumar M. (2023). Surface-engineered extracellular vesicles in cancer immunotherapy. Cancers 15, 2838.

Jones P., Carter D., Lowe D. and Fougerolles A.D. (2022). Engineered

exosomes: A transformative therapeutic modality. Cell Gene Ther. Insights 08, 571-581.

Kabani M. and Melki R. (2015). Sup35p in its soluble and prion states is packaged inside extracellular vesicles. mBio 6, e01017-15.

Kabani M., Pilard M. and Melki R. (2020). Glucose availability dictates the export of the soluble and prion forms of Sup35p via periplasmic or extracellular vesicles. Mol. Microbiol. 114, 322-332.

Kamerkar S., Lebleu V.S., Sugimoto H., Yang S., Ruivo C.F., Melo S.A., Lee J.J. and Kalluri R. (2017). Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer. Nature 546, 498-503.

Kang J., Mun D., Chun Y., Park D., Kim H., Yun N. and Joung B. (2023). Engineered small extracellular vesicle-mediated NOX4 siRNA delivery for targeted therapy of cardiac hypertrophy. J. Extracell. Vesicles 12, e12371.

Keysberg C., Hertel O., Hoffrogge R., Reich S., Hornung N., Holzmann K. and Otte K. (2024). Hyperthermic shift and cell engineering increase small extracellular vesicle production in HEK293F cells. Biotechnol. Bioeng. 121, 942-958.

Kim H.Y., Kim T.J., Kang L., Kim Y., Kang M.K., Kim J., Ryu J.H., Hyeon T., Yoon B., Ko S. and Kim B. (2020). Mesenchymal stem cell-derived magnetic extracellular nanovesicles for targeting and treatment of ischemic stroke. Biomaterials 243, 119942.

Kim H.K., Cho J., Kim E., Kim J., Yang J., Kim K., Lee J., Shin Y., Palomera L.F., Park J., Baek S.H., Bae H., Cho Y., Han J., Sul J.H., Lee J., Park J.H., Cho Y.W., Lee W. and Jo D. (2022a). Engineered small extracellular vesicles displaying ACE2 variants on the surface protect against SARS-CoV-2 infection. J. Extracell. Vesicles 11, e12179.

Kim J.S., Li S.Y., Zhang S.Y. and Wang J.X. (2022b). Plant-derived exosome-like nanoparticles and their therapeutic activities. Asian J. Pharm. Sci. 17, 53-69.

Kong W., Li X., Guo X., Sun Y., Chai W., Chang Y., Huang Q., Wang P. and Wang X. (2024). Ultrasound-assisted CRISPRi-exosome for epigenetic modification of α-synuclein gene in a mouse model of Parkinson's disease. ACS Nano 18, 7837-7851.

Kooijmans S.A.A., Fliervoet L.A.L., van der Meel R., Fens M.H.A.M., Heijnen H.F.G., van Bergen En Henegouwen P.M.P., Vader P. and Schiffelers R.M. (2016). PEGylated and targeted extracellular vesicles display enhanced cell specificity and circulation time. J. Control Release 224, 77-85.

Kopparapu P.K., Deshmukh M., Hu Z., Mohammad M., Maugeri M., Götz F., Valadi H. and Jin T. (2021). Lipoproteins are responsible for the pro-inflammatory property of *Staphylococcus aureus* extracellular vesicles. Int. J. Mol. Sci. 22, 7099.

Krylova S.V. and Feng D. (2023). The machinery of exosomes: Biogenesis, release, and uptake. Int. J. Mol. Sci. 24, 1337.

Lai J.J., Chau Z.L., Chen S., Hill J.J., Korpany K.V., Liang N., Lin L., Lin Y., Liu J.K., Liu Y., Lunde R. and Shen W. (2022). Exosome processing and characterization approaches for research and technology development. Adv. Sci. 9, e2103222.

Lambertz U., Oviedo Ovando M.E., Vasconcelos E.J.R., Unrau P.J., Myler P.J. and Reiner N.E. (2015). Small RNAs derived from tRNAs and rRNAs are highly enriched in exosomes from both old and new world Leishmania providing evidence for conserved exosomal RNA Packaging. BMC Genomics 16, 151.

Leidal A.M., Huang H.H., Marsh T., Solvik T., Zhang D., Ye J., Kai F., Goldsmith J., Liu J.Y., Huang Y., Monkkonen T., Vlahakis A., Huang E.J., Goodarzi H., Yu L., Wiita A.P. and Debnath J. (2020). The LC3-conjugation machinery specifies the loading of RNA-binding proteins into extracellular vesicles. Nat. Cell Biol. 22, 187-199.

Li X., Shao C., Shi Y. and Han W. (2018). Lessons learned from the blockade of immune checkpoints in cancer immunotherapy. J. Hematol. Oncol. 11, 31.

Li Z., Zhou X., Wei M., Gao X., Zhao L., Shi R., Sun W., Duan Y., Yang G. and Yuan L. (2019). *In vitro* and *in vivo* RNA inhibition by CD9-HuR functionalized exosomes encapsulated with miRNA or CRISPR/dCas9. Nano Lett. 19, 19-28.

Li C., Wang W., Sun Y., Ni Y., Qin F., Li X., Wang T., Guo M. and Sun G. (2022a). Selective sorting and secretion of hY4 RNA fragments into extracellular vesicles mediated by methylated YBX1 to promote lung cancer progression. J. Exp. Clin. Cancer Res. 41, 136.

Li Q., Huang Z., Wang Q., Gao J., Chen J., Tan H., Li S., Wang Z., Weng X., Yang H., Pang Z., Song Y., Qian J. and Ge J. (2022b). Targeted immunomodulation therapy for cardiac repair by platelet membrane engineering extracellular vesicles via hitching peripheral monocytes. Biomaterials 284, 121529.

Li Q., Hu W., Huang Q., Yang J., Li B., Ma K., Wei Q., Wang Y., Su J., Sun M., Cui S., Yang R., Li H., Fu X. and Zhang C. (2023). MiR146a-loaded engineered exosomes released from silk fibroin patch promote diabetic wound healing by targeting IRAK1. Signal Transduct. Target. Ther. 8, 62.

Liang Y., Duan L., Lu J. and Xia J. (2021). Engineering exosomes for targeted drug delivery. Theranostics 11, 3183-3195.

Liang Y., Xu X., Xu L., Iqbal Z.Y., Ouyang K., Zhang H., Wen C., Duan L. and Xia J. (2022). Chondrocyte-specific genomic editing enabled by hybrid exosomes for osteoarthritis treatment. Theranostics 12, 4866-4878.

Liang W., Sagar S., Ravindran R., Najor R.H., Quiles J.M., Chi L., Diao R.Y., Woodall B.P., Leon L.J., Zumaya E., Duran J., Cauvi D.M., De Maio A.N., Adler E.D. and Gustafsson Å.B. (2023). Mitochondria are secreted in extracellular vesicles when lysosomal function is impaired. Nat. Commun. 14, 5031.

Lin S., Yu C., Tsai J. and Shyong Y. (2024). Delivery of extracellular vesicles loaded with immune checkpoint inhibitors for immunotherapeutic management of glioma. Mater. Today Bio. 28, 101244.

Liu H., Geng Z. and Su J. (2022). Engineered mammalian and bacterial extracellular vesicles as promising nanocarriers for targeted therapy. Extracell. Vesicles Circ. Nucl. Acids 3, 63-86.

Liu H., Zhang H., Wang S.C., Cui J., Weng W.Z., Liu X.R., Tang H., Hu Y., Li X.Q., Zhang K., Zhou F.J., Jing Y.Y. and Su J.C. (2023a). Bone-targeted bioengineered bacterial extracellular vesicles delivering siRNA to ameliorate osteoporosis. Composites Part B: Engineering 255, 110610.

Liu P., Shi J., Sheng D., Lu W., Guo J., Gao L., Wang X., Wu S., Feng Y., Dong D., Huang X. and Tang H. (2023b). Mitopherogenesis, a form of mitochondria-specific ectocytosis, regulates sperm mitochondrial quantity and fertility. Nat. Cell Biol. 25, 1625-1636.

Liu Q., Li D., Pan X. and Liang Y. (2023c). Targeted therapy using engineered extracellular vesicles: Principles and strategies for membrane modification. J. Nanobiotechnology 21, 334.

Liu C., Yazdani N., Moran C.S., Salomon C., Seneviratne C.J., Ivanovski S. and Han P. (2024a). Unveiling clinical applications of bacterial extracellular vesicles as natural nanomaterials in disease diagnosis and therapeutics. Acta Biomater. 180, 18-45.

Liu S., Xiao X., Zhang L., Wang J., Zhao W., Liu H., Liao R., Li Z., Xu M., Guo J., Zhou B., Du C., Peng Q. and Jiang N. (2024b).

Reprogramming exosomes to escape from immune surveillance for mitochondrial protection in hepatic ischemia-reperfusion injury. Theranostics 14, 116-132.

Lu W., Zhang J., Wu Y., Sun W., Jiang Z. and Luo X. (2023a). Engineered NF-κB siRNA-encapsulating exosomes as a modality for therapy of skin lesions. Front. Immunol. 14, 1109381.

Lu X., Xu G., Lin Z., Zou F., Liu S., Zhang Y., Fu W., Jiang J., Ma X. and Song J. (2023b). Engineered exosomes enriched in netrin-1 modRNA promote axonal growth in spinal cord injury by attenuating inflammation and pyroptosis. Biomater. Res. 27, 3.

Lu X., Xu Z., Shu F., Wang Y., Han Y., Yang X., Shi P., Fan C., Wang L., Yu F., Sun Q., Cheng F. and Chen H. (2024). Reactive oxygen species responsive multifunctional fusion extracellular nanovesicles: Prospective treatments for acute heart transplant rejection. Adv. Mater. 35, e2406758.

Ma S., Song L., Bai Y., Wang S., Wang J., Zhang H., Wang F., He Y., Tian C. and Qin G. (2023). Improved intracellular delivery of exosomes by surface modification with fluorinated peptide dendrimers for promoting angiogenesis and migration of HUVECs. RSC Adv. 13, 11269-11277.

Ma C., Liu K., Wang F., Fei X., Niu C., Li T. and Liu L. (2024). Neutrophil membrane-engineered Panax ginseng root-derived exosomes loaded miRNA 182-5p targets NOX4/Drp-1/NLRP3 signal pathway to alleviate acute lung injury in sepsis: Experimental studies. Int. J. Surg. 110, 72-86.

Ma Y., Dong S., Grippin A.J., Teng L., Lee A.S., Kim B.Y.S. and Jiang W. (2025). Engineering therapeutical extracellular vesicles for clinical translation. Trends Biotechnol. 43, 61-82.

Marina C.L., Bürgel P.H., Agostinho D.P., Zamith-Miranda D., Las-Casas L.D.O., Tavares A.H., Nosanchuk J.D. and Bocca A.L. (2020). Nutritional conditions modulate *C. neoformans* extracellular vesicles' capacity to elicit host immune response. Microorganisms. 8, 1815.

Medina-Castellanos E., Salgado-Bautista D.A., Martínez-Andrade J.M., Cadena-Nava R.D. and Riquelme M. (2023). Nanosized extracellular vesicles released by *Neurospora crassa* hyphae. Fungal Genet. Biol. 165, 103778.

Medvedeva E.S., Baranova N.B., Mouzykantov A.A., Grigorieva T.Y., Davydova M.N., Trushin M.V., Chernova O.A. and Chernov V.M. (2014). Adaptation of mycoplasmas to antimicrobial agents: *Acholeplasma laidlawii* extracellular vesicles mediate the export of ciprofloxacin and a mutant gene related to the antibiotic target. ScientificWorldJournal 2014, 150615.

Mi B., Chen L., Xiong Y., Yang Y., Panayi A.C., Xue H., Hu Y., Yan C., Hu L., Xie X., Lin Z., Zhou W., Cao F., Xiao X., Feng Q. and Liu G. (2022). Osteoblast/osteoclast and immune cocktail therapy of an exosome/drug delivery multifunctional hydrogel accelerates fracture repair. ACS Nano 16, 771-782.

Mishra A., Singh P., Qayoom I., Prasad A. and Kumar A. (2021). Current strategies in tailoring methods for engineered exosomes and future avenues in biomedical applications. J. Mater. Chem. B. 9, 6281-6309.

Mun D., Kang J., Kim H., Yun N. and Joung B. (2024). Small extracellular vesicle-mediated CRISPR-Cas9 RNP delivery for cardiac-specific genome editing. J. Control Release 370, 798-810.

Naito T., Jingushi K., Ueda K. and Tsujikawa K. (2021). Azurocidin is loaded into small extracellular vesicles via its N-linked glycosylation and promotes intravasation of renal cell carcinoma cells. FEBS Lett. 595, 2522-2532.

Nakase I. and Futaki S. (2015). Combined treatment with a pH-sensitive fusogenic peptide and cationic lipids achieves enhanced cytosolic delivery of exosomes. Sci. Rep. 5, 10112.

Nemidkanam V., Banlunara W. and Chaichanawongsaroj N. (2024). *Kaempferia parviflora* extracellular vesicle loaded with clarithromycin for the treatment of *Helicobacter pylori* Infection. Int. J. Nanomed. 19, 1967-1983.

Nkosi D., Sun L., Duke L.C., Patel N., Surapaneni S.K., Singh M. and Meckes D.G. (2020). Epstein-Barr virus LMP1 promotes syntenin-1- and hrs-induced extracellular vesicle formation for its own secretion to increase cell proliferation and migration. mBio 11, e00589-20.

Ofir G. and Sorek R. (2017). Vesicles spread susceptibility to phages. Cell 168, 13-15.

Paganini C., Capasso Palmiero U., Pocsfalvi G., Touzet N., Bongiovanni A. and Arosio P. (2019). Scalable production and isolation of extracellular vesicles: available sources and lessons from current industrial bioprocesses. Biotechnol. J. 14, e1800528.

Pan J., Wang Z., Huang X., Xue J., Zhang S., Guo X. and Zhou S. (2023). Bacteria-derived outer-membrane vesicles hitchhike neutrophils to enhance ischemic stroke therapy. Adv. Mater. 35, e2301779.

Patel D.B., Gray K.M., Santharam Y., Lamichhane T.N., Stroka K.M. and Jay S.M. (2017). Impact of cell culture parameters on production and vascularization bioactivity of mesenchymal stem cell-derived extracellular vesicles. Bioeng. Transl. Med. 2, 170-179.

Pei W., Zhang Y., Zhu X., Zhao C., Li X., Lü H. and Lv K. (2024). Multitargeted immunomodulatory therapy for viral myocarditis by engineered extracellular vesicles. ACS Nano 18, 2782-2799.

Peng W., Xie Y., Liu Y., Xu J., Yuan F., Li C., Qin T., Lu H., Duan C. and Hu J. (2023). Targeted delivery of CD163+ macrophage-derived small extracellular vesicles via RGD peptides promote vascular regeneration and stabilization after spinal cord injury. J. Control. Release 361, 750-765.

Peres Da Silva R., Puccia R., Rodrigues M.L., Oliveira D.L., Joffe L.S., César G.V., Nimrichter L., Goldenberg S. and Alves L.R. (2015). Extracellular vesicle-mediated export of fungal RNA. Sci. Rep. 5, 7763.

Pham T.C., Jayasinghe M.K., Pham T.T., Yang Y.Q., Wei L.K., Usman W.M., Chen H., Pirisinu M., Gong J., Kim S., Peng B., Wang W., Chan C., Ma V., Nguyen N.T.H., Kappei D., Nguyen X.H., Cho W.C., Shi J. and Le M.T.N. (2021). Covalent conjugation of extracellular vesicles with peptides and nanobodies for targeted therapeutic delivery. J. Extracell. Vesicles 10, e12057.

Qi H., Liu C., Long L., Ren Y., Zhang S., Chang X., Qian X., Jia H., Zhao J., Sun J., Hou X., Yuan X. and Kang C. (2016). Blood exosomes endowed with magnetic and targeting properties for cancer therapy. ACS Nano 10, 3323-3333.

Qin Q., Shan Z., Xing L., Jiang Y., Li M., Fan L., Zeng X., Ma X., Zheng D., Wang H., Wang H., Liu H., Liang S., Wu L. and Liang S. (2024). Synergistic effect of mesenchymal stem cell-derived extracellular vesicle and miR-137 alleviates autism-like behaviors by modulating the NF-κB pathway. J. Transl. Med. 22, 446.

Rädler J., Gupta D., Zickler A. and Andaloussi S.E. (2023). Exploiting the biogenesis of extracellular vesicles for bioengineering and therapeutic cargo loading. Mol. Ther. 31, 1231-1250.

Rayner S., Bruhn S., Vallhov H., Andersson A., Billmyre R.B. and Scheynius A. (2017). Identification of small RNAs in extracellular vesicles from the commensal yeast *Malassezia sympodialis*. Sci. Rep 7, 39742.

Ruan H., Li Y., Wang C., Jiang Y., Han Y., Li Y., Zheng D., Ye J., Chen G., Yang G., Deng L., Guo M., Zhang X., Tang Y. and Cui W. (2023). Click chemistry extracellular vesicle/peptide/chemokine nanocarriers for treating central nervous system injuries. Acta Pharm. Sin. B 13, 2202-2218.

Rutter B.D. and Innes R.W. (2017). Extracellular vesicles isolated from the leaf apoplast carry stress-response proteins. Plant Physiol. 173, 728-741.

Salazar-Puerta A.I., Rincon-Benavides M.A., Cuellar-Gaviria T.Z., Aldana J., Vasquez Martinez G., Ortega-Pineda L., Das D., Dodd D., Spencer C.A., Deng B., Mccomb D.W., Englert J.A., Ghadiali S., Zepeda-Orozco D., Wold L.E., Gallego-Perez D. and Higuita-Castro N. (2023). Engineered extracellular vesicles derived from dermal fibroblasts attenuate inflammation in a murine model of acute lung injury. Adv. Mater. 35, e2210579.

Salunkhe S., Dheeraj, Basak M., Chitkara D. and Mittal A. (2020). Surface functionalization of exosomes for target-specific delivery and in vivo imaging & tracking: Strategies and significance. J. Control. Release 326, 599-614.

Sartorio M.G., Pardue E.J., Scott N.E. and Feldman M.F. (2023). Human gut bacteria tailor extracellular vesicle cargo for the breakdown of diet- and host-derived glycans. Proc. Natl. Acad. Sci. USA 120, e2306314120.

Shi R., Lv R., Dong Z., Cao Q., Wu R., Liu S., Ren Y., Liu Z., van der Mei H.C., Liu J. and Busscher H.J. (2023). Magnetically-targetable outer-membrane vesicles for sonodynamic eradication of antibiotic-tolerant bacteria in bacterial meningitis. Biomaterials 302, 122320.

Shirazi S., Huang C., Kang M., Lu Y., Ravindran S. and Cooper L.F. (2021). The importance of cellular and exosomal miRNAs in mesenchymal stem cell osteoblastic differentiation. Sci. Rep. 11, 5953.

Shrivastava S., Ray R.M., Holguin L., Echavarria L., Grepo N., Scott T.A., Burnett J. and Morris K.V. (2021). Exosome-mediated stable epigenetic repression of HIV-1. Nat. Commun. 12, 5541.

Si K., Dai Z., Li Z., Ye Z., Ding B., Feng S., Sun B., Shen Y. and Xiao Z. (2023). Engineered exosome-mediated messenger RNA and single-chain variable fragment delivery for human chimeric antigen receptor T-cell engineering. Cytotherapy 25, 615-624.

Silva A.M., Lázaro-Ibáñez E., Gunnarsson A., Dhande A., Daaboul G., Peacock B., Osteikoetxea X., Salmond N., Friis K.P., Shatnyeva O. and Dekker N. (2021). Quantification of protein cargo loading into engineered extracellular vesicles at single-vesicle and single-molecule resolution. J. Extracell. Vesicles 10, e12130.

Smyth T., Petrova K., Payton N.M., Persaud I., Redzic J.S., Graner M.W., Smith-Jones P. and Anchordoquy T.J. (2014). Surface functionalization of exosomes using click chemistry. Bioconjug. Chem. 25, 1777-1784.

Song J., Song B., Yuan L. and Yang G. (2022). Multiplexed strategies toward clinical translation of extracellular vesicles. Theranostics 12, 6740-6761.

Spokeviciute B., Kholia S. and Brizzi M.F. (2024). Chimeric antigen receptor (CAR) T-cell therapy: Harnessing extracellular vesicles for enhanced efficacy. Pharmacol. Res. 208, 107352.

Statello L., Maugeri M., Garre E., Nawaz M., Wahlgren J., Papadimitriou A., Lundqvist C., Lindfors L., Collén A., Sunnerhagen P., Ragusa M., Purrello M., Di Pietro C., Tigue N., Valadi H. and Gerber A.P. (2018). Identification of RNA-binding proteins in exosomes capable of interacting with different types of RNA: RBP-facilitated transport of RNAs into exosomes. PLoS One 13, e0195969.

Stephan M.S., Broeker N.K., Saragliadis A., Roos N., Linke D. and Barbirz S. (2020). *In vitro* analysis of O-antigen-specific bacteriophage P22 inactivation by *Salmonella* outer membrane vesicles. Front. Microbiol. 11, 510638.

Sterzenbach U., Putz U., Low L.H., Silke J., Tan S.S. and Howitt J. (2017). Engineered exosomes as vehicles for biologically active proteins. Mol. Ther. 25, 1269-1278.

Sun L., Fan M., Huang D., Li B.Q., Xu R., Gao F. and Chen Y. (2021). Clodronate-loaded liposomal and fibroblast-derived exosomal hybrid system for enhanced drug delivery to pulmonary fibrosis. Biomaterials 271, 120761.

Sun F., Sun Y., Wang X., Zhu J., Chen S., Yu Y., Zhu M., Xu W. and Qian H. (2024a). Engineered mesenchymal stem cell-derived small extracellular vesicles for diabetic retinopathy therapy through HIF-1α/EZH2/PGC-1α pathway. Bioact. Mater. 33, 444-459.

Sun H., Dong J., Fu Z., Lu X., Chen X., Lei H., Xiao X., Chen S., Lu J., Su D., Xiong Y., Fang Z., Mao J., Chen L. and Wang X. (2024b). TSG6-Exo@CS/GP attenuates endometrium fibrosis by inhibiting macrophage activation in a murine IUA model. Adv. Mater. 36, 2308921.

Tian T., Zhang H., He C., Fan S., Zhu Y., Qi C., Huang N., Xiao Z., Lu Z., Tannous B.A. and Gao J. (2018). Surface functionalized exosomes as targeted drug delivery vehicles for cerebral ischemia therapy. Biomaterials 150, 137-149.

Tian Y., Zhang F., Qiu Y., Wang S., Li F., Zhao J., Pan C., Tao Y., Yu D. and Wei W. (2021). Reduction of choroidal neovascularization via cleavable VEGF antibodies conjugated to exosomes derived from regulatory T cells. Nat. Biomed. Eng. 5, 968-982.

Tian T., Liang R., Erel-Akbaba G., Saad L., Obeid P.J., Gao J., Chiocca E.A., Weissleder R. and Tannous B.A. (2022). Immune checkpoint inhibition in GBM primed with radiation by engineered extracellular vesicles. ACS Nano 16, 1940-1953.

Toyofuku M., Cárcamo-Oyarce G., Yamamoto T., Eisenstein F., Hsiao C., Kurosawa M., Gademann K., Pilhofer M., Nomura N. and Eberl L. (2017). Prophage-triggered membrane vesicle formation through peptidoglycan damage in *Bacillus subtilis*. Nat. Commun. 8, 481.

Toyofuku M., Schild S., Kaparakis-Liaskos M. and Eberl L. (2023). Composition and functions of bacterial membrane vesicles. Nat. Rev. Microbiol. 21, 415-430.

Tu W., Hu X., Wan R., Xiao X., Shen Y., Srikaram P., Avvaru S.N., Yang F., Pi F.M., Zhou Y., Wan M. and Gao P. (2024). Effective delivery of miR-511-3p with mannose-decorated exosomes with RNA nanoparticles confers protection against asthma. J. Control. Release 365, 602-616.

Valdez A.F., de Souza T.N., Bonilla J.J.A., Zamith-Miranda D., Piffer A.C., Araujo G.R.S., Guimarães A.J., Frases S., Pereira A.K., Fill T.P., Estevao I.L., Torres A., Almeida I.C., Nosanchuk J.D. and Nimrichter L. (2023). Traversing the cell wall: The chitinolytic activity of *Histoplasma capsulatum* extracellular vesicles facilitates their release. J. Fungi. 9, 1052.

Wan Y., Li L., Chen R., Han J., Lei Q., Chen Z., Tang X., Wu W., Liu S. and Yao X. (2024). Engineered extracellular vesicles efficiently deliver CRISPR-Cas9 ribonucleoprotein (RNP) to inhibit herpes simplex virus1 infection *in vitro* and *in vivo*. Acta Pharm. Sin. B. 14, 1362-1379.

Wang J., Ding Y., Wang J., Hillmer S., Miao Y., Lo S.W., Wang X., Robinson D.G. and Jiang L. (2010). EXPO, an exocyst-positive organelle distinct from multivesicular endosomes and autophagosomes, mediates cytosol to cell wall exocytosis in

Arabidopsis and tobacco cells. Plant Cell 22 4009-4030.

Wang B., Zhuang X., Deng Z., Jiang H., Mu J., Wang Q., Xiang X., Guo H., Zhang L., Dryden G., Yan J., Miller D. and Zhang H. (2014). Targeted drug delivery to intestinal macrophages by bioactive nanovesicles released from grapefruit. Mol. Ther. 22, 522-534.

Wang Q., Yu J., Kadungure T., Beyene J., Zhang H. and Lu Q. (2018). ARMMs as a versatile platform for intracellular delivery of macromolecules. Nat. Commun. 9, 960.

Wang Z., Wei C., Hao X., Deng W., Zhang L., Wang Z. and Wang H. (2019). Genome-wide identification and characterization of transfer RNA-derived small RNAs in *Plasmodium falciparum*. Parasites Vectors 12, 36.

Wang Y., Jiang M., Zheng X., He Y., Ma X., Li J. and Pu K. (2023). Application of exosome engineering modification in targeted delivery of therapeutic drugs. Biochem. Pharmacol. 215, 115691.

Wang Y., Li S., Wang T., Zou M. and Peng X. (2024). Extracellular vesicles from mycoplasma gallisepticum: Modulators of macrophage activation and virulence. J. Infect. Dis. 229, 1523-1534.

Welsh J.A., Goberdhan D.C.I., O'Driscoll L., Buzas E.I., Blenkiron C., Bussolati B., Cai H., Di Vizio D., Driedonks T.A.P., Erdbrügger U., Falcon-Perez J.M., Fu Q., Hill A.F., Lenassi M., Lim S.K., Mahoney M.G., Mohanty S., Möller A., Nieuwland R., Ochiya T., Sahoo S., Torrecilhas A.C., Zheng L., Zijlstra A., Abuelreich S., Bagabas R., Bergese P., Bridges E.M., Brucale M., Burger D., Carney R.P., Cocucci E., Crescitelli R., Hanser E., Harris A.L., Haughey N.J., Hendrix A., Ivanov A.R., Jovanovic-Talisman T., Kruh-Garcia N.A., Ku'Ulei-Lyn Faustino V., Kyburz D., Lässer C., Lennon K.M., Lötvall J., Maddox A.L., Martens-Uzunova E.S., Mizenko R.R., Newman L.A., Ridolfi A., Rohde E., Rojalin T., Rowland A., Saftics A., Sandau U.S., Saugstad J.A., Shekari F., Swift S., Ter-Ovanesyan D., Tosar J.P., Useckaite Z., Valle F., Varga Z., van der Pol E., van Herwijnen M.J.C., Wauben M.H.M., Wehman A.M., Williams S., Zendrini A., Zimmerman A.J., Consortium M., Théry C. and Witwer K.W. (2024). Minimal information for studies of extracellular vesicles (MISEV2023): From basic to advanced approaches. J. Extracell. Vesicles. 13, e12404.

Wowk P.F., Zardo M.L., Miot H.T., Goldenberg S., Carvalho P.C. and Mörking P.A. (2017). Proteomic profiling of extracellular vesicles secreted from *Toxoplasma gondii*. Proteomics 17.

Wozniak A.L., Adams A., King K.E., Dunn W., Christenson L.K., Hung W. and Weinman S.A. (2020). The RNA binding protein FMR1 controls selective exosomal miRNA cargo loading during inflammation. J. Cell Biol. 219, e201912074.

Wu L., Wang L., Liu X., Bai Y., Wu R., Li X., Mao Y., Zhang L., Zheng Y., Gong T., Zhang Z. and Huang Y. (2022). Milk-derived exosomes exhibit versatile effects for improved oral drug delivery. Acta Pharm. Sin. B. 12, 2029-2042.

Wu Q., Glitscher M., Tonnemacher S., Schollmeier A., Raupach J., Zahn T., Eberle R., Krijnse-Locker J., Basic M. and Hildt E. (2023). Presence of intact Hepatitis B Virions in exosomes. Cell. Mol. Gastroenterol. Hepatol. 15, 237-259.

Xie X., Cao Y., Dai L. and Zhou D. (2023). Bone marrow mesenchymal stem cell-derived exosomal lncRNA KLF3-AS1 stabilizes Sirt1 protein to improve cerebral ischemia/reperfusion injury via miR-206/USP22 axis. Mol. Med. 29, 3.

Xie J., Li Q. and Nie S. (2024a). Bacterial extracellular vesicles: An emerging postbiotic. Trends Food Sci. Technol. 143, 104275.

Xie L., Chen J., Hu H., Zhu Y., Wang X., Zhou S., Wang F. and Xiang M. (2024b). Engineered M2 macrophage-derived extracellular vesicles with platelet membrane fusion for targeted therapy of atherosclerosis. Bioact. Mater. 35, 447-460.

Xie Q., Hao Y., Li N., Song H., Chen X., Zhou Z., Wang J., Zhang Y., Li H., Han P. and Wang X. (2024c). Cellular uptake of engineered extracellular vesicles: biomechanisms, engineered strategies, and disease treatment. Adv. Healthc. Mater. 13, e2302280.

Xie X., Cheng P., Hu L., Zhou W., Zhang D., Knoedler S., Liu G., Xiong Y., Xue H., Hu Y., Kern B., Obed D., Panayi A.C., Chen L., Yan C., Lin Z., Dai G., Mi B., Zhang Y. and Liu G. (2024d). Bone-targeting engineered small extracellular vesicles carrying anti-miR-6359-CGGGAGC prevent valproic acid-induced bone loss. Signal Transduct. Target. Ther. 9, 24.

Xiong Y., Lou P., Xu C., Han B., Liu J. and Gao J. (2024). Emerging role of extracellular vesicles in veterinary practice: Novel opportunities and potential challenges. Front. Vet. Sci. 11, 1335107.

Xu F., Fei Z., Dai H., Xu J., Fan Q., Shen S., Zhang Y., Ma Q., Chu J., Peng F., Zhou F., Liu Z. and Wang C. (2022). Mesenchymal stem cell-derived extracellular vesicles with high PD-L1 expression for autoimmune diseases treatment. Adv. Mater. 34, e2106265.

Xu L.M., Xu X., Liang Y.J., Wen C.N., Ouyang K., Huang J.D., Xiao Y., Deng X., Xia J. and Duan L. (2023). Osteoclast-targeted delivery of anti-miRNA oligonucleotides by red blood cell extracellular vesicles. J. Control Release 358, 259-272.

Yaghoobi A., Nazerian Y., Meymand A.Z., Ansari A., Nazerian A. and Niknejad H. (2023). Hypoxia-sensitive miRNA regulation *via* CRISPR/dCas9 loaded in hybrid exosomes: A novel strategy to improve embryo implantation and prevent placental insufficiency during pregnancy. Front. Cell. Dev. Biol. 10, 1082657.

Yang Z., Liu X., Zhao F., Yao M., Lin Z., Yang Z., Liu C., Liu Y., Chen X. and Du C. (2022). Bioactive glass nanoparticles inhibit osteoclast differentiation and osteoporotic bone loss by activating lncRNA NRON expression in the extracellular vesicles derived from bone marrow mesenchymal stem cells. Biomaterials 283, 121438.

Yang Z., Ji P., Li Z., Zhang R., Wei M., Yang Y., Yuan L., Han Y. and Yang G. (2023). Improved extracellular vesicle-based mRNA delivery for familial hypercholesterolemia treatment. Theranostics 13, 3467-3479.

Yang Z., Zhang Z., Li L., Jing Z., Ma Y., Lan T., Li Y., Lin Z., Fang W., Zhang J., Zhang J., Liang X., Wu B., Zheng Y. and Zhang X. (2024). Bioengineered artificial extracellular vesicles presenting PD-L1 and Gal-9 ameliorate new-onset type 1 diabetes. Diabetes 73, 1325-1335.

Yaron S., Kolling G.L., Simon L. and Matthews K.R. (2000). Vesicle-mediated transfer of virulence genes from *Escherichia coli* O157:H7 to other enteric bacteria. Appl. Environ. Microbiol. 66, 4414-4420.

You Y., Tian Y., Yang Z.G., Shi J.F., Kwak K.J., Tong Y.H., Estania A.P., Cao J., Hsu W., Liu Y., Chiang C., Schrank B.R., Huntoon K., Lee D., Li Z., Zhao Y., Zhang H., Gallup T.D., Ha J., Dong S., Li X., Wang Y., Lu W., Bahrani E., Lee L.J., Teng L., Jiang W., Lan F., Kim B.Y.S. and Lee A.S. (2023). Intradermally delivered mRNA-encapsulating extracellular vesicles for collagen-replacement therapy. Nat. Biomed. Eng. 7, 887-900.

Yu X., Bai Y., Han B., Ju M., Tang T., Shen L., Li M., Yang L., Zhang Z., Hu G., Chao J., Zhang Y. and Yao H. (2022). Extracellular vesicle-mediated delivery of circDYM alleviates CUS-induced depressive-like behaviours. J. Extracell. Vesicles 11, e12185.

Zeng B., Li Y., Xia J., Xiao Y., Khan N., Jiang B., Liang Y. and Duan L. (2024a). Micro Trojan horses: Engineering extracellular vesicles crossing biological barriers for drug delivery. Bioeng. Transl. Med. 9,

e10623.

Zeng W., Zheng L., Li Y., Yang J., Mao T., Zhang J., Liu Y., Ning J., Zhang T., Huang H., Chen X. and Lu F. (2024b). Engineered extracellular vesicles for delivering functional Cas9/gRNA to eliminate hepatitis B virus cccDNA and integration. Emerg. Microbes Infect. 13, 2284286.

Zhang Y., Dou Y., Liu Y., Di M., Bian H., Sun X. and Yang Q. (2023a). Advances in therapeutic applications of extracellular vesicles. Int. J. Nanomed. 18, 3285-3307.

Zhang Y.Y., Ge T., Huang M., Qin Y., Liu T., Mu W., Wang G., Jiang L., Li T., Zhao L. and Wang J. (2023b). Extracellular vesicles expressing CD19 antigen improve expansion and efficacy of CD19-targeted CAR-T cells. Int. J. Nanomed. 18, 49-63.

Zhang H., Yan W., Wang J., Xie S., Tao W.A., Lee C., Zhang X., Zhang G., Liu Y., Wei D., Hu J., Liu H., Liu F., Nie Y., Chen X., Xu H., Xia J. and Wang S. (2024a). Surface functionalization of exosomes for chondrocyte-targeted siRNA delivery and cartilage regeneration. J. Control. Release 369, 493-505.

Zhang L., Liu M., Sun Q., Cheng S., Chi Y., Zhang J., Wang B., Zhou L. and Zhao J. (2024b). Engineering M2 type macrophage-derived exosomes for autoimmune hepatitis immunotherapy via loading siRIPK3. Biomed. Pharmacother. 171, 116161.

Zhang Y., Ding N., Li Y., Ouyang M., Fu P., Peng Y. and Tan Y. (2024c). Transcription factor FOXM1 specifies chromatin DNA to extracellular vesicles. Autophagy 20, 1054-1071.

Zhao B., Lin H., Jiang X., Li W., Gao Y., Li M., Yu Y., Chen N. and Gao J. (2024a). Exosome-like nanoparticles derived from fruits, vegetables, and herbs: innovative strategies of therapeutic and drug delivery. Theranostics 14, 4598-4621.

Zhao J., Zhu W., Mao Y., Li X., Ling G., Luo C. and Zhang P. (2024b). Unignored intracellular journey and biomedical applications of extracellular vesicles. Adv. Drug Deliv. Rev. 212, 115388.

Zheng G., Ma H.W., Xiang G.H., He G.L., Cai H.C., Dai Z.H., Chen Y.L., Lin Y., Xu H.Z., Ni W.F., Xu C., Liu H.X. and Wang X.Y. (2022a). Bone-targeting delivery of platelet lysate exosomes ameliorates glucocorticoid-induced osteoporosis by enhancing bone-vessel coupling. J. Nanobiotechnology 20, 220.

Zheng W., He R., Liang X., Roudi S., Bost J., Coly P.M., van Niel G. and Andaloussi S.E.L. (2022b). Cell-specific targeting of extracellular vesicles through engineering the glycocalyx. J. Extracell. Vesicles 11, e12290.

Zheng W., Rädler J., Sork H., Niu Z., Roudi S., Bost J.P., Görgens A., Zhao Y., Mamand D.R., Liang X., Wiklander O.P.B., Lehto T., Gupta D., Nordin J.Z. and El Andaloussi S. (2023a). Identification of scaffold proteins for improved endogenous engineering of extracellular vesicles. Nat. Commun. 14, 4734.

Zheng X., Xing Y., Sun K., Jin H., Zhao W. and Yu F. (2023b). Combination therapy with resveratrol and celastrol using folic acid-functionalized exosomes enhances the therapeutic efficacy of sepsis. Adv. Healthc. Mater. e2301325.

Zhou X., Deng X., Liu M., He M., Long W., Xu Z., Zhang K., Liu T., So K., Fu Q. and Zhou L. (2023). Intranasal delivery of BDNF-loaded small extracellular vesicles for cerebral ischemia therapy. J. Control. Release 357, 1-19.

Zhu Y., Huang R., Wang D., Yu L., Liu Y., Huang R., Yin S., He X., Chen B., Liu Z., Cheng L. and Zhu R. (2023). EVs-mediated delivery of CB2 receptor agonist for Alzheimer's disease therapy. Asian J. Pharm. Sci. 18, 100835.

Zhuang M., Du D., Pu L., Song H., Deng M., Long Q., Yin X., Wang Y. and Rao L. (2019). SPION-decorated exosome delivered BAY55-9837 targeting the pancreas through magnetism to improve the blood GLC response. Small 15, e1903135.

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