

REVIEW

Open Access



Encapsulation and assessment of therapeutic cargo in engineered exosomes: a systematic review

Zhen Chen^{1,2,3}, Min Xiong⁴, Jiaqi Tian^{1,2}, Dandan Song^{1,2}, Shuyin Duan⁵ and Lin Zhang^{1,2*}

Abstract

Exosomes are nanoscale extracellular vesicles secreted by cells and enclosed by a lipid bilayer membrane containing various biologically active cargoes such as proteins, lipids, and nucleic acids. Engineered exosomes generated through genetic modification of parent cells show promise as drug delivery vehicles, and they have been demonstrated to have great therapeutic potential for treating cancer, cardiovascular, neurological, and immune diseases, but systematic knowledge is lacking regarding optimization of drug loading and assessment of delivery efficacy. This review summarizes current approaches for engineering exosomes and evaluating their drug delivery effects, and current techniques for assessing exosome drug loading and release kinetics, cell targeting, biodistribution, pharmacokinetics, and therapeutic outcomes are critically examined. Additionally, this review synthesizes the latest applications of exosome engineering and drug delivery in clinical translation. The knowledge compiled in this review provides a framework for the rational design and rigorous assessment of exosomes as therapeutics. Continued advancement of robust characterization methods and reporting standards will accelerate the development of exosome engineering technologies and pave the way for clinical studies.

Keywords Engineered exosomes, Drug encapsulation, Efficacy assessment, Clinical translation

*Correspondence:

Lin Zhang

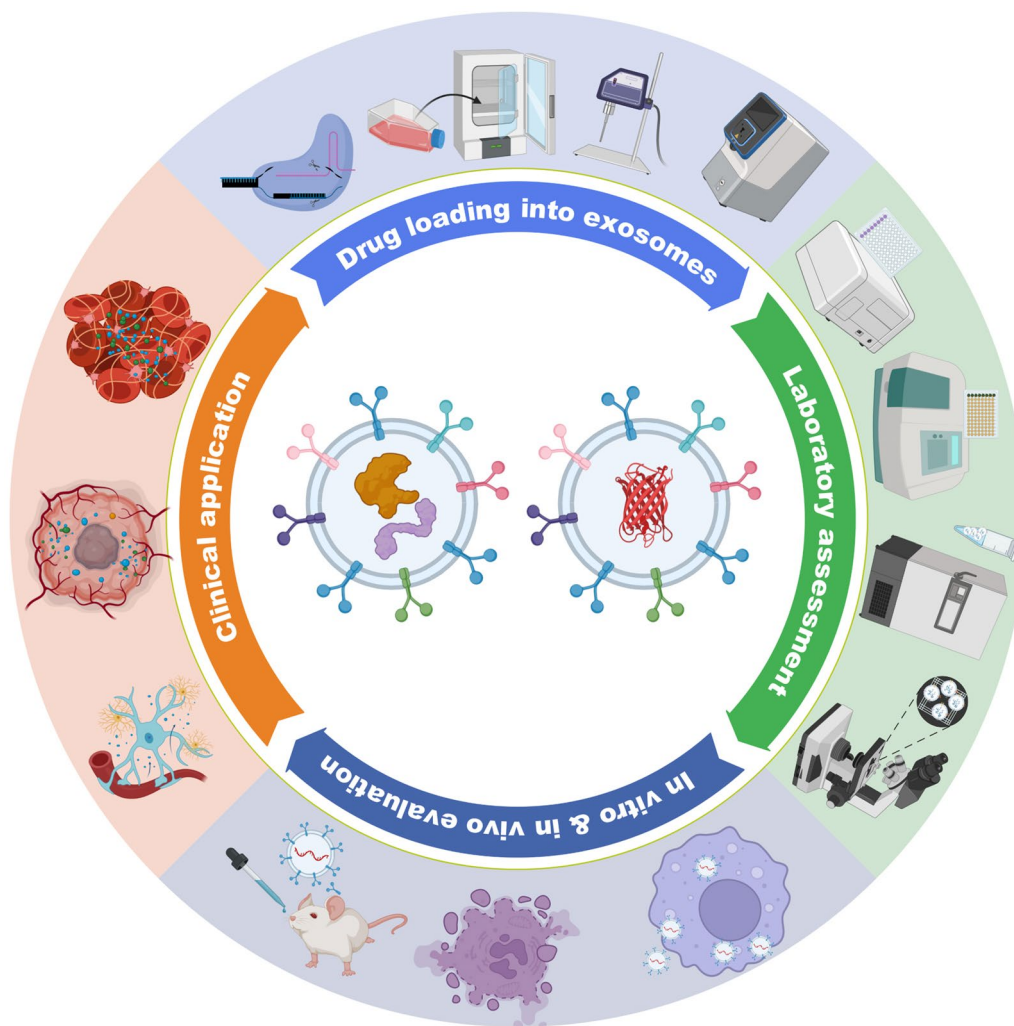
zhanglin8901@163.com

Full list of author information is available at the end of the article



© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

Graphical Abstract



Introduction

Exosomes are nanoscale extracellular vesicles secreted by cells that have recently emerged as promising drug delivery vehicles. Functionally, exosomes possess a lipid bilayer membrane that encloses bioactive cargoes including proteins, lipids, and nucleic acids. With high biocompatibility and low immunogenicity, exosomes can facilitate targeted drug transport with reduced immune clearance [1]. However, the clinical potential of natural exosomes is limited by their short circulation half-lives, variability based on cell source, and insufficient drug loading capacity. To overcome these limitations, researchers have developed engineered exosomes through bioengineering techniques to enhance drug encapsulation and targeting. Generally, engineered exosomes hold increased drug loading and stability compared to natural exosomes by

preventing exosome aggregation, adsorption, and rapid clearance, and the engineering of exosomes to create tailored and efficient drug delivery systems has become an intense area of investigation across biomedical and biotechnology fields.

Bioengineering techniques can enhance exosome production, stability, and purity, and engineered exosomes generated through genetic modification of parent cells have emerged as promising drug delivery vehicles (Fig. 1). Besides, these engineered exosomes offer numerous advantages, such as drug protection, increased stability and bioavailability, reduced toxicity, and improved targeting ability. As a result of these benefits, engineered exosomes are currently being explored as efficient drug carriers in the field of therapeutic delivery [2]. Although significant technical progress has been made in exosome

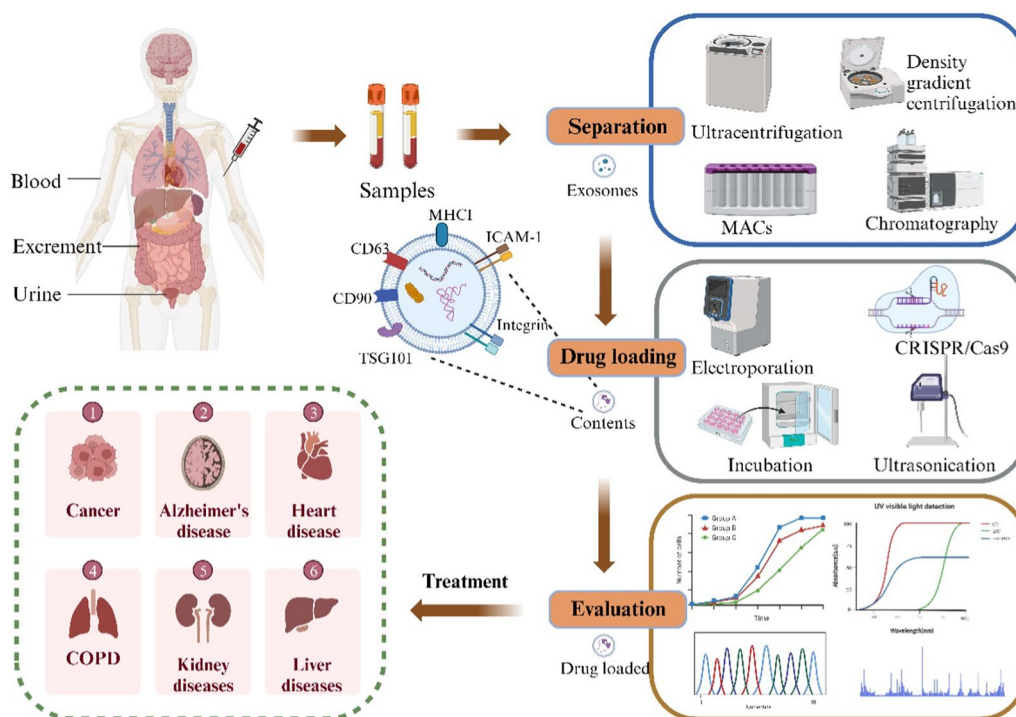


Fig. 1 Overview of the process for engineering exosomes for therapeutic applications. Natural exosomes are first isolated from body fluids, then they are engineered to carry specific cargoes or express ligands on their surface. The drug loading capability of the engineered exosomes is evaluated before studying their potential clinical uses

Table 1 Summary and comparison of different exosome isolation methods

Methods	Advantages	Disadvantages
Ultracentrifugation	Obtaining exosomes with high concentration suitable for large-scale isolation	Complex instrument operation and low efficiency of exosome isolation and extraction
Filtration	High exosome purity, short time expenditure, and ability to fractionate exosomes by size	Membrane pore size needs to be selected based on sample characteristics, and the filtration rate may be affected by pore size and sample concentration
Differential centrifugation	The extracted exosomes have high purity, which ensures the structural integrity of the exosomes	Multiple centrifugation steps required, resulting in cumbersome workflow
Density gradient centrifugation	Efficient isolation of exosomes of different densities	Preparing density gradient solutions requires technical expertise and experience. Centrifugation speed and duration need to be properly controlled, otherwise separation efficiency may be impacted
Immunomagnetic bead separation	Selective isolation of target exosomes	Selection and modification of target-specific antibodies is required, and complex procedure may impact exosome structure and function
Size exclusion chromatography	Simple procedure concurrently isolates exosomes while excluding larger exosomes, protein aggregates, and cell debris	Membrane damage or disruption during isolation may impact exosome function and properties

engineering, yet demonstrations on the evaluation of exosome therapeutic efficacy, standardized methods for fabrication, drug loading, and quantification remain underdeveloped, and the lack of robust methods has hindered the clinical translation of engineered exosomes. Therefore, this review synthesizes recent progresses in

isolation, drug encapsulation, and characterization of engineered exosomes with special focus on evaluating their drug delivery capacity. The establishment of reliable engineering guidelines and assessment strategies will facilitate the clinical development of exosome engineering technologies. At last, we outline current challenges

and future directions in translating engineered exosomes from bench to bedside.

Exosome isolation, purification, and drug encapsulation

Isolation and purification of exosomes

The isolation and purification of exosomes involve a variety of techniques, each with its own set of advantages and limitations. These techniques include ultracentrifugation, filtration, differential centrifugation, density gradient centrifugation, immunomagnetic separation, and size exclusion chromatography (Table 1). The choice of method often results in a trade-off between exosome purity and yield [3]. For instance, while ultracentrifugation might yield a high volume of exosomes, it may not provide the same level of purity as immunomagnetic separation. Conversely, methods that offer high purity, such as size exclusion chromatography, might not yield as many exosomes. Therefore, the selection of an appropriate method depends on the specific requirements of the study or application, and it is crucial to consider both the advantages and limitations of each method to make an informed decision.

Early exosome research lack standardization and relies on study-specific protocols. Recently, commercial kits have become more popular, but they often produce exosome preparations of suboptimal purity and quality control [4, 5]. Therefore, establishing standardized guidelines for isolation and benchmarking methods using defined purity and yield metrics would improve compatibility and reproducibility across studies.

Ultracentrifugation is the most widely used exosome isolation technique, applicable to cell culture medium and diverse biofluids such as plasma, saliva, and urine. This method first removes cells and debris via centrifugation, then exosomes are pelleted by high-speed centrifugation. Varying centrifugation speed and duration isolate exosomes of defined size and density ranges. For example, Zhu et al. successfully extracted and identified exosomes from human neural stem cell using ultracentrifugation, which were demonstrated to inhibit the activation of microglia and promote the differentiation of endogenous neural stem cells into neurons [6]. However, a major limitation of ultracentrifugation is vesicle membrane damage and loss during high g-force spinning, which greatly hinders its recovery and application.

Filtration is a method that isolates exosomes from samples containing large particulates. This process involves the passage of these samples through membranes with defined pore sizes. Notably, this technique allows for the isolation of exosomes within specific size ranges. It is particularly effective when applied to cell culture medium and urine, as it can effectively remove non-vesicular

debris while allowing exosomes to pass through. A notable application of this technique was demonstrated by Tang et al. [7], who successfully extracted exosomes from HepG2 cells using filtration. They found that these exosomes could deliver chemotherapeutics to cisplatin-resistant cells, thereby conferring drug resistance. However, a significant limitation of filtration is the requirement for multiple centrifugation steps both before and after filtration, which can make the protocol quite cumbersome.

Differential centrifugation separates exosomes based on substance densities. In this process, solutions of decreasing density are layered and then centrifuged at varying forces. This results in the pelleting of exosomes into specific density fractions. A practical application of this technique was demonstrated by Wan et al. [8], who used differential centrifugation to isolate M2 type tumor-associated macrophage (TAM)-derived exosomes. They discovered the intercellular transfer of the MSTRG.292666.16 via extracellular vesicles, which conferred osimertinib resistance in non-small cell lung cancer cells. This revealed a new mechanism of clinical osimertinib resistance in lung cancer. However, one of the challenges of differential centrifugation is the need for precise control over the centrifugation speed and time to ensure proper density-based separation of exosomes.

Based on the decreasing density of the suspension, the density gradient centrifugation method separates exosomes from non-vesicular particles, including proteins, lipids, and RNA aggregates. During centrifugation, the exosome containing components migrate to their equilibrium zone, allowing the isolation of highly purified exosomes from other extracellular material. Using density gradient centrifugation, Tian et al. successfully isolated exosomes derived from irradiated glioblastoma cells and demonstrated that these exosomes could promote tumor growth by increasing FoxP3 expression of differentiating Th1 cells [9]. However, the shortness of this technique is the need for substantial technical expertise to prepare density gradients properly and precise control over centrifugation speed and time to achieve separation. Improper gradient preparation or centrifugation conditions can result in failed fractionation of exosomes from contaminating particles.

Immunomagnetic separation is a technique that uses antibody-coated magnetic beads to selectively isolate exosomes displaying specific surface proteins. This method is known for its high specificity and selectivity. For instance, Guo et al. introduced the immunomagnetic separation method as an emerging strategy for rapid isolation and mild release of exosomes from the 293T cell culture supernatant [10], Arnau et al. isolated exosomes using this method and detected overexpressed exosomal

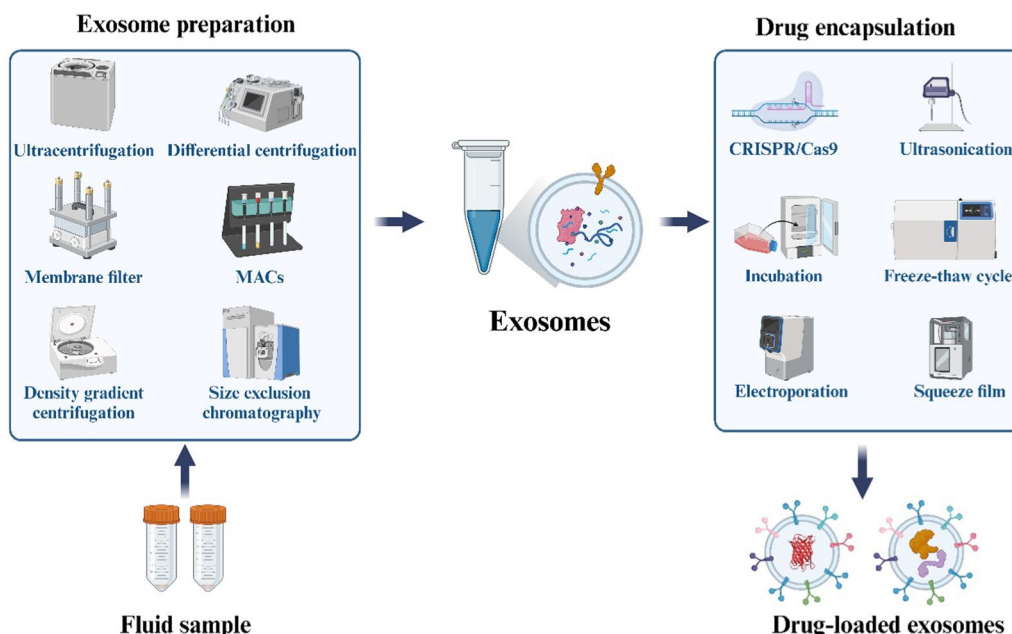


Fig. 2 Methods for drug loading into engineered exosomes. Drug loading approaches for exosomes are divided into endogenous and exogenous methods. Endogenous loading primarily utilizes CRISPR/Cas9 technology to package cargo intracellularly. Exogenous loading methods include incubation, electroporation, sonication, freeze–thaw cycles, and extrusion to load cargo after vesicle isolation

GAPDH transcripts [11]. However, the major limitation of immunomagnetic separation is the need for extensive antibody screening and modification, which adds a significant level of complexity to the method. Additionally, this technique can alter vesicle structure and function due to antibody binding.

Size exclusion chromatography is a method that separates exosomes based on their sizes. In this process, larger exosomes are excluded from alternative-column pores and elute earlier, while smaller proteins and aggregates enter the pores and elute later. Dhananjie et al. conducted a comparison between the precipitation and size exclusion chromatography methods in terms of exosome yields and purity [12]. They discovered that a combination of precipitation followed by size exclusion chromatography resulted in high exosome yields with good purity. On the other hand, Bai et al. tested different size exclusion chromatography-based tandem strategies for plasma exosome enrichment through proteomic analysis [13]. Their findings indicated that the size exclusion chromatography-based tandem methods were superior to the direct size exclusion chromatography method in terms of the purity of exosome isolation from human plasma. However, this technique has its limitations. One major concern is the potential disruption of exosome membranes during separation, which can impair their biological activities. Additionally, size exclusion chromatography is both time-consuming and costly. These

factors must be taken into consideration when using this method for exosome separation.

Drug loading into exosomes

Drug loading methods for engineered exosomes can be broadly categorized into two main technical approaches: endogenous loading and exogenous loading (Fig. 2).

Endogenous loading method

This method involves transfecting donor cells to express the desired proteins, nucleic acids, or drug molecules using genetic engineering techniques. Subsequently, exosomes containing the bioactive cargo are isolated from the donor cell culture supernatant. An example of this is the CRISPR/Cas9 system for targeted gene editing, which has shown therapeutic promise for cancer [14, 15]. However, its clinical translation has been largely limited by barriers such as immunogenicity. This is where engineered exosomes come into play, as they can overcome these limitations. When exosomes are packaged with the CRISPR/Cas9 system, they directly emerge with the membranes of cancer cells, enabling efficient non-invasive gene editing. A study by Kim et al. demonstrated that CRISPR/Cas9-loaded exosomes could induce apoptosis in ovarian cancer cells by suppressing the expression of poly (ADP-ribose) polymerase-1 [16], which underscores the potential for precision anti-cancer therapy using exosomes engineered with gene editing machinery.

Exosomes are also being investigated as cancer vaccines with advantages over traditional chemotherapies such as higher specificity, controllability, and free of toxicity [17–19]. A recent study demonstrated that, when co-administered with a cancer vaccine, exosomes released by M1-polarized macrophages could boost vaccine immunogenicity and anti-tumor immune responses in mouse models, which shines light on the therapeutic potential of engineered exosomes to develop optimized anti-cancer vaccines [20].

Exogenous loading method

This method first isolates exosomes, then incorporates drugs or bioactive cargo externally using various techniques. Compared to the endogenous loading method, the exogenous loading method has several advantages. It offers simpler protocols, greater stability, and improved scalability for large-scale production. Common exogenous loading approaches include co-incubation, electroporation, sonication, freeze–thaw cycles, and extrusion. These techniques provide a versatile and efficient way to load therapeutic agents into exosomes for targeted delivery.

In co-incubation method, exosomes are isolated from cell culture supernatant then incubated with drugs or bioactive molecules. The drug-loaded exosomes are finally purified by ultracentrifugation or filtration. To the greatest extent, this method maintains the integrity of the exosome membranes and is particularly suitable for small hydrophobic molecules. For instance, Zhao et al. loaded docetaxel (DTX) into M1-polarized macrophage-derived exosomes (M1-Exo) using the co-incubation method and they successfully developed a DTX-M1-Exo drug delivery system. Evidenced by *in vitro* experiments data, they demonstrated that the DTX-M1-Exo could effectively target and kill breast cancer cells, which highlights the potential of the co-incubation method in creating targeted drug delivery systems using exosomes [21].

Electroporation is a technique where an electric field is applied to increase exosome membrane permeability and induce temporary hydrophilic pores, which enables the loading of hydrophilic small molecules like amino acids and peptides. A study by Yan et al. utilized this method to encapsulate miR-31-5p mimics in milk-derived exosomes, which were used as carriers for miR-31-5p delivery. The use of exosomes for miRNA delivery offers several advantages over direct miRNA delivery, such as enhanced cell uptake and resisted enzymatic degradation of miRNAs, as well as increased miRNA bioavailability, which results in improved efficacy for wound healing in diabetics [22]. However, electroporation may cause exosome aggregation and reduce their stability.

Sonication is a method that uses probe sonication to create transient pores in the exosome membrane. This process allows the diffusion of small hydrophilic molecules into the exosomes. Generally, the exosome membrane can be restored after a 1-h incubation at 37 °C post-sonication. Utilizing this method, Gao et al. loaded berberine into M2 macrophage-derived exosomes (Exo-Ber), and the newly generated Exo-Ber was found to reduce the M1 marker iNOS and suppress the expression of M2 marker CD206, which resulted in the promotion of macrophage/microglia polarization from the M1 to the M2 phenotype [23]. In addition, Britta et al. compared the loading capacity to hydrophilic compounds and impact on vesicle functions between different encapsulation methods, they found increased loading capacity in order saponin \leq sonication $<$ fusion $<$ freeze–thawing \leq osmotic shock [24]. Notably, it's important to consider the specific conditions and parameters of sonication to ensure the integrity and functionality of the exosomes.

For the freeze–thaw cycle method, exosomes and drugs are first incubated at room temperature, then undergo 3–5 freeze–thaw cycles between -80 °C and room temperature. After these cycles, the drug-loaded exosomes are purified by ultracentrifugation, but this method can affect the stability of the exosomes and their uptake by cells. Cheng et al. found in HEK 293T exosomes that higher temperature and increased freeze–thaw cycles altered vesicle stability and reduced cell uptake [25]. Therefore, while the freeze–thaw cycle method can be effective for loading drugs into exosomes, careful consideration must be given to the specific conditions to maintain the functionality of the exosomes.

The extrusion method starts by dissolving or suspending the drug to be encapsulated in a solvent such as saline, glycerol or ethanol. The drug solution is then mixed with the exosome suspension, and the mixture passes through a lipid extruder multiple times, which loads the drug into the exosomes. Zhang et al. utilized this method to generate drug-loaded exosomes derived from human umbilical cord MSCs [26]. By conducting proteomic analysis on these exosomes, they identified 1,669 encapsulated proteins. Further experiments demonstrated that these drug-loaded exosomes were able to facilitate wound healing by promoting cell proliferation, migration, and angiogenesis. However, the extrusion method can potentially damage exosomes and impact drug loading efficiency if not properly optimized. Parameters such as extrusion pressure, number of passes, and exosome concentration need to be carefully controlled to avoid disrupting exosome integrity and to achieve efficient drug encapsulation.

Table 2 Engineered exosome as delivery vehicles for diverse drug types

Drug types	Drugs	Disease types	In vitro	In vivo	References
RNAs	miR-145-5p	Pancreatic ductal adenocarcinoma	Cancer cell proliferation and invasion↓	Cancerous cells apoptosis↑	[27]
	miR-183-5p	Breast cancer	Growth and metastasis of cancer cells↓	Tumor growth↓	[28]
	miR-1827	Colorectal	Cancer cell proliferation, migration, and invasion↓	Tumor growth and liver metastasis↓	[29]
	Cas9 mRNA	Breast cancer	Cancer cell growth↓	Tumor growth↓	[30]
	piRNA-18	Colon cancer	Cancer cell proliferation↓	Tumor proliferation, migration, and invasion↓	[31]
Proteins	circABC10	Colorectal	Cancer cell growth↓	Tumor growth↓	[32]
	CIITA	Lymphoma	T cell response↑	T cell response↑	[33]
	NF-κB	Prostate cancer	Androgen receptor inhibition↑	Tumor growth↓	[34]
Compounds	TRAIL	Lymphoma	Leukocyte apoptosis↑	No effect on tumor growth	[35]
	Paclitaxel	Breast cancer	T cell ↑	Tumor growth↓	[36]
	Doxorubicin	Triple-negative breast cancer	Cancer cell growth↓	Tumor growth↓	[37]
	Curcumin	Glioblastoma	Glioblastoma cell growth↓	–	[38]

↑ increase; ↓ decrease

Decoration of exosome for drug delivery and targeted therapy

Exosomes are promising drug delivery vehicles capable of carrying small molecules, proteins, nucleic acids, and other bioactive molecules (Table 2). However, native exosomes exhibit broad biodistribution and lack targeting specificity. Therefore, a major research focus has been developing strategies to decorate the exosomal surface with targeting moieties to direct their delivery to desired cell types and tissues. This section we review recent approaches for functionalization and targeting of engineered exosomes.

Targeting ligand display

Display of targeting ligands on the exosome surface enables directed delivery to cells expressing the cognate receptors. Ligands can be attached directly to exosomal membrane proteins or lipids using chemical conjugation or fusion protein approaches. Alternatively, parental cells can be engineered to express ligand-anchored proteins that get incorporated into secreted exosomes.

Peptide ligands typically possess the ability to bind selective receptors or epitopes on target cells. These peptides can be fused to exosomal membrane proteins, a process that equips the exosomes with the ability to target specific cells. As a result, these modified exosomes exhibit enhanced functionality in intercellular communication. For instance, Lamp2b protein was fused with α v integrin-binding iRGD peptide or EGFR-binding GE11 peptide to exosomes displaying these targeting peptides on the surface. The iRGD and GE11 exosomes showed preferential accumulation in α v integrin-positive and

EGFR-overexpressing breast cancer cells [39, 40]. Moreover, a cardiac homing peptide (CHP) was anchored onto exosomes via a streptavidin–biotin linkage, and those CHP-displaying exosomes showed enhanced cardiac accumulation after intravenous injection [41, 42], which demonstrated that these peptide ligands could enable functional targeting of exosomes to specific tissues and disease sites.

Surface display of antibody fragments that bind unique cellular epitopes is another common targeting approach. For example, Lamp2b can fuse with an anti-HER2 nanobody and direct exosomes to HER2-overexpressing breast cancer cells, while exosomes displaying anti-EGFR nanobody are found to suppress EGFR signaling and reduce proliferation in triple negative breast cancer cells [43]. Single chain variable fragments (scFv) against tumor antigens have also been anchored using hydrophobic membrane intercalating peptides or enzymatic lipidation, and the scFv-decorated exosomes are found to bind and get internalized by target cancer cells [44]. Furthermore, researchers also generated exosomes displaying scFv against folate receptor, which increased delivery of chemotherapeutic cargo to folate receptor-positive cancer cells [45].

Aptamer ligands are short nucleic acids that bind specific molecules. Previous reports showed that bivalent RNA aptamers against PSMA were conjugated to exosomes using cholesterol anchors, and elevated PSMA-dependent uptake in prostate cancer cells was shown [46]. Also, tetravalent RNA aptamers against EGFR conjugated exosome using click chemistry were proved to enhance targeting in EGFR-overexpressing

triple negative breast cancer [47]. Notably, DNA aptamers against nucleolin surface protein were anchored onto exosomes using a biotin-NeutrAvidin connector [48], and this system was proved to be capable of specifically delivering exosomal cargo into leukemia cells in vivo, which, in combination with most recent findings, highlighted aptamers as adaptable targeting ligands to direct exosomes to cancer.

Glycan-binding proteins on immune cells play a crucial role in the removal of exosomes from circulation. One strategy to enhance delivery is to shield the exosome glycocalyx, thereby evading immune clearance and simultaneously displaying targeting glycans. A previous study implemented a metabolic glycoengineering approach to exosomes, which enabled them to present targeting glycans, and the resulted engineered glycoexosomes exhibited lacNAc sugars and demonstrated an affinity for dendritic cells via the DC-SIGN receptor [49]. In a different approach, linear polyglycerol sulfate was applied as a coating to the exosome surfaces, which effectively shielded the immune-activating glycans, while still allowing for the display of targeting mannose moieties [50]. Collectively, this innovative approach significantly increased the potential of exosome to reduce immune elimination while simultaneously enabling directed targeting.

Conjugation of lipids ligands presenting headgroups that engage biological targets provides a direct means for exosomal surface decoration. For example, phospholipids displaying folate groups were inserted into exosome membranes using hydrophobic interactions, and the folate-lipids were found to mediate specific uptake in cancer cells expressing the folate receptor [51]. Several researchers synthetically incorporated glycolipids presenting CD47 "don't eat me" protein and an $\alpha\beta3$ -integrin targeting peptide, and these glycolipid-exosomes evaded phagocytosis and homed to angiogenic blood vessels [52]. More recently, Gong et al. generated exosomes displaying hyaluronic acid by expressing a membrane anchor protein fused with a hyaluronic acid-binding domain [53], and they demonstrated that the hyaluronic acid-displaying exosomes showed enhanced anti-hepatic fibrosis effect by interacting with CD44 receptors on tumor cells.

Modulation of endogenous proteins

Exosomal membrane natively contains proteins such as tetraspanins, integrins, and lectins. These proteins can influence the exosome biodistribution through their interactions with target cell receptors. By genetically modulating parental cells to either overexpress or knockdown specific proteins, the targeting capabilities of engineered exosomes can be significantly enhanced.

Tetraspanins comprise a family of scaffolding proteins that mediate vesicle-cell fusion and signaling. Take CD63-rich exosomes as an example, they display tropism towards target cells expressing Tspan8, while knockdown of Tspan8 in mice can reduce exosome uptake in liver and increase accumulation in spleen, brain and heart [54, 55]. Hence, modulating tetraspanin levels can alter exosome biodistribution. Tetraspanins also bind tissue-specific lectins, which support exosomes adhesion to dendritic cells by engaging DC-SIGN receptor, and exosomal CD81 binds hepatocyte asialoglycoprotein receptor through galectin domains [56]. Therefore, strategies to overexpress tetraspanins could therefore enhance targeting.

Integrins are cell adhesion molecules, they play a crucial role in facilitating cell-matrix and cell-cell interactions. Interestingly, exosomes display integrins that determine their capture by recipient cells. For instance, $\alpha6\beta4$ and $\alpha6\beta1$ integrins present on tumor cell-derived exosomes have been found to bind to lung epithelial cells, thereby promoting metastasis [57, 58]. In addition, the $\beta1$ integrin present on exosomes has been shown to influence organotropic metastasis in models of breast and pancreatic cancer. Studies have shown that the knockdown of αv integrin on exosomes reduces liver tropism, while its overexpression increases hepatic localization [59, 60]. Consequently, it is reasonable to conclude that by modulating the levels of exosomal integrins, it is possible to alter their biodistribution profile, which opens up new avenues for improving the targeting capabilities of exosomes, potentially enhancing the efficacy of exosome-based therapies.

Galectins are β -galactoside-binding proteins that can facilitate glycan-dependent interactions between cells and vesicles. One such galectin, galectin-5, when expressed, triggers the secretion of galectin-5-enriched exosomes from Kupffer cells. These exosomes show a preference for hepatocellular carcinoma cells, adhering to their surface glycoproteins [61]. However, when galectin-5 is inhibited, this binding of exosomes is also suppressed, leading to an inhibition of cancer progression. Similarly, the overexpression of another galectin, galectin-9, stimulates the release of galectin-9-expression exosomes from tumor cells, and these exosomes promote angiogenesis [62].

Targeting motif display

Cell-binding motifs can be displayed on exosome surface using protein engineering approaches, and these approaches commonly include the use of viral targeting motifs, cell-penetrating peptides, and protein transfection domains. Notably, each of these elements plays a unique role in enhancing the ability of exosomes to target

and penetrate cells, which makes them valuable tools in the field of targeted drug delivery and disease therapy.

Viral proteins contain short peptide sequences that bind host cell receptors to allow viral entry. Fusing these targeting peptides to exosomal proteins can reroute their tropism. Yu et al. demonstrated this by expressing a peptide from the rabies virus glycoprotein (RVG) on the Lamp2b protein, then this RVG-Lamp2b was displayed on the surfaces of exosomes and was verified to mediate specific delivery of exosomes into neural cells by binding to the acetylcholine receptor [63, 64]. In a similar vein, a peptide from the hepatitis C virus envelope glycoprotein E2 was found to bind hepatocellular carcinoma cells through interaction with the CD81 receptor, and exosomes displaying this H77 peptide showed enhanced accumulation in the liver in vivo [65, 66].

Cationic cell-penetrating peptides (CPPs) like transactivator of transcription (TAT) can translocate lipid membranes through electrostatic interactions and insert into exosomes when fused to proteins [67]. Haney et al. attached TAT peptide to catalase enzyme and loaded them into exosomes for treating Parkinson's disease [68, 69], where TAT-catalase exosomes were found to display neurotropism and antioxidant effects in the brain.

Certain protein known as protein transfection domains possess an inherent capacity to cross cell membranes. The transgenic membrane permeable (Tango) peptide is one such example, it consists of a membrane translocation sequence derived from the zebrafish voltage-dependent anion channel. When this Tango peptide was fused to the mCherry fluorescent protein and anchored into exosome membranes using a laminin domain, the resulting Tango exosome demonstrated increased cellular internalization compared to unmodified exosomes, indicating that protein transfection domains could potentially enhance exosomal uptake and facilitate the delivery of encapsulated cargo across plasma membranes [70]. However, it's important to note that the tissue targeting ability of these domains may still require further optimization.

Assessment of drug encapsulation in exosomes

Assessment criteria

Evaluation of drug loading into exosomes focuses on several aspects, including drug release efficiency, drug stability, drug delivery efficiency, cellular uptake efficiency, therapeutic efficacy, and biosafety evaluation. For Drug release efficiency, it refers to the percentage of drugs released from exosome and indicates the loading and controlled release capabilities. Methods to quantify drug release efficiency include UV-vis spectrophotometry, high performance liquid chromatography (HPLC), fluorescence staining, Tinopal staining, magnetic resonance

imaging (MRI), and filtration. Chen et al. developed a graphene oxide-based nanocomposite coated with chitooligosaccharides and polyglutamic acid (GO-CO-PGA) as an exosome-based drug delivery system. Using UV-vis spectrophotometry, they confirmed sustained drug loading efficacy by 73% [71]. Similarly, Sun et al. loaded curcumin into exosomes and examined its efficacy using HPLC [72]. Değirmenci et al. electroporated exosomes derived from normal epithelial breast cells with lapatinib and verified the drug loading efficacy using HPLC, they found that compared to free drug, the lapatinib-loaded exosomes showed higher anti-proliferative effects and enhanced apoptosis induction ability in breast cancer cells [73].

Drug stability describes the ability of a loaded drug to maintain its molecular structure, activity, physicochemical properties, and biological function under certain conditions without being influenced by external factors over time. Methods to assess drug stability include high performance liquid chromatography-mass spectrometry (HPLC-MS), flow cytometry, electron microscopy, and molecular dynamic simulations. Sharma et al. used an immunoinformatic approach to design a multi-epitope tuberculosis vaccine from immunogenic exosomal proteins, and they demonstrated that the vaccine elicited cellular and humoral immunity and provided broad population coverage by compensating for genetic variations. In particular, molecular dynamics simulations were used to model, refine, and dock the vaccine structure to the TLR4 immune receptor [74].

The efficiency of drug delivery is gauged by the quantity of drug that exosomes successfully transport to target cells within a specified timeframe, which is influenced by several factors, including the properties of the exosome suspension, the concentration of the drug, its stability, and the target organ. Various methods are employed to assess drug delivery efficiency, such as fluorescent labeling, biofluorescence imaging, fractionation, transmission electron microscopy (TEM), and scanning electron microscopy (SEM). In a study aimed at promoting cartilage regeneration, Lee et al. utilized the freeze-thaw cycle method to load exosomes with miR-140 [75]. They found that these miR-140-loaded exosomes induced membrane fusion and released miRNA into the cytoplasm. Furthermore, TEM was used to confirm the morphology of the exosomes and the delivery efficiency of miR-140. On a related note, Zhu et al. engineered tumor-exocytosis exosome/AIE luminogenic hybrid nanovesicles for use in photodynamic therapy [76]. They used electroporation to load the photosensitizer DCPy into exosomes. The quantity of drugs packaged into the exosomes was then confirmed using fluorescent labeling.

Cellular uptake efficiency defines the efficacy of exosome drug absorption and utilization by target cells after uptake. It depends on various factors, such as drug properties, exosome loading capacity, exosome/cell membrane permeability, receptor affinity, and metabolic capacity. To assess cellular uptake efficiency, different methods can be used, such as fluorescent/radioisotope labeling, confocal microscopy, and cytological staining. For example, Zhu et al. developed an intraocular lens (IOL) surface modified with exosomes derived from lens epithelial cells (LECs) and loaded with doxorubicin (Dox), an anti-proliferative drug [77]. They used confocal microscopy to demonstrate that the exosome-functionalized IOL enhanced the cellular uptake of Dox by LECs due to the homologous targeting feature of exosome, which resulted in superior anti-proliferation effect and effective posterior capsular opacification (PCO) prevention. A recent development in the evaluation of exosome cellular uptake efficiency is the creation of fluorine-engineered exosomes (exo@FPG3), which are generated through surface engineering of exosomes with fluorinated peptide dendrimers (FPG3), and they are proved to enhance the intracellular delivery and biological activity of exosomes [78]. The study found that the intracellular uptake of exo@FPG3 was energy-dependent and clathrin-mediated endocytosis was a key pathway for exo@FPG3 to enter HUVECs. Moreover, Yang et al. investigated the cellular uptake efficiency of curcuma rhizoma exosome-like nanoparticles (CELNs) loaded with astragalus components (AC) in Caco-2 cell models, and they found that AC-CELNs exhibited superior uptake and transmembrane transport capacity compared to free AC [79].

Therapeutic efficacy assessment involves laboratory assessment of disease status in experimental subjects treated with drug-loaded exosomes to determine if the expected therapeutic outcomes are achieved, it is crucial for establishing the scientific merit, rationale, and safety of a treatment regimen. Better therapeutic efficacy indicates more potent disease treatment by the drug-loaded exosomes. Methods for evaluating therapeutic efficacy include cell viability assays, molecular profiling, animal studies, and clinical trials. Since MSCs have low retention and survival in infarcted hearts, Huang et al. investigated whether exosomes derived from MSCs could enhance treatment of acute myocardial infarction. They found that intramyocardial delivery of exosomes followed by intravenous MSC infusion significantly improved cardiac function, reduced infarct size, and increased neovascularization compared to exosome alone or MSC monotherapy [80]. Xie et al.

presented a novel technique for label-free detection of cellular HER2 using machine learning-driven SERS, and they applied their method to dynamically monitor the therapeutic efficacy of drug-loaded exosomes targeting HER2+ breast cancer cells [81]. They showed that their method could capture the variations in HER2 expression and cell viability during the treatment, which could facilitate the therapeutic decision-making and management of breast cancer. Sana et al. investigated the use of exosomes as a natural delivery platform for bleomycin, where they prepared exosomes loaded with bleomycin (Exo-BLM) from cancer cells and tested their effects on tumor cells *in vitro* and *in vivo* [82]. Their findings showed that Exo-BLM had high cancer targeting ability and enhanced antitumor activity, and reduced toxicity compared to free bleomycin in a mouse model. Similarly, using a non-invasive liquid-biopsy-based assay, Ting et al. developed a novel approach for assessing the therapeutic efficacy of neoadjuvant chemotherapy (NACT) in patients with advanced gastric cancer (AGC), which was demonstrated to potentially facilitate precision treatment of NACT for patients with AGC [83].

Since exosomes are recognized as promising drug delivery vehicles regarding their ability to traverse biological barriers, a comprehensive biosafety evaluation is imperative prior to clinical translation. This rigorous assessment should encompass several areas: (1) *In vitro* cytotoxicity and functional assays can determine potential toxicity and adverse effects of exosomes on cells; (2) animal models enable evaluation of *in vivo* systemic toxicity, immunogenicity, and other safety risks; (3) biodistribution studies are critical for tracking exosome accumulation and clearance kinetics in organs and tissues following administration; (4) pharmacokinetic and pharmacodynamic analyses elucidate exosome stability, metabolism, elimination, and delivery of drug cargo to target sites; and (5) drug interaction studies reveal impacts of exosomes on the safety and efficacy of concomitant medications. For example, Kim et al. evaluated exosomes loaded with the chemotherapy drug paclitaxel and demonstrated that the drug-loaded exosomes are free of cytotoxicity or immunogenicity, but can accumulate in and deliver drug to tumor sites [84]. Jiang et al. loaded exosomes engineered to express TRAIL with the drug triptolide as targeted melanoma therapy. *In vitro*, the TRAIL-expressing exosomes enhanced tumor cell uptake, inhibited cancer cell proliferation, invasion, and migration, and induced apoptosis. *In vivo*, the TRAIL-expressing exosomes suppressed tumor progression and reduced triptolide toxicity with ideal biosafety [85].

Table 3 Summary of methods for drug loading analysis in engineered exosomes

Evaluation methods	Advantages	Disadvantages
Morphological observation	Directly observing exosome morphology, size, distribution, and drug loading	Requiring specialized equipment and expertise; sample preparation can be complex, with possible artifacts introduced during processing that may affect sample integrity
UV-visible spectroscopy	Simple, rapid technique to directly quantify contents in exosome samples	Only measuring absorption spectra, does not directly quantify drug loading into exosomes
Protein quantification	Simple, rapid, direct measurements of protein in exosome samples	Only measuring total protein content, does not directly quantify drug loading into exosomes
Fluorescence microscopy	Directly observation of drug encapsulation and quantify exosome uptake	Complex, requiring specialized equipment, extensive sample preparation, and optimization, and potential of introducing artifacts
Stability testing	Assessing stability of exosomes under stressed conditions, providing parameters on exosome physical, chemical, and biological stability	Time-consuming and require stringent storage conditions

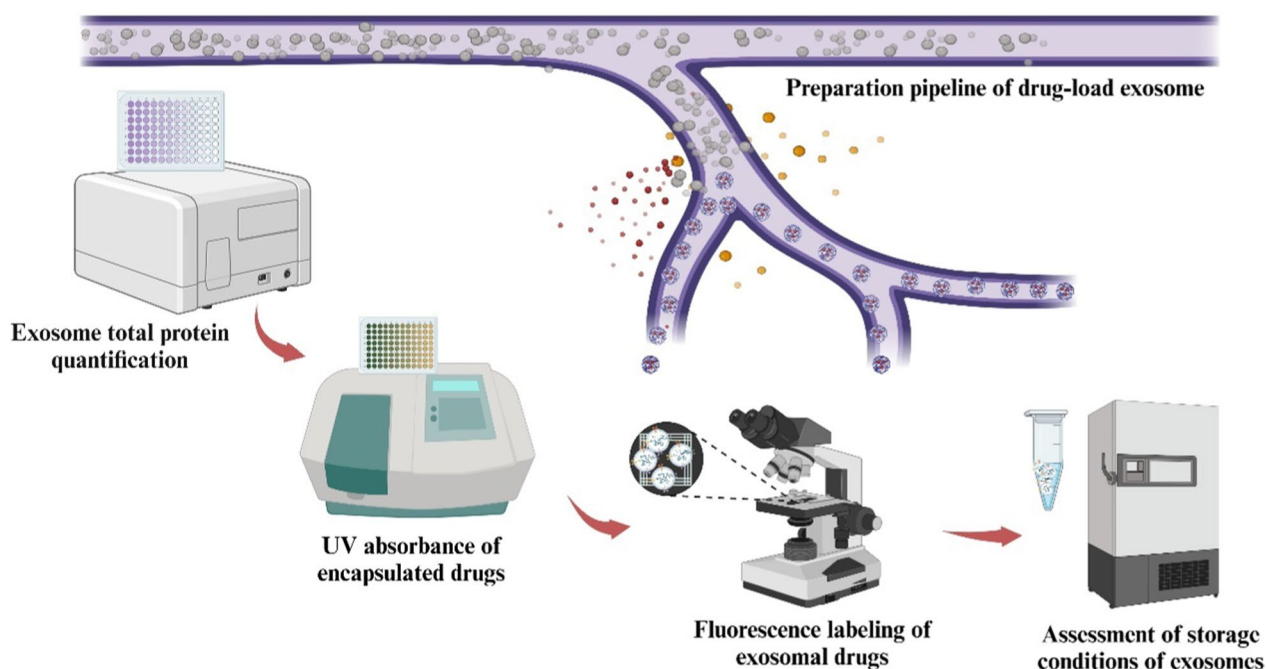


Fig. 3 Laboratory detection and evaluation of engineered exosomes. After harvesting drug-loaded exosomes, the total protein is first quantified to determine exosome yield. Next, UV absorbance of the exosome suspension is measured to quantify drug loading efficacy. The engineered exosomes are then fluorescently labeled, enabling evaluation of their biodistribution and drug release capabilities *in vitro* and *in vivo*. Finally, storage conditions are optimized by studying the engineered exosomes after freezing for various periods

Evaluation methods

Laboratory testing

TEM enables direct visualization of exosomes such as their morphology, size distribution, cargo abundance, and other physical characteristics. Zhu et al. imaged triple-negative breast cancer cell-derived exosomes by TEM and found proteins involved in extracellular matrix interactions and metastasis [86]. Also, Yu et al. visualized milk-derived and drug-loaded exosomes by TEM,

they showed superior osteogenic potential of exosomes encapsulating icariin both *in vitro* and *in vivo* [87]. Liu et al. engineered MRI-trackable exosomes by expressing a ferritin-lactadherin fusion protein in parent cells, they demonstrated that the genetic modification had no effect on exosome morphology using TEM, and further MR imaging enabled *in vitro* tracking and *in vivo* monitoring of exosomes [88].

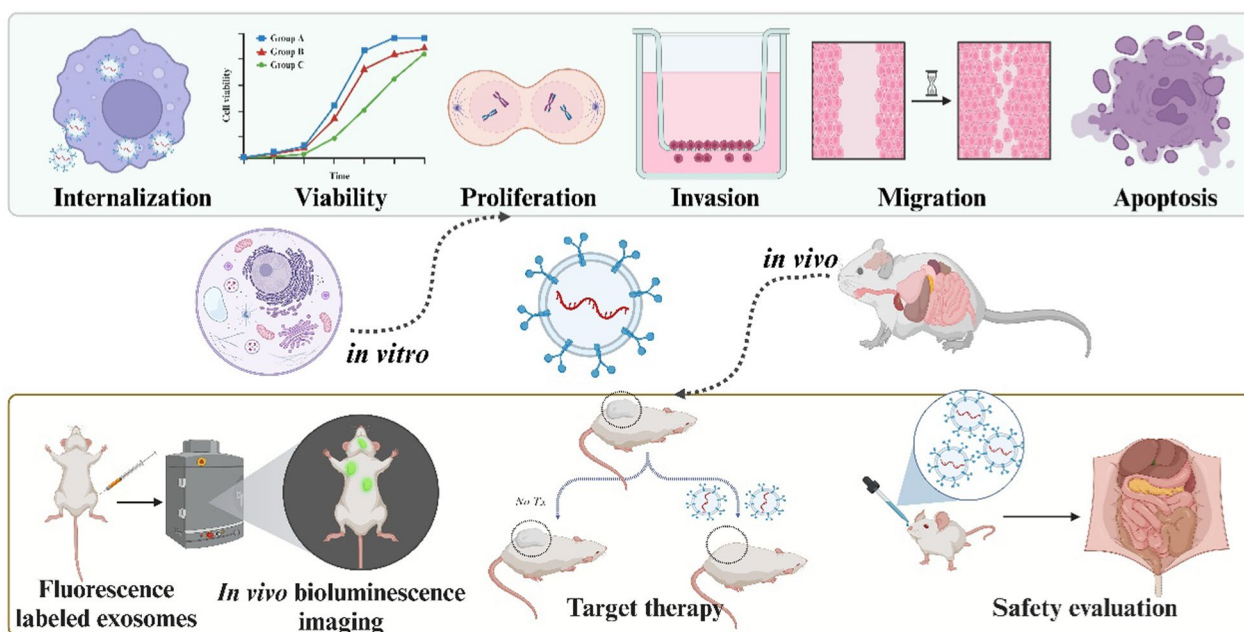


Fig. 4 Cellular experiments and animal models for testing engineered exosomes. In vitro cell studies assess exosome-mediated drug delivery by quantifying drug abundance within cells, measuring changes in cell viability or functionality after exosome treatment. In vivo animal studies establish disease models in rodents or other organisms to test exosome biodistribution and therapeutic efficacy

To detect drugs encapsulated in exosomes, researchers commonly employ UV–vis spectroscopy. Tanziela et al. (2022) reported a novel drug delivery platform based on exosomes isolated from glioblastoma cells (U87), where they detected the UV–vis absorption and fluorescence spectra from prepared solution of AgNCs [89]. Additionally, Zheng et al. confirmed the assembly of gold nanorods and aptamers on exosomes for targeted cancer photothermal therapy using UV–vis spectroscopy, among which the UV–vis characterization enabled investigation of the photothermal properties of modified exosome [90].

Protein quantification method can rapidly detect the total protein content loaded in exosomes. Although these methods do not provide information on specific protein cargoes, they allow quick measurement of the overall protein loading. By quantifying total protein, these methods enable fast detection of the presence of protein-based drugs in exosomes. Haney et al. studied exosome delivery of the antioxidant enzyme catalase as a potential therapy for Parkinson’s disease. They first isolated exosomes from macrophages using differential centrifugation. To load the exosomes with catalase, they tested passive incubation, freeze–thaw, sonication, and extrusion methods. Western blot analysis showed that sonication and extrusion loading resulted in higher levels of catalase protein in the exosomes compared to passive incubation [91].

Prior to loading into exosomes, the drug can be fluorescently labeled, then fluorescence microscopy was employed to visualize the intensity within exosomes and further determine the successful loading and quantify/visualize cargo distribution. This method is applicable to fluorescently taggable drugs but costly, and it can also visualize interactions between labeled exosomes and cells to elucidate delivery mechanisms. For example, He et al. used fluorescence microscopy to visualize the uptake of PKH26-labeled exosomes by chondrocytes in vitro [69], they found that the injected labeled exosomes accumulated in the joint and attenuated cartilage damage and pain versus controls, and these exosomes could increase collagen II synthesis and improve pain thresholds over 6 weeks.

Stability testing evaluates exosome stability and drug release kinetics by measuring the drug release over time, thereby this method can confirm the drug loading, stability, and guides storage recommendations for drug-loaded exosomes. In area of breast cancer treatment, Moumita et al. performed stability testing of docetaxel-loaded exosomes by measuring the release kinetics, particle size, zeta-potential, and encapsulation efficiency. As a result, they demonstrated promising anticancer efficacy of docetaxel-loaded exosomes against 4T1 breast cancer cells [92]. Other studies found exosomes protected cargoes from enzymatic degradation in blood and from environmental stressors like high temperature and low

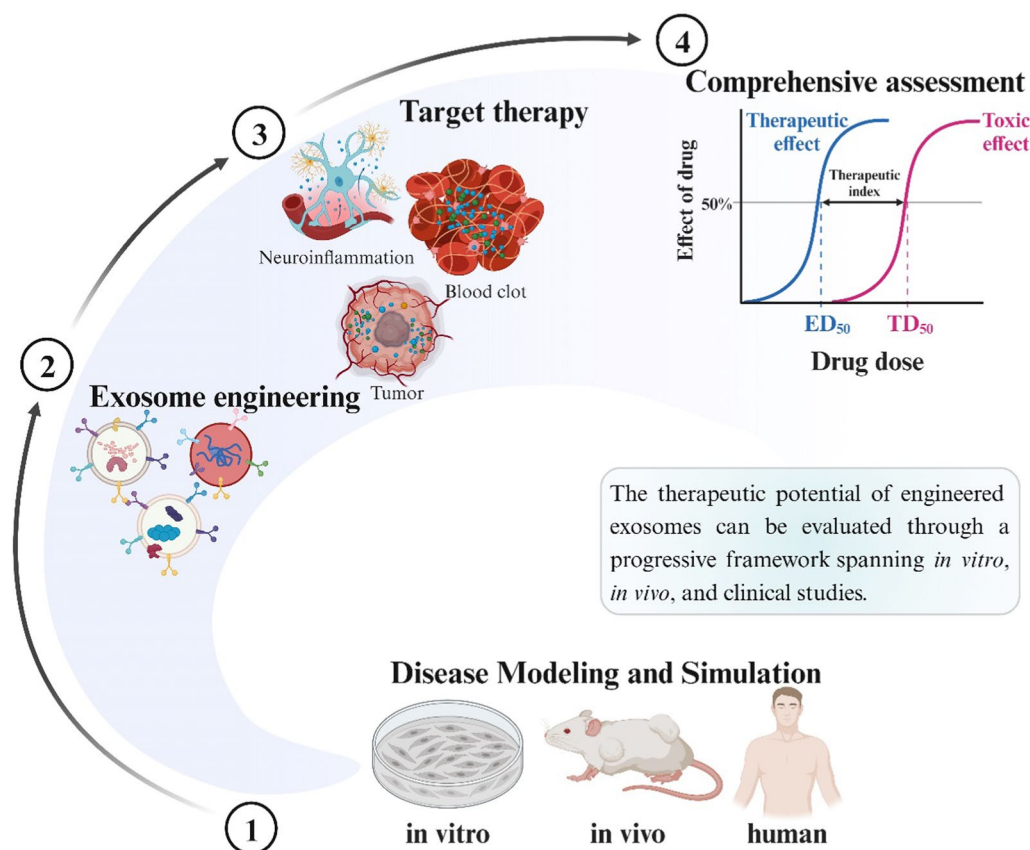


Fig. 5 Schematic diagram showing the therapeutic evaluation of engineered exosomes through a progressive framework spanning *in vitro*, *in vivo*, and clinical studies

pH [93–96]. Furthermore, exosomes were found to have the ability in improving drug pharmacokinetics through increased circulation half-life, and these exosomes could be maintained for 24 h at 37 °C (Table 3 and Fig. 3)[97].

Cell experiments

Cell experiments are fundamental for validating exosome drug loading capacity and assessing exosome pharmacological activity (Fig. 4). Methodology, drug-loaded exosomes are added to cultured cells, then the changes in drug levels, cell viability, function, and other endpoints are quantified to evaluate the drug delivery efficacy, which enables the investigation of drug affinity, pharmacology, and mechanisms. For instance, drug-loaded exosomes had shown promise for cancer therapy, and available studies had demonstrated that they could modulate cell viability, apoptosis, migration, proliferation, invasion, angiogenesis, metastasis, and drug resistance across cancer types [98–102]. However, as factors like cell type, density, timing, and detection methods can influence the evaluation results, standardizing assays improves reliability are urgently needed. For instance,

immunomodulatory exosomes, such as those loaded with STING agonists or IL-12, can activate anti-tumor immunity and are currently in clinical trial [103], while exosomes delivering KRAS-G12D siRNA can also silence this oncogenic mutation and reduce mutant cancer cell growth []. Hopefully, exosomes loaded with paclitaxel or Dox were demonstrated to overcome drug resistance and improve cytotoxicity compared to free drugs.

Animal experiments

Animal experiments are crucial for evaluating drug-loaded exosomes before human testing, as preclinical studies enable assessment of pharmacokinetics, dynamics, and toxicity as required by regulations. Animal models, such as mice and rats, possess similar physiologies to humans and are generally cost-effective and easily managed. Usually, researchers administer drug-loaded exosomes into these testing animals, then they monitor the biodistribution of the drug-loaded exosomes and observe the effects on the animals, including physiological and behavioral changes as well as histological effects [104–107]. The use of small

animal models thus allows for controlled evaluation of drug-loaded exosomes before advancing to human testing. For instance, exosomes loaded with chemotherapy drugs inhibited tumor growth in colon and glioblastoma cancer models, and those exosomes were found in tumors with enhanced efficacy over free drug [108, 109]. Moreover, using cardiovascular disease models, Cheng et al. revealed accumulation of angiogenic drug-loaded exosomes in heart tissue, which was demonstrated to promote vessel growth [110].

Clinical applications

Cancer

The therapeutic potential of drug-loaded exosomes for cancer treatment can be evaluated through a progressive framework spanning *in vitro*, *in vivo*, and clinical studies (Fig. 5). Initial characterization involves assessment of exosome properties such as size, morphology, surface markers, and drug encapsulation and release. *In vitro* cancer cell studies focus on cellular uptake of exosomes, cytotoxicity, and anti-proliferation effects. While *in vivo* animal models enable examination of the biodistribution, pharmacokinetics, tumor accumulation, and anti-tumor efficacy of the drug-loaded exosomes. Additionally, the toxicity of exosome-loaded drugs is also monitored through changes in body weight, blood counts, biochemistry, and histopathology [82, 104, 111].

Early-stage human trials primarily concentrate on the safety, maximum tolerated dose, pharmacokinetics, and preliminary efficacy of drug-loaded exosomes [112]. Larger randomized controlled trials are then needed to firmly establish clinical safety and efficacy parameters. The evaluation process is methodically structured, beginning with basic lab studies that assess formulation and biological interactions, followed by preclinical animal models that examine tumor-targeting and efficacy. The final step involves phased human trials that thoroughly assess clinical safety and therapeutic potential. This stepped evaluation provides a comprehensive characterization of drug-loaded exosome formulations as cancer nanomedicines.

In two review article by Xu et al. and Lee et al., they both indicated that various techniques could effectively load chemotherapeutic drugs into exosomes, with electroporation and membrane permeabilization approaches yielding high encapsulation efficiency. *In vitro* studies demonstrated enhanced cellular uptake, cytotoxicity and anti-proliferation effects of drug-loaded exosomes across diverse cancer cell lines, while animal models revealed superior pharmacokinetics, including extended circulation, tumor localization and anti-tumor efficacy compared to free drug or nanoparticle formulations [113, 114]. However, some toxicity issues like altered blood cell

counts and inflammation have been noted and require careful evaluation. Early phase human trials display feasibility and preliminary signs of safety and efficacy, but significant clinical development on drug-loaded exosomes is still needed.

Neurological diseases

In line with the application of exosomes in cancer treatment, a systematic framework has also been developed for treating nervous system diseases [115–117]. This process begins with the biophysical and biochemical characterization of exosomes, which includes assessing the exosome size, morphology, cargo loading, and interactions with nervous system cells. Subsequent animal studies are conducted to examine the biodistribution of these exosomes, their ability to cross the blood–brain barrier, target engagement, therapeutic effects, and toxicity parameters. The next phase involves early human trials that focus on feasibility, maximum tolerated dose, pharmacokinetics, and preliminary proof-of-concept for safety and efficacy. Following this, larger controlled studies are carried out to rigorously evaluate the clinical effectiveness of exosomes on relevant neurological outcome measures. Throughout these assessment procedures, multifaceted techniques tailored to each stage are employed to systematically validate stable and efficient drug encapsulation, nervous system delivery, therapeutic effects, and clinical safety.

Given the prevalence of neurological diseases like Alzheimer's and Parkinson's and the current lack of effective treatments, exosomes have shown promising therapeutic effects in both *in vitro* and *in vivo* models of neurodegenerative diseases, which positioned them as a novel strategy for neural repair. Recent studies have revealed that those drug-loaded exosomes can promote neural growth and regeneration by releasing bioactive molecules like neurotrophic factors and miRNAs. Besides, some exosomes can also target neural cells by modifying their surface proteins to enhance treatment effects [118]. Collectively, the potential of exosomes in the treatment of neurological diseases is promising, and ongoing research continues to explore their full therapeutic potential.

Cardiovascular diseases

Exosomes are emerging as therapeutic strategy for delivering drugs and treating various cardiovascular diseases. By engineering exosomes to carry targeted drug payloads, researchers can leverage the innate biology of exosomes to deliver therapies to specific sites in the body. Over the past years, there has been significant progress in developing exosome-based therapies for major cardiovascular diseases. Take myocardial infarction as an example, it is commonly known as a heart attack and

results from blockage of blood flow to the heart muscle leading to cell death. Several studies have shown promise for exosome therapy to reduce damage and improve healing after myocardial infarction. For example, stem cell-derived exosomes containing specific miRNAs and circRNAs were found to reduce apoptosis and increase proliferation of cardiomyocytes in AC16 cell and mouse models of myocardial infarction [119, 120]. Additionally, exosomes have been engineered to deliver pro-angiogenic factors to stimulate new blood vessel growth after infarction. In 2022, Hu et al. prepared exosomes carrying islet-1 (ISL1) which improved the survival and angiogenesis of endothelial cells and accelerated the recovery of myocardial infarction in a mouse model [121]. Similar with myocardial infarction, emerging progresses were also reported in pulmonary hypertension, atherosclerosis, and heart failure. One study loaded exosomes with miR-211 and found they could contribute to pulmonary hypertension via attenuating CaMK1/PPAR- γ axis in rats [122]. In other work, Li et al. engineered exosomes with platelet membrane phenotype to inherit the targeting ability towards injured endothelial cells which were proved to be able to regress atherosclerosis in mice [123]. While still an emerging field, additional work is needed to scale up manufacturing and evaluate clinical safety and efficacy through human trials. With continued research, exosome nanotherapies could provide transformative new paradigms in cardiovascular treatment.

Immune system diseases

Immune disorders are well defined as abnormal self-attack of normal tissues due to immune system dysfunction, and diseases related to immune disorders include rheumatoid arthritis, lupus, myasthenia gravis, and multiple sclerosis. Casually, these diseases are associated with varying degrees of tissue/organ damage [124]. For instance, You et al. engineered stem cell-derived exosomes to display molecules targeting macrophage reprogramming in rheumatoid arthritis, using flow cytometry and immunofluorescence microscopy, they tracked in vitro and in vivo the transportation of exosomes. The in vivo stability monitoring demonstrated that exosomes could precisely regulate macrophage activity and functionality [97]. Additionally, Wu et al. employed exosomes to deliver bryostatin-1, and they reported that the bryostatin-1-loaded exosomes could promote remyelination and neuroprotection in a cuprizone-induced demyelination model of multiple sclerosis. Hopefully, the bryostatin-1-loaded exosomes were found to effectively protect neurons and stimulate remyelination as a novel treatment strategy [125]. Fang et al. engineered exosomes to display CD40 receptor, and they

showed the ability of exosomes in modulating B cell activation and demonstrated it could alleviate systemic lupus erythematosus nephritis [126].

Conclusions and perspectives

Conclusions

Exosomes have emerged as promising biological nanoparticles for targeted drug delivery due to their biocompatibility, stability, and ability to encapsulate diverse cargoes. However, standardized methods are still lacking for the fabrication, optimization, and analysis of drug-loaded exosomes. This review highlights current techniques to address these gaps, including: (1) endogenous and exogenous loading methods that enable encapsulation of cargoes into exosomes; (2) characterization criteria such as quantification of drug loading, stability, release kinetics, cell uptake, biodistribution, pharmacokinetics, and therapeutic outcomes in preclinical models to evaluate exosomes as delivery vectors; and (3) advances in isolation, modification, cargo loading, and rigorous lab and animal testing that are paving the way for clinical translation of exosomes.

In summary, the current review synthesizes cutting-edge bioengineering strategies enabling the production of exosomes with enhanced drug delivery capabilities, and the establishment of robust protocols for manufacturing, optimization, and analysis will accelerate the development of exosomes as next-generation therapeutics. In the future, continued progress in this emerging field would firmly hold great promise in leveraging exosomes as targeted, multifunctional nanocarriers to address unmet clinical needs across diverse disease contexts.

Future perspectives

The exciting potential of exosomes has garnered tremendous interest, but realizing this potential in the clinic remains a challenge. There are several critical priorities that need to be addressed to advance exosomes towards clinical translation. These include:

- Scalable production methods must be developed to enable manufacturing of quality-controlled exosomes at therapeutic quantities using bioreactors and defined cell sources;
- Drug loading techniques should be optimized to maximize stable encapsulation of bioactive cargoes via endogenous and exogenous methods, improving delivery capacity;
- Mechanistic understanding is needed of how exosomes traverse barriers, interact with targets, and deliver cargo intracellularly after uptake;

- Versatile isolation and characterization methods require validation and standardization for reproducibility across research groups;
- Rigorous preclinical biosafety assessments are imperative to evaluate risks prior to human trials;
- Pilot clinical studies should demonstrate feasibility, biodistribution, and preliminary efficacy before scaling up.

The realization of exosomes for clinical nanomedicine will require continued cross-disciplinary collaboration between bioengineers, pharmacologists, and clinicians. Archiving the above goals through systematic applied research will unleash the full potential of exosomes to address diverse unmet medical needs.

Abbreviations

TAM	Tumor-associated macrophage
AC	Astragalus components
AGC	Advanced gastric cancer
CELNs	Curcuma rhizoma exosome-like nanoparticles
CHP	Cardiac homing peptide
CPPs	Cell-penetrating peptides
Dox	Doxorubicin
DTX	Docetaxel
exo@FPG3	Fluorine-engineered exosomes
Exo-BLM	Exosomes loaded with bleomycin
Exos-Ber	Berberine into M2 macrophage-derived exosomes
FPG3	Fluorinated peptide dendrimers
HPLC	High performance liquid chromatography
HPLC-MS	High performance liquid chromatography-mass spectrometry
IOL	Intraocular lens
ISL1	Isl1-1
LECs	Lens epithelial cells
M1-Exo	M1-polarized macrophage-derived exosomes
MRI	Magnetic resonance imaging
NACT	Neoadjuvant chemotherapy
PCO	Posterior capsular opacification
RVG	Rabies virus glycoprotein
scFv	Single chain variable fragments
SEM	Scanning electron microscopy
TAT	Trans-acting activator of transcription
TEM	Transmission electron microscopy

Acknowledgements

The authors would like to express their sincere gratitude to all of the researchers whose work contributed to and informed this review.

Author contributions

LZ and ZC conceived the study. ZC and JT conducted the literature search and screened records for eligibility. ZC, JT, and DS extracted and analyzed the data. ZC, MX, and LZ interpreted the results. ZC, LZ, and SD drafted the manuscript. All authors provided critical feedback on the draft and approved the final version for submission.

Funding

This work was financially supported by the National Natural Science Foundation of China (82003405), the Natural Science Foundation of Shandong (ZR2020QH290), and the Taishan Scholar Program of Shandong Province (No. tsqn202211359). The funders had no role in study design, data collection, and interpretation, or the decision to submit the work for publication.

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Clinical Medical Research Center for Women and Children Diseases, Key Laboratory of Birth Regulation and Control Technology of National Health Commission of China, Shandong Provincial Maternal and Child Health Care Hospital Affiliated to Qingdao University, Jinan 250001, China. ²Key Laboratory of Birth Defect Prevention and Genetic Medicine of Shandong Health Commission, Jinan 250001, China. ³School of Public Health, Weifang Medical University, Weifang 261000, China. ⁴School of Public Health, North China University of Science and Technology, Tangshan 063000, China. ⁵School of Public Health, Shandong First Medical University & Shandong Academy of Medical Sciences, Jinan 250001, China.

Received: 9 September 2023 Accepted: 7 December 2023

Published online: 03 January 2024

References

- Joorabloo A, Liu T. Engineering exosome-based biomimetic nanovehicles for wound healing. *J Controlled Release*. 2023;356:463–80.
- Marar C, Starich B, Wirtz D. Extracellular vesicles in immunomodulation and tumor progression. *Nat Immunol*. 2021;22:560–70.
- Chernyshev VS, Chuprova-Netochin RN, Tsydenzhapova E, Svirshchevskaya EV, Poltavtseva RA, Merdalimova A, et al. Asymmetric depth-filtration: a versatile and scalable method for high-yield isolation of extracellular vesicles with low contamination. *J Extracell Vesicles*. 2022;11: e12256.
- Omrani M, Beyrampour-Basmenji H, Jahanban-Esfahlan R, Talebi M, Raeisi M, Serej ZA, et al. Global trend in exosome isolation and application: an update concept in management of diseases. *Mol Cell Biochem*. 2023. <https://doi.org/10.1007/s11010-023-04756-6>.
- Yakubovich EI, Polischouk AG, Evtushenko VI. Principles and problems of exosome isolation from biological fluids. *Biochemistry*. 2022;16:115–26.
- Zhu Z-H, Jia F, Ahmed W, Zhang G-L, Wang H, Lin C-Q, et al. Neural stem cell-derived exosome as a nano-sized carrier for BDNF delivery to a rat model of ischemic stroke. *Neural Regen Res*. 2022;18:404–9.
- Tang Z, He J, Zou J, Yu S, Sun X, Qin L. Cisplatin-resistant HepG2 cell-derived exosomes transfer cisplatin resistance to cisplatin-sensitive cells in HCC. *PeerJ*. 2021;9: e11200.
- Wan X, Xie B, Sun H, Gu W, Wang C, Deng Q, et al. Exosomes derived from M2 type tumor-associated macrophages promote osimertinib resistance in non-small cell lung cancer through MSTRG.292666.16-miR-6836-5p-MAPK8IP3 axis. *Cancer Cell Int*. 2022;22:83.
- Tian Y, Liu C, Li Z, Ai M, Wang B, Du K, et al. Exosomal B7-H4 from irradiated glioblastoma cells contributes to increase FoxP3 expression of differentiating Th1 cells and promotes tumor growth. *Redox Biol*. 2022;56: 102454.
- Guo X, Hu F, Zhao S, Yong Z, Zhang Z, Peng N. Immunomagnetic separation method integrated with the Strep-Tag II system for rapid enrichment and mild release of exosomes. *Anal Chem*. 2023;95:3569–76.
- Pallares-Rusiñol A, Moura SL, Martí M, Pividori MI. Electrochemical genosensing of overexpressed GAPDH transcripts in breast cancer exosomes. *Anal Chem*. 2023;95:2487–95.
- Chandrasekera D, Shah R, van Hout I, De Jonge W, Bunton R, Parry D, et al. Combination of precipitation and size exclusion chromatography as an effective method for exosome like extracellular vesicle isolation from pericardial fluids. *Nanotheranostics*. 2023;7:345–52.

13. Bai H, Wang X, Zhang B, Liu W. A comparison of size exclusion chromatography based tandem strategies for plasma exosome enrichment and proteomic analysis. *Anal Methods*. 2023;15:6245.
14. Lin Y-Q, Feng K-K, Lu J-Y, Le J-Q, Li W-L, Zhang B-C, et al. CRISPR/Cas9-based application for cancer therapy: challenges and solutions for non-viral delivery. *J Controlled Release*. 2023;361:727–49.
15. Ye Y, Shi Q, Yang T, Xie F, Zhang X, Xu B, et al. In vivo visualized tracking of tumor-derived extracellular vesicles using CRISPR-Cas9 system. *Technol Cancer Res T*. 2022;21:15330338221085370.
16. Kim SM, Yang Y, Oh SJ, Hong Y, Seo M, Jang M. Cancer-derived exosomes as a delivery platform of CRISPR/Cas9 confer cancer cell tropism-dependent targeting. *J Controlled Release*. 2017;266:8–16.
17. Meng S, Whitt AG, Stamp BF, Eaton JW, Li C, Yaddanapudi K. Exosome-based cancer vaccine for prevention of lung cancer. *Stem Cell Investigation*. 2023;10:2.
18. Feng C, Tan P, Nie G, Zhu M. Biomimetic and bioinspired nano-platforms for cancer vaccine development. *Exploration*. 2023;3:20210263.
19. Dyball LE, Smales CM. Exosomes: biogenesis, targeting, characterization and their potential as “plug & play” vaccine platforms. *Biotechnol J*. 2022;17:2100646.
20. Gunassekaran GR, Poongkavithai Vadevo SM, Baek M-C, Lee B. M1 macrophage exosomes engineered to foster M1 polarization and target the IL-4 receptor inhibit tumor growth by reprogramming tumor-associated macrophages into M1-like macrophages. *Biomaterials*. 2021;278: 121137.
21. Zhao Y, Zheng Y, Zhu Y, Li H, Zhu H, Liu T. Docetaxel-loaded M1 macrophage-derived exosomes for a safe and efficient chemoinmunotherapy of breast cancer. *J Nanobiotechnology*. 2022;20:359.
22. Yan C, Chen J, Wang C, Yuan M, Kang Y, Wu Z, et al. Milk exosomes-mediated miR-31-5p delivery accelerates diabetic wound healing through promoting angiogenesis. *Drug Deliv*. 2022;29:214–28.
23. Gao Z-S, Zhang C-J, Xia N, Tian H, Li D-Y, Lin J-Q, et al. Berberine-loaded M2 macrophage-derived exosomes for spinal cord injury therapy. *Acta Biomater*. 2021;126:211–23.
24. Hettich BF, Bader JJ, Leroux J-C. Encapsulation of hydrophilic compounds in small extracellular vesicles: loading capacity and impact on vesicle functions. *Adv Healthc Mater*. 2022;11:2100047.
25. Cheng Y, Zeng Q, Han Q, Xia W. Effect of pH, temperature and freezing-thawing on quantity changes and cellular uptake of exosomes. *Protein Cell*. 2019;10:295–9.
26. Zhang Z, Mi T, Jin L, Li M, Zhanghuang C, Wang J, et al. Comprehensive proteomic analysis of exosome mimetic vesicles and exosomes derived from human umbilical cord mesenchymal stem cells. *Stem Cell Res Ther*. 2022;13:312.
27. Ding Y, Cao F, Sun H, Wang Y, Liu S, Wu Y, et al. Exosomes derived from human umbilical cord mesenchymal stromal cells deliver exogenous miR-145-5p to inhibit pancreatic ductal adenocarcinoma progression. *Cancer Lett*. 2019;442:351–61.
28. Guo J, Duan Z, Zhang C, Wang W, He H, Liu Y, et al. Mouse 4T1 breast cancer cell-derived exosomes induce proinflammatory cytokine production in macrophages via miR-183. *J Immunol*. 2020;205:2916–25.
29. Chen J, Li Z, Yue C, Ma J, Cao L, Lin J, et al. Human umbilical cord mesenchymal stem cell-derived exosomes carrying miR-1827 downregulate SUCNR1 to inhibit macrophage M2 polarization and prevent colorectal liver metastasis. *Apoptosis*. 2023;28:549–65.
30. Usman WM, Pham TC, Kwok YY, Vu LT, Ma V, Peng B, et al. Efficient RNA drug delivery using red blood cell extracellular vesicles. *Nat Commun*. 2018;9:2359.
31. Liu Q, Chen Q, Zhou Z, Tian Z, Zheng X, Wang K. piRNA-18 inhibition cell proliferation, migration and invasion in colorectal cancer. *Biochem Genet*. 2023;61:1881–1897.
32. Xie Y, Liu JB, Li JM, Zhang C, Lu CX, Wen ZJ. Effects of silencing circRNA ABCB10 expression on biological properties of colorectal cancer cells. *Zhonghua Zhong Liu Za Zhi*. 2021;43:449–56.
33. Accolla RS, Ramia E, Tedeschi A, Forlani G. CLITA-driven MHC class II expressing tumor cells as antigen presenting cell performers: toward the construction of an optimal anti-tumor vaccine. *Front Immunol*. 2019;10:1806.
34. Morel KL, Hamid AA, Clohessy JG, Pandell N, Ellis L, Sweeney CJ. NF- κ B blockade with oral administration of dimethylaminoparthenolide (DMAPT), delays prostate cancer resistance to androgen receptor (AR) inhibition and inhibits AR variants. *Mol Cancer Res*. 2021;19:1137–45.
35. Hesry V, Piquet-Pellorce C, Travert M, Donaghy L, Jégou B, Patard J-J, et al. Sensitivity of prostate cells to TRAIL-induced apoptosis increases with tumor progression: DR5 and caspase 8 are key players. *Prostate*. 2006;66:987–95.
36. Chen C, Shen M, Wan X, Sheng L, He Y, Xu M, et al. Activated T cell-derived exosomes for targeted delivery of AXL-siRNA loaded paclitaxel-poly-L-lysine prodrug to overcome drug resistance in triple-negative breast cancer. *Chem Eng J*. 2023;468: 143454.
37. Wang X, Chen T, Li C, Li W, Zhou X, Li Y, et al. CircRNA-CREIT inhibits stress granule assembly and overcomes doxorubicin resistance in TNBC by destabilizing PKR. *J Hematol Oncol*. 2022;15:122.
38. Mousavi SM, Hosseindoost S, Mahdian SMA, Vousoughi N, Rajabi A, Jafari A, et al. Exosomes released from U87 glioma cells treated with curcumin and/or temozolomide produce apoptosis in naive U87 cells. *Pathol Res Pract*. 2023;245: 154427.
39. Vadevo SMP, Gurung S, Lee H-S, Gunassekaran GR, Lee S-M, Yoon J-W, et al. Peptides as multifunctional players in cancer therapy. *Exp Mol Med*. 2023;55:1099–109.
40. He J, Ren W, Wang W, Han W, Jiang L, Zhang D, et al. Exosomal targeting and its potential clinical application. *Drug Deliv Transl Res*. 2022;12:2385–402.
41. Vandergriff A, Huang K, Shen D, Hu S, Hensley MT, Caranasos TG, et al. Targeting regenerative exosomes to myocardial infarction using cardiac homing peptide. *Theranostics*. 2018;8:1869–78.
42. Mondal J, Pillarisetti S, Junnuthula V, Saha M, Hwang SR, Park I, et al. Hybrid exosomes, exosome-like nanovesicles and engineered exosomes for therapeutic applications. *J Control Release*. 2023;353:1127–49.
43. Gurrieri E, D’Agostino VG. Strategies to functionalize extracellular vesicles against HER2 for anticancer activity. *Extracell Vesicles Circ Nucleic Acids*. 2022;3:93–101.
44. Si K, Ye Z, Ali DJ, Ding B, He C, Dai Z, et al. Co-delivery of PDL1-blocking scFv and chemotherapeutics using engineered exosomes for cancer therapy. *J Drug Deliv Sci Technol*. 2023;82: 104337.
45. Frigerio B, Bizzoni C, Jansen G, Leamon CP, Peters GJ, Low PS, et al. Folate receptors and transporters: biological role and diagnostic/therapeutic targets in cancer and other diseases. *J Exp Clin Oncol*. 2019;38:125.
46. Razlansari M, Jafarinejad S, Rahdar A, Shirvaliloo M, Arshad R, Fathi-Karkan S, et al. Development and classification of RNA aptamers for therapeutic purposes: an updated review with emphasis on cancer. *Mol Cell Biochem*. 2023;478:1573–98.
47. Liu X, Duan D, Wang Y, Liu J, Duan D. Advancements in 3WJ-based RNA nanotechnology and its application for cancer diagnosis and therapy. *Front Biosci-Landmark*. 2022;27:61.
48. Yan H, Li Y, Cheng S, Zeng Y. Advances in analytical technologies for extracellular vesicles. *Anal Chem*. 2021;93:4739–74.
49. Grzesik K, Janik M, Hoja-Lukowicz D. The hidden potential of glycomarkers: Glycosylation studies in the service of cancer diagnosis and treatment. *Biochim Biophys Acta*. 2023;1878: 188889.
50. Ferber S, Tiram G, Sousa-Herves A, Eldar-Boock A, Krivitsky A, Scomparin A, et al. Co-targeting the tumor endothelium and P-selectin-expressing glioblastoma cells leads to a remarkable therapeutic outcome. *eLife*. 2017;6: e25281.
51. Kar R, Dhar R, Mukherjee S, Nag S, Gorai S, Mukerjee N, et al. Exosome-based smart drug delivery tool for cancer theranostics. *ACS Biomater Sci Eng*. 2023;9:577–94.
52. Li Z, Li Y, Gao J, Fu Y, Hua P, Jing Y, et al. The role of CD47-SIRP α immune checkpoint in tumor immune evasion and innate immunotherapy. *Life Sci*. 2021;273: 119150.
53. Gong L, Zhou H, Zhang Y, Wang C, Fu K, Ma C, et al. Preparation of Phyllygenin-Hyaluronic acid composite milk-derived exosomes and its anti-hepatic fibrosis effect. *Mater Today Bio*. 2023;23: 100804.
54. Ginini L, Billan S, Fridman E, Gil Z. Insight into extracellular vesicle-cell communication: from cell recognition to intracellular fate. *Cells*. 2022;11:1375.
55. Choi H, Choi Y, Yim HY, Mirzaaghasi A, Yoo J-K, Choi C. Biodistribution of exosomes and engineering strategies for targeted delivery of therapeutic exosomes. *Tissue Eng Regen Med*. 2021;18:499–511.

56. Kuipers ME, Nolte-t Hoen EN, van der Ham AJ, Ozir-Fazalalikhhan A, Nguyen DL, de Korne CM, et al. DC-SIGN mediated internalisation of glycosylated extracellular vesicles from *Schistosoma mansoni* increases activation of monocyte-derived dendritic cells. *J Extracell Vesicles*. 2020;9:1753420.
57. Li S, Sampson C, Liu C, Piao H, Liu H-X. Integrin signaling in cancer: bidirectional mechanisms and therapeutic opportunities. *Cell Commun Signal*. 2023;21:266.
58. Li X, Tang M, Zhu Q, Wang X, Lin Y, Wang X. The exosomal integrin $\alpha 5 \beta 1$ /AEP complex derived from epithelial ovarian cancer cells promotes peritoneal metastasis through regulating mesothelial cell proliferation and migration. *Cell Oncol*. 2020;43:263–77.
59. Bhatia R, Chang J, Munoz JL, Walker ND. Forging new therapeutic targets: efforts of tumor derived exosomes to prepare the pre-metastatic niche for cancer cell dissemination and dormancy. *Biomedicines*. 2023;11:1614.
60. Huang L, Wang F, Wang X, Su C, Wu S, Yang C, et al. M2-like macrophage-derived exosomes facilitate metastasis in non-small-cell lung cancer by delivering integrin $\alpha v \beta 3$. *MedComm*. 2023;4: e191.
61. Parada N, Romero-Trujillo A, Georges N, Alcayaga-Miranda F. Camouflage strategies for therapeutic exosomes evasion from phagocytosis. *J Adv Res*. 2021;31:61–74.
62. Zhang C, Huang D, Baloche V, Zhang L, Xu J, Li B, et al. Galectin-9 promotes a suppressive microenvironment in human cancer by enhancing STING degradation. *Oncogenesis*. 2020;9:1–14.
63. Muteeb G, Zia Q, Alshoabi A. Engineered exosomes as nano-vectors against neurodegenerative disorders. In: Jahan S, Siddiqui AJ, editors. *Applications of stem cells and derived exosomes in neurodegenerative disorders*. Singapore: Springer Nature; 2023. p. 291–327. https://doi.org/10.1007/978-981-99-3848-3_12.
64. Yu Y, Li W, Mao L, Peng W, Long D, Li D, et al. Genetically engineered exosomes display RVG peptide and selectively enrich a neprilysin variant: a potential formulation for the treatment of Alzheimer's disease. *J Drug Target*. 2021;29:1128–38.
65. Bunz M, Ritter M, Schindler M. HCV egress—unconventional secretion of assembled viral particles. *Trends Microbiol*. 2022;30:364–78.
66. Kalemera MD, Capella-Pujol J, Chumbe A, Underwood A, Bull RA, Schinkel J, et al. Optimized cell systems for the investigation of hepatitis C virus E1E2 glycoproteins. *J Gen Virol*. 2021;102:jgv001512.
67. Ali A, Mishra R, Kaur H, Chandra BA. HIV-1 Tat: an update on transcriptional and non-transcriptional functions. *Biochimie*. 2021;190:24–35.
68. Keighron CN, Avazzadeh S, Goljanek-Whysall K, McDonagh B, Howard L, Ritter T, et al. Extracellular vesicles, cell-penetrating peptides and miRNAs as future novel therapeutic interventions for Parkinson's and Alzheimer's Disease. *Biomedicines*. 2023;11:728.
69. He L, He T, Xing J, Zhou Q, Fan L, Liu C, et al. Bone marrow mesenchymal stem cell-derived exosomes protect cartilage damage and relieve knee osteoarthritis pain in a rat model of osteoarthritis. *Stem Cell Res Ther*. 2020;11:276.
70. Liu X, Guo Q, Gao G, Cao Z, Guan Z, Jia B, et al. Exosome-transmitted circCABIN1 promotes temozolomide resistance in glioblastoma via sustaining ErbB downstream signaling. *J Nanobiotechnology*. 2023;21:45.
71. Chen Q, Che C, Liu J, Gong Z, Si M, Yang S, et al. Construction of an exosome-functionalized graphene oxide based composite bionic smart drug delivery system and its anticancer activity. *Nanotechnology*. 2022;33:175101.
72. Sun D, Zhuang X, Xiang X, Liu Y, Zhang S, Liu C, et al. A novel nanoparticle drug delivery system: the anti-inflammatory activity of curcumin is enhanced when encapsulated in exosomes. *Mol Ther*. 2010;18:1606–14.
73. Değirmenci NS, Uslu M, Kirbaş OK, Şahin F, Uçar EÖ. Lapatinib loaded exosomes as a drug delivery system in breast cancer. *J Drug Deliv Sci Tec*. 2022;75:103584.
74. Sharma R, Rajput VS, Jamal S, Grover A, Grover S. An immunoinformatics approach to design a multi-epitope vaccine against *Mycobacterium tuberculosis* exploiting secreted exosome proteins. *Sci Rep*. 2021;11:13836.
75. Won Lee G, Thangavelu M, Joung Choi M, Yeong Shin E, Sol Kim H, Seon Baek J, et al. Exosome mediated transfer of miRNA-140 promotes enhanced chondrogenic differentiation of bone marrow stem cells for enhanced cartilage repair and regeneration. *J Cell Biochem*. 2020;121:3642–52.
76. Zhu D, Duo Y, Suo M, Zhao Y, Xia L, Zheng Z, et al. Tumor-exocytosed exosome/aggregation-induced emission luminogen hybrid nanovesicles facilitate efficient tumor penetration and photodynamic therapy. *Angew Chem Int Ed Engl*. 2020;59:13836–43.
77. Zhu S, Huang H, Liu D, Wen S, Shen L, Lin Q. Augmented cellular uptake and homologous targeting of exosome-based drug loaded IOL for posterior capsular opacification prevention and biosafety improvement. *Bioact Mater*. 2022;15:469–81.
78. Ma S, Song L, Bai Y, Wang S, Wang J, Zhang H, et al. Improved intracellular delivery of exosomes by surface modification with fluorinated peptide dendrimers for promoting angiogenesis and migration of HUVECs. *RSC Adv*. 2023;13:11269–77.
79. Yang X, Peng Y, Wang Y, Zheng Y, He Y, Pan J, et al. Curcuma rhizoma exosomes-like nanoparticles loaded astragalus components improve the absorption and enhance anti-tumor effect. *J Drug Deliv Sci Technol*. 2023;81: 104274.
80. Huang P, Wang L, Li Q, Xu J, Xu J, Xiong Y, et al. Combinatorial treatment of acute myocardial infarction using stem cells and their derived exosomes resulted in improved heart performance. *Stem Cell Res Ther*. 2019;10:300.
81. Xie Y, Wen Y, Su X, Zheng C, Li M. Label-free plasmon-enhanced spectroscopic HER2 detection for dynamic therapeutic surveillance of breast cancer. *Anal Chem*. 2022;94:12762–71.
82. Shaikh S, Younis M, Yingying S, Tanziela T, Yuan L. Bleomycin loaded exosomes enhanced antitumor therapeutic efficacy and reduced toxicity. *Life Sci*. 2023;330: 121977.
83. Guo T, Tang X-H, Gao X-Y, Zhou Y, Jin B, Deng Z-Q, et al. A liquid biopsy signature of circulating exosome-derived mRNAs, miRNAs and lncRNAs predict therapeutic efficacy to neoadjuvant chemotherapy in patients with advanced gastric cancer. *Mol Cancer*. 2022;21:216.
84. Kim MS, Haney MJ, Zhao Y, Mahajan V, Deygen I, Klyachko NL, et al. Development of exosome-encapsulated paclitaxel to overcome MDR in cancer cells. *Nanomedicine*. 2016;12:655–64.
85. Jiang L, Gu Y, Du Y, Tang X, Wu X, Liu J. Engineering exosomes endowed with targeted delivery of triptolide for malignant melanoma therapy. *ACS Appl Mater Interfaces*. 2021;13:42411–28.
86. Zhu Y, Tao Z, Chen Y, Lin S, Zhu M, Ji W, et al. Exosomal MMP-1 transfers metastasis potential in triple-negative breast cancer through PAR1-mediated EMT. *Breast Cancer Res Treat*. 2022;193:65–81.
87. Yu X, Dong M, Wang L, Yang Q, Wang L, Han W, et al. Nanotherapy for bone repair: milk-derived small extracellular vesicles delivery of icariin. *Drug Deliv*. 2023;30:2169414.
88. Liu T, Zhu Y, Zhao R, Wei X, Xin X. Visualization of exosomes from mesenchymal stem cells in vivo by magnetic resonance imaging. *Magn Reson Imaging*. 2020;68:75–82.
89. Tanziela T, Shaikh S, ur Rehman F, Semcheddine F, Jiang H, Lu Z, et al. Cancer-exocytosed exosomes loaded with bio-assembled AgNCs as smart drug carriers for targeted chemotherapy. *Chem Eng J*. 2022;440:135980.
90. Zheng L, Zhang B, Chu H, Cheng P, Li H, Huang K, et al. Assembly and in vitro assessment of a powerful combination: aptamer-modified exosomes combined with gold nanorods for effective photothermal therapy. *Nanotechnology*. 2020;31: 485101.
91. Haney MJ, Klyachko NL, Zhao Y, Gupta R, Plotnikova EG, He Z, et al. Exosomes as drug delivery vehicles for Parkinson's disease therapy. *J Control Release*. 2015;207:18–30.
92. Basak M, Sahoo B, Chaudhary DK, Narisepalli S, Tiwari S, Chitkara D, et al. Human umbilical cord blood-mesenchymal stem cell derived exosomes as an efficient nanocarrier for docetaxel and miR-125a: formulation optimization and anti-metastatic behaviour. *Life Sci*. 2023;322: 121621.
93. Rodríguez-Comas J, Castaño C, Ortega MA, Tejedera A, Fernandez-González M, Novials A, et al. Immunoaffinity-based microfluidic platform for exosomal microRNA isolation from obese and lean mouse plasma. *Adv Mater Technol*. 2023;8:2300054.
94. Wang Y, Wu J, Xia S-W, Zhao F, Ding Q, Ye X-M, et al. miR-27a-3p relieves heat stress-induced mitochondrial damage and aberrant milk protein synthesis through MEK/ERK pathway in BMECs. *Cell Stress Chaperones*. 2023;28:265–74.
95. Khatami SH, Karami N, Taheri-Anganeh M, Taghvi S, Tondro G, Khorsand M, et al. Exosomes: promising delivery tools for overcoming

- blood-brain barrier and glioblastoma therapy. *Mol Neurobiol*. 2023;60:4659–78.
96. Lim J, Kang B, Son HY, Mun B, Huh Y-M, Rho HW, et al. Microfluidic device for one-step detection of breast cancer-derived exosomal mRNA in blood using signal-amplifiable 3D nanostructure. *Biosens Bioelectron*. 2022;197: 113753.
 97. You DG, Lim GT, Kwon S, Um W, Oh BH, Song SH, et al. Metabolically engineered stem cell-derived exosomes to regulate macrophage heterogeneity in rheumatoid arthritis. *Sci Adv*. 2021;7:eabe0083.
 98. Zhao S, Pan T, Deng J, Cao L, Vicencio JM, Liu J, et al. Exosomal transfer of miR-181b-5p confers senescence-mediated doxorubicin resistance via modulating BCLAF1 in breast cancer. *Br J Cancer*. 2023;128:665–77.
 99. Miyazaki K, Wada Y, Okuno K, Murano T, Morine Y, Ikemoto T, et al. An exosome-based liquid biopsy signature for pre-operative identification of lymph node metastasis in patients with pathological high-risk T1 colorectal cancer. *Mol Cancer*. 2023;22:2.
 100. Luo J, Yang T, Wu J, Lai H, Zou L, Chen W, et al. Exosomal PGAM1 promotes prostate cancer angiogenesis and metastasis by interacting with ACTG1. *Cell Death Dis*. 2023;14:1–14.
 101. Yu D, Chang Z, Liu X, Chen P, Zhang H, Qin Y. Macrophage-derived exosomes regulate gastric cancer cell oxaliplatin resistance by wrapping circ 0008253. *Cell Cycle*. 2023;22:705–17.
 102. Wang L, Chen X, Meng F, Huang T, Wang S, Zheng Z, et al. α 2,6-Sialylation promotes hepatocellular carcinoma cells migration and invasion via enhancement of nSmase2-mediated exosomal miRNA sorting. *J Physiol Biochem*. 2023;79:19–34.
 103. Elsharkasy OM, Nordin JZ, Hagey DW, de Jong OG, Schifflers RM, Andaloussi SE, et al. Extracellular vesicles as drug delivery systems: why and how? *Adv Drug Deliv Rev*. 2020;159:332–43.
 104. Zhang X, Wang J, Liu N, Wu W, Li H, Lu W, et al. Umbilical cord blood-derived M1 macrophage exosomes loaded with cisplatin target ovarian cancer in vivo and reverse cisplatin resistance. *Mol Pharmaceutics*. 2023;20:5440–53.
 105. Mehryab F, Rabbani S, Shekari F, Nazari A, Goshtasbi N, Haeri A. Sirolimus-loaded exosomes as a promising vascular delivery system for the prevention of post-angioplasty restenosis. *Drug Deliv Transl Res*. 2023. <https://doi.org/10.1007/s13346-023-01390-z>.
 106. Kumar DN, Chaudhuri A, Kumar D, Singh S, Agrawal AK. Impact of the drug loading method on the drug distribution and biological efficacy of exosomes. *AAPS PharmSciTech*. 2023;24:166.
 107. Ahmed F, Tamma M, Pathigadapa U, Reddanna P, Yenuganti VR. Drug loading and functional efficacy of cow, buffalo, and goat milk-derived exosomes: a comparative study. *Mol Pharm*. 2022;19:763–74.
 108. Shekh R, Ahmad A, Tiwari RK, Saeed M, Shukla R, Al-Thubiani WS, et al. High therapeutic efficacy of 5-Fluorouracil-loaded exosomes against colon cancer cells. *Chem Biol Drug Des*. 2023;101:962–76.
 109. Cui J, Wang X, Li J, Zhu A, Du Y, Zeng W, et al. Immune exosomes loading self-assembled nanomicelles traverse the blood-brain barrier for chemo-immunotherapy against glioblastoma. *ACS Nano*. 2023;17:1464–84.
 110. Cheng G, Zhu D, Huang K, Caranasos TG. Minimally invasive delivery of a hydrogel-based exosome patch to prevent heart failure. *J Mol Cell Cardiol*. 2022;169:113–21.
 111. Liu X, Zhang G, Yu T, Liu J, Chai X, Yin D, et al. CL4-modified exosomes deliver lncRNA DARS-AS1 siRNA to suppress triple-negative breast cancer progression and attenuate doxorubicin resistance by inhibiting autophagy. *Int J Biol Macromol*. 2023;250: 126147.
 112. Cook N, Hansen AR, Siu LL, Abdul Razak AR. Early phase clinical trials to identify optimal dosing and safety. *Mol Oncol*. 2015;9:997–1007.
 113. Xu M, Yang Q, Sun X, Wang Y. Recent advancements in the loading and modification of therapeutic exosomes. *Front Bioeng Biotechnol*. 2020;8:586130.
 114. Lee J, Lee J-H, Chakraborty K, Hwang J, Lee Y-K. Exosome-based drug delivery systems and their therapeutic applications. *RSC Adv*. 2022;12:18475–92.
 115. Shetgaonkar GG, Marques SM, DCruz CEM, Vibhahari RJA, Kumar L, Shirodkar RK. Exosomes as cell-derivative carriers in the diagnosis and treatment of central nervous system diseases. *Drug Deliv Transl Res*. 2022;12:1047–79.
 116. Jiang Y, Wang F, Wang K, Zhong Y, Wei X, Wang Q, et al. Engineered exosomes: a promising drug delivery strategy for brain diseases. *Curr Med Chem*. 2022;29:3111–24.
 117. Qu M, Lin Q, Huang L, Fu Y, Wang L, He S, et al. Dopamine-loaded blood exosomes targeted to brain for better treatment of Parkinson's disease. *J Controlled Release*. 2018;287:156–66.
 118. Shen Y, Cai J. The importance of using exosome-loaded miRNA for the treatment of spinal cord injury. *Mol Neurobiol*. 2023;60:447–59.
 119. Wang T, Li T, Niu X, Hu L, Cheng J, Guo D, et al. ADSC-derived exosomes attenuate myocardial infarction injury by promoting miR-205-mediated cardiac angiogenesis. *Biol Direct*. 2023;18:6.
 120. Fan M, Liang T, Xie F, Ma P, Li J. Exosomal circ_HIPK3 reduces apoptosis in H2O2-induced AC16 cardiomyocytes through miR-33a-5p/IRS1 axis. *Transpl Immunol*. 2023;80: 101862.
 121. Hu X, Ning X, Zhao Q, Zhang Z, Zhang C, Xie M, et al. Islet-1 mesenchymal stem cells-derived exosome-incorporated angiogenin-1 hydrogel for enhanced acute myocardial infarction therapy. *ACS Appl Mater Interfaces*. 2022;14:36289–303.
 122. Zhang S, Liu J, Zheng K, Chen L, Sun Y, Yao Z, et al. Exosomal miR-211 contributes to pulmonary hypertension via attenuating CaMK1/PPAR- γ axis. *Vasc Pharmacol*. 2021;136: 106820.
 123. Li Q, Huang Z, Pang Z, Wang Q, Gao J, Chen J, et al. Targeted delivery of platelet membrane modified extracellular vesicles into atherosclerotic plaque to regress atherosclerosis. *Chem Eng J*. 2023;452: 138992.
 124. McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. *N Engl J Med*. 2011;365:2205–19.
 125. Wu X-Y, Liao B-Y, Xiao D, Wu W-C, Xiao Y, Alexander T, et al. Encapsulation of bryostatin-1 by targeted exosomes enhances remyelination and neuroprotection effects in the cuprizone-induced demyelinating animal model of multiple sclerosis. *Biomater Sci*. 2022;10:714–27.
 126. Fang T, Li B, Li M, Zhang Y, Jing Z, Li Y, et al. Engineered cell membrane vesicles expressing CD40 alleviate system lupus nephritis by intervening B cell activation. *Small Methods*. 2023;7: e2200925.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

