Liu *et al. Molecular Cancer* (2025) 24:166
https://doi.org/10.1186/s12943-025-02372-0

Molecular Cancer

REVIEW

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Exosomes in cancer nanomedicine: biotechnological advancements and innovations

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Abstract

Exosomes, as natural intercellular messengers, are gaining prominence as delivery vehicles in nanomedicine, offering a superior alternative to conventional synthetic nanoparticles for cancer therapeutics. Unlike lipid, polymer, or metallic nanoparticles, which often face challenges related to immunogenicity, targeting precision, and off-tumor toxicity, exosomes can effectively encapsulate a diverse range of therapeutic agents while exhibiting low toxicity, favorable pharmacokinetics, and organotropic properties. This review examines recent advancements in exosome bioengineering over the past decade. Innovations such as microfluidics-based platforms, nanoporation, fusogenic hybrids, and genetic engineering have significantly improved loading efficiencies, production scalability, and pharmacokinetics of exosomes. These advancements facilitate tumor-specific cargo delivery, resulting in substantial improvements in retention and efficacy essential for clinical success. Moreover, enhanced biodistribution, targeting, and bioavailability—through strategies such as cell selection, surface modifications, membrane composition alterations, and biomaterial integration—suggests a promising future for exosomes as an ideal nanomedicine delivery platform. We also highlight the translational impact of these strategies through emerging clinical trials. Additionally, we outline a framework for clinical translation that focuses on: cargo selection, organotropic cell sourcing, precision loading methodologies, and route-specific delivery optimization. In summary, this review emphasizes the potential of exosomes to overcome the pharmacokinetic and safety challenges that have long impeded oncology drug development, thus enabling safer and more effective cancer treatments.

Keywords Engineered exosomes, Extracellular vesicles, Nanomedicine, Cancer, Drug delivery

Background

Cancer nanomedicine utilizes nanotechnology to deliver therapeutics, such as nucleic acids, proteins, and small molecule drugs, to tumor tissues. Following the success of mRNA vaccines in nanoparticles by companies such as Moderna and Pfizer-BioNTech against COVID-19, interest in engineered nanomedicine for cancer treatment has surged [1, 2]. Although numerous nanomedicine delivery vehicles have been proposed and tested in both preclinical and clinical settings (Fig. 1), a significant challenge in this field remains: most current delivery vehicles struggle to efficiently and selectively reach tumor cells. This inefficiency can

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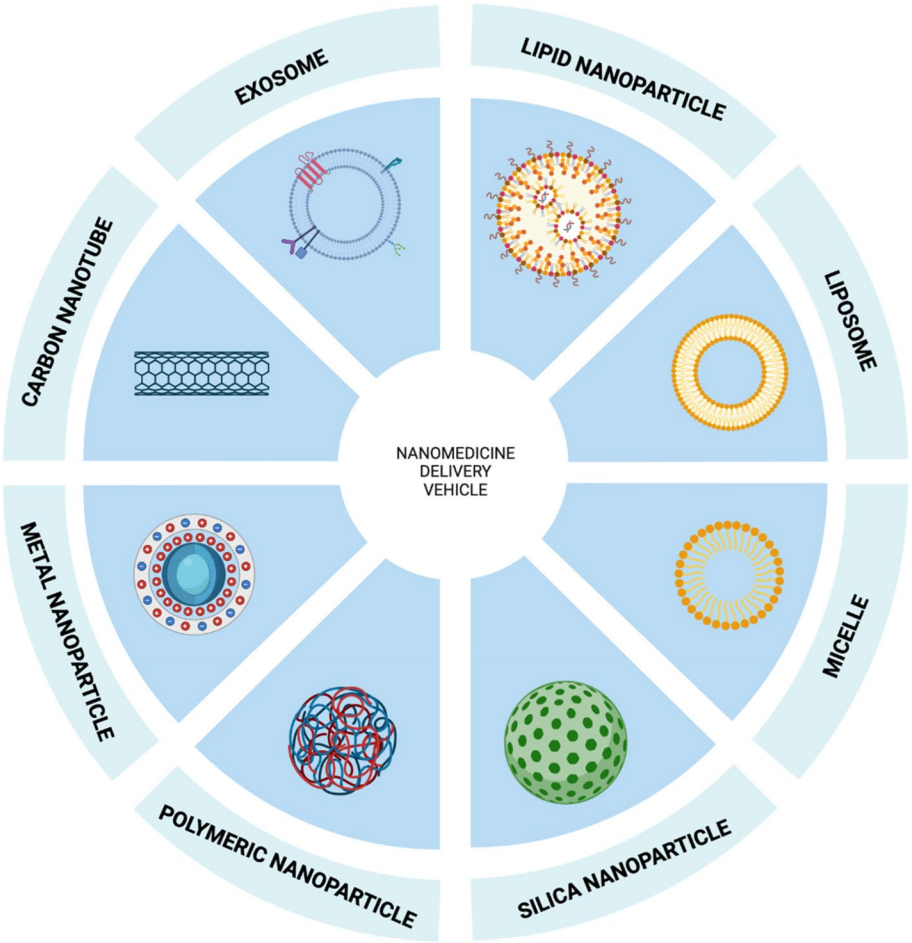

Fig. 1 Nanoparticle Classification Overview. Diagram depicting the various nanoparticle classes that are used to deliver cancer nanomedicine

contribute to suboptimal therapeutic outcomes, including drug resistance, increased toxicity, and reduced efficacy. Given these challenges, there is growing attention on extracellular vesicles (EVs), particularly exosomes, as next-generation drug delivery platforms in oncology. EVs are secreted by cells to eliminate unwanted cellular material and serve as messengers for intercellular communication over both short and long distances. EVs contain various subtypes. The EV subtypes, ranked by decreasing diameter, include exopher (3.5–4 μm), large oncosomes (1–10 μm), migrasomes (500–3,000 nm), apoptotic bodies (100–5,000 nm), microvesicles (200–1,000 nm), exosomes (50–200 nm), exomeres (< 50 nm), and supermeres (< 35 nm) [3–6]. Many subtypes, such as microvesicles and exosomes, play crucial roles in vital physiological processes, including blood coagulation and cell–cell communication [7–9]. Amongst the EVs, exosomes are the most well-characterized, with robust research into its roles in various physiological and cancer pathophysiological processes as well as research into engineering them into the ideal nanomedicine delivery vehicle [10, 11].

While previous literature reviews on cancer exosome nanomedicine have primarily focused on targets, molecular pathways, and the tumor microenvironment, this review emphasizes the technological and translational aspects of exosomes, providing a unique perspective that complements existing research [12, 13]. We explore recent advances in exosome engineering and the clinical translation of exosome-based therapies. A general comparison of exosomes with conventional nanoparticles will first be given, highlighting the distinct features that make exosomes particularly promising drug delivery vehicles for cancer treatment. Next, we will delve into the latest strategies for drug loading, targeted delivery, and exosome functionalization, as well as an overview of current clinical trials, followed by a conclusion on key challenges and future directions for clinical application.

Comparative advantages of exosomes as advanced nanoparticle drug delivery systems

Exosomes are increasingly recognized as the superior alternative to conventional nanoparticles for cancer treatment. This advantage stems from their unique properties that address the limitations prevalent among traditional nanoparticles such as liposomes, lipid nanoparticles (LNPs), micelles, polymeric nanoparticles, metallic nanoparticles, silica nanoparticles, and carbon nanotubes (CNTs) [14]. These nanoparticles are typically classified as either organic or inorganic. Organic nanoparticles generally exhibit lower toxicity and fewer adverse reactions; however, they often suffer from poor stability and suboptimal biodistribution, requiring many modifications to achieve effective cargo delivery [2]. Inorganic nanoparticles tend to be more toxic and are associated with more adverse reactions, but they offer greater stability and possess other intrinsic properties that allow for better control over cargo release [14]. Both types typically lack key properties that define an ideal nanoparticle delivery vehicle. While these nanoparticles are typically synthesized in Good Manufacturing Practice (GMP) settings, significant issues related to toxicity, biodistribution, and targeting persist [14, 15]. This section will explore various advantages of exosomes, which position them as preferred delivery vehicles for cancer therapeutics compared to traditional nanoparticles (Table 1).

Immunogenicity, toxicity & adverse reactions

Nanoparticle drug delivery is based on the principle that delivering drugs via nanoparticles is generally safer than administering unbound free drugs, as it can mitigate off-target toxicity and reduce organ damage, including cardiac toxicity [16]. An ideal nanomedicine delivery vehicle should be biodegradable, biocompatible, and safe. Among nanoparticles, exosomes most closely meet these criteria. Organic nanoparticles such as liposomes, LNPs, micelles and polymeric nanoparticles are generally biodegradable and biocompatible, however, they can be recognized by the innate system as foreign to cause inflammation and be opsonized [2, 14, 17]. In some cases, these nanoparticles can trigger adverse reactions such as hypersensitivity reactions and anaphylaxis [2, 14, 18]. For liposomes and LNPs, their lipid compositions require a fine balance of surface charges to allow for better stability while not causing cationic toxicity and triggering immune reactions. This is because lipids used for liposomes and LNPs can help with the stability of the nanoparticle during circulation. However, in the acidic tumor environment, surface charges of the nanoparticles can be changed to cause harmful effects [17]. While the safety risks associated with these nanoparticles are generally low, the repeated administrations often necessary for cancer therapy can lead to cumulative toxicity and an increased risk of adverse reactions [2]. The immunogenicity of certain organic nanoparticles can also cause the nanoparticles to be rapidly cleared out of the body via the reticuloendothelial system (RES) [14]. Inorganic nanoparticles, such as metallic nanoparticles, silica nanoparticles, and CNTs, often pose greater problems for safety due to their poor biodegradability, potentials for organ damage, risks of long-term exposure with repeated administrations, and dose-dependent toxicities [19–22]. Most metallic and silica nanoparticles exhibit limited biodegradability or are non-biodegradable, and those biodegradable variants may still induce oxidative stress and cellular damage as a result of metal ion accumulation. [19, 20]. For other inorganic nanoparticles like CNTs that are generally inert and biocompatible, research has also found them to be toxic following interaction with the immune cells, leading to cytotoxicity, granuloma development, and chronic inflammation [23–25]. Another safety concern with inorganic nanoparticles, such as silica nanoparticles, is that variations in patient populations and differences in particle formulations could lead to significant harmful effects. For instance, some formulations of the silica nanoparticle have been recently reported to cause liver damage and metabolic dysregulation, which can aggravate patients with or in risk of fatty liver diseases [26]. Another recent report showed that some formulations of silica nanoparticles with a diameter of 50 nm could cause severe lung damage while those with a diameter of 3 μm did not [22].

As for exosomes, they are completely biodegradable and biocompatible as they are a vital part of normal physiology [27]. They also present minimal safety concerns due to their low immunogenicity and lack of toxicity and adverse reactions [28]. The choice of source for exosomes can further reduce immunogenicity and safety issues, particularly when derived from less differentiated cells or from allogeneic or autologous sources [29–31]. This unique feature distinguishes exosomes, as no other nanoparticles allow for sourcing from safer options without significantly compromising their functional or structural integrity.

Targeting & modification

Traditional nanoparticles accumulate at tumor sites via the enhanced permeability and retention (EPR) effect, which is a key mechanism for passive tumor targeting. However, recent research indicates that relying solely on the EPR effect for endogenous targeting is insufficient and highly inefficient, with some formulations demonstrating that less than 1% of administered nanoparticles actually reach tumor tissues [32, 33]. For both organic and inorganic nanoparticles, the most significant limitation is

Table 1 Comparison of major types of nanoparticles

Nanoparticle formulations		Immunogenicity, toxicity & adverse reactions	Biodegradability & biocompatibility	Endogenous targeting	Modifications	Drug delivery properties
EXOSOME		Little to no immunogenicity, potentials for toxicity, and potentials for adverse reactions.	Highly biodegradable and highly biocompatible.	Possess endogenous targeting and tumor homing capabililties.	Amenable to most surface functionalization methods. Additionally, sources of exosomes can be modifiable.	High biostability, low off-target toxicity, highly efficient cellular uptake and cargo release. Long biological half-life, controlled release and sustained-release can be achieved.
LIPOSOME		Immunogenicity issues present, can trigger immune reaction and inflammation. Potential cationic toxicity.	Highly biodegradable and generally biocompatible.	No endogenous targeting, rely only on EPR effect. Require modification for active targeting.	Amenable to most surface functionalization methods. Lipid composition and nanoformulation modifiable.	Limited biostability, off-target toxicity present, leakage and premature cargo release can occur. Good biological half-life and controlled release can be achieved.
LIPID NANOPARTICLE (LNP)		Immunogenicity issues present, can trigger immune reaction and inflammation. Potential cationic toxicity and cytokine induction.	Generally biodegradable, excluding certain ionizable lipids, and generally biocompatible.	No endogenous targeting, rely only on EPR effect. Require modification for active targeting.	Amenable to most surface functionalization methods. Lipid composition, precise particle size and nanoformulation modifiable.	Good biostability, low off-target toxicity, good cellular uptake efficiency and cargo release. Controlled release can be achieved.
MICELLE		Low to no immunogenicity issues, toxicity, and adverse reactions.	Highly biodegradable and some biocompatiblity issues.	No endogenous targeting, rely only on EPR effect. Require modification for active targeting.	Amenable to most surface functionalization methods as well as chemical modification and polymer modification.	Poor biostability, prone to disassembly, leakage, and premature cargo release. Controlled release can be achieved.
POLYMERIC NANOPARTICLE	NATURAL (e.g. CHITOSAN, DEXTRAN)	Low immunogenicity. Toxicity and adverse reaction potential includes allergic and other immune reactions.	Highly biodegradable and generally biocompatible.	No endogenous targeting, rely only on EPR effect. Require modification for active targeting.	Amenable to most surface functionalization methods as well as chemical modification and polymer composition modification.	Poor biostability, prone to disassembly, leakage, and premature release of cargo. Possess rapid and efficient cellular uptake. Controlled release can be achieved. High batch-to-batch variability with reproducibility issues.
	SYNTHETIC (e.g. PEG, PLGA)	Immunogenicity issue present. Toxicity and adverse reactions potentials include oxidative stress, apoptosis, peroxidation, inflammation, and immune activation.	Generally poor biodegradability and biocompatibility.	No endogenous targeting, rely only on EPR effect. Require modification for active targeting.	Amenable to most surface functionalization methods as well as chemical modification and polymer composition modification.	Good biostability, low off-target toxicity, poor cellular uptake efficiency. Controlled and sustained-release can be achieved.

Table 1 (continued)

Nanoparticle formulations		Immunogenicity, toxicity & adverse reactions	Biodegradability & biocompatibility	Endogenous targeting	Modifications	Drug delivery properties
METALLIC NANOPARTICLE	METAL (e.g. GOLD)	Immunogenicity potential is present. Generally inert but toxicity and adverse reaction potentials include dose-dependent toxicity, oxidative stress, and inflammation.	Not biodegradable. Generally inert and biocompatible.	No endogenous targeting, rely only on EPR effect. Require modification for active targeting.	Amenable to extensive and precise physical modification, nanoformulation modification, and most surface functionalizations.	High biostability, off-target toxicity present, highly efficient cellular uptake. Due to the intrinsic principle of the nanoparticles, synergistic anti-tumor effect can be achieved. Controlled and sustained-release can be achieved.
	METAL OXIDE (e.g. IRON OXIDE)	Low immunogenicity. Toxicity and adverse reactions potential includes mutagenic effects, possess long-term exposure effects, genotoxicity, cytotoxicity, and oxidative stress.	Can be biodegradable and generally biocompatible.	No endogenous targeting, rely only on EPR effect. Can be magnetically guided for targeting in addition to modifications.	Amenable to most surface functionalization methods as well as amenable to some physical and chemical modifications.	Good biostability, low off-target toxicity, highly precise cellular uptake and cargo release due to highly precise magnetic guiding/targeting. Controlled and sustained-release can be achieved.
SILICA NANOPARTICLE (e.g. MSN)		Immunogenicity issues present. Toxicity and adverse reactions potential include organ injuries, hemolysis, and immune activation.	Poorly biodegradable, excretion takes a significant amount of time and debris prone to particle bioaccumulation. Generally biocompatible.	No endogenous targeting, rely only on EPR effect. Require modification for active targeting.	Amenable to most surface functionaliation methods with the unique addition of a stimuli-responsive cap on the pores.	High biostability due to stimuli-responsive capping. Off-target toxicity is present. Highly efficient and precise cargo release. Controlled and sustained-release can be achieved.
CARBON NANOTUBE (CNT)		Immunogenicity issues present. Toxicity and adverse reactions potential include cytotoxicity, granuloma, chronic inflammation, and harmful impurities in CNTs.	Poorly biodegradable. Generally biocompatible, similar to metallic nanoparticles.	No endogenous targeting, rely only on EPR effect. Can be magnetically guided for targeting in addition to modifications.	Amenable to extensive and precise physical modification, nanoformulation modification, and most surface functionalizations.	High biostability, off-target toxicity present. Good cargo release and controlled release can be achieved.

their lack of endogenous targeting capabilities, resulting in low efficacy and off-target toxicities. While modifications can enable site-directed active targeting of tumor tissues, these alterations can be costly and may introduce new safety or efficacy concerns. [14]. Common modifications to these nanoparticles include surface functionalization aimed at improving biostability while reducing immunogenicity and toxicity. Active targeting modifications typically involve the conjugation of ligands, such as antibodies, proteins, aptamers, and specific peptides, to the surface, facilitating site-directed targeting of tumor tissues [34, 35]. These strategies often target surface antigens that are overexpressed in cancer. However, their effectiveness can be limited by significant intra-tumor and inter-tumor heterogeneity, which reduces the overall efficacy and translatability of the modified nanoparticles [36]. Additionally, surface modifications can sometimes compromise the structural integrity of the nanoparticles, potentially impacting their drug delivery properties and uptake efficiencies [37]. Other modifications such as using polyethylene glycol (PEG) help to improve the circulation time and reduce the immunogenicity of various nanoparticles. For example, without PEGylation, liposomes, LNPs, micelles, and polymeric nanoparticles will have short circulation time and can be cleared out of the body in a short amount of time [14, 38]. However, safety concerns regarding PEG have risen in recent years because PEG is not biodegradable, can accumulate, and trigger adverse reactions such as hypersensitivity reactions via activating the complement system and causing anaphylaxis [3–5, 39].

Exosomes exhibit distinct endogenous targeting profiles and tumor homing capabilities depending on their sources [40]. To enhance their circulation time within the body, various common modification approaches are employed. Notably, exosomes can circumvent the need for PEGylation by utilizing surface CD47 expression to reduce clearance through the RES and significantly improve their biological half-life. CD47 serves as a "don't eat me" signal recognized by phagocytes in the RES, preventing exosome recognition and allowing for extended circulation to deliver their cargo to cancer cells [41–45]. CD47 expression can be introduced by genetically modifying the source cells before exosome isolation, enabling this modification without significantly affecting the structural or functional integrity of the exosomes [30, 45].

Cargo delivery profiles

Ideal nanoparticle delivery systems should exhibit controlled and sustained-release profiles, ensuring no leakage or premature cargo release. Mechanisms for controlled release can be triggered by environmental factors such as pH, temperature, magnetic fields, and enzymatic activity [14]. Organic nanoparticles, due to their lower levels of biostability, are more prone to breakage, leakage, and premature cargo release. Lipid compositions could be a factor that affects cargo release because certain lipids are prone to hydrolysis and peroxidation of the ester linkage, reducing delivery efficiency [46]. Through modifications, some levels of controlled and sustained-release profiles can be achieved in liposomes and LNPs [2, 14]. Micelles could be disassembled in the mucus or in contact with epithelial cells, while polymeric nanoparticles could be disassembled and prematurely release their cargo due to the dilution following systemic administration [9]. Inorganic nanoparticles typically possess superior cargo release profiles by having greater stability that prevents leakage or premature cargo release as well as having mechanisms for stimuli-responsive cargo release [47–49]. Additionally, silica nanoparticles are highly porous with high surface area, which offer them advantages in highly efficient drug loading and release [35]. Modifications can be made to the silica nanoparticles to cap the cargo in the pores and only release at specific conditions. Metal oxide nanoparticles are often magnetic and can be guided via external magnetic fields to allow for precise control and release of the cargo to the tumor [21]. Similar to inorganic nanoparticles, exosomes possess great stability and do not show leakage or premature cargo release [45]. Compared to other organic nanoparticles, cargo release in exosomes can be accelerated in acidic conditions such as those found in the tumor microenvironment, late endosomes, and lysosomes [50, 51]. Furthermore, exosomes are naturally enriched with transmembrane and membrane-anchored proteins that could enhance uptake into target cells [45].

Versatility & hybrid systems

Nanoparticles can be tunable, allowing adjustments in various aspects of their composition and properties, including structure, internal architecture, and surface charge [47]. Regarding organic nanoparticles, the versatility typically lies in the compositional changes, such as using ionizable lipids instead of cationic lipids in liposomes and LNPs to reduce cationic toxicity [52]. LNPs can also have control over the internal architecture, allowing design changes to best suit the desired cargo and target [2, 14]. Similarly, for metallic nanoparticles and CNTs, precise control over shape, size, and nanostructure also enables design modifications that optimize them for specific cargo and targets [53, 54]. For both silica nanoparticles and CNTs, their superior surface area allows for a wide variety of surface functionalization. These modifications were often made to mimic the endogenous properties of exosomes [54]. This

idea is further explored in various approaches that utilize hybrid systems combining traditional nanoparticles with exosomes to enhance their cargo delivery properties [55–57]. For example, gold nanoparticles can be encapsulated in exosomes to improve targeting and accumulation in tumors, while iron oxide nanoparticles can be encapsulated to leverage exosomal immune evasion properties, combined with MRI-guided controlled release to enhance therapeutic efficacy [56–58].

Taken together, exosomes represent an ideal nanomedicine vehicle, fulfilling most essential criteria, including inherent targeting capabilities, safety, versatility, effective and efficient cargo release profiles, and biodegradability—attributes where conventional synthetic platforms frequently encounter limitations.

Exosome sources, isolation & characterization

The properties and cargo profiles of exosomes vary significantly based on their cellular origins, highlighting the importance of cell source selection for exosome production. Optimized isolation techniques are essential for obtaining high-quality exosomes with substantial yields. Thorough characterization is also crucial to ensure their consistency and functionality for clinical applications. This section will examine exosome sources, isolation methods, and key characterization parameters vital for effective cargo loading.

Sources

Exosome biogenesis involves the formation of intraluminal vesicles (ILVs) through the ESCRT-dependent inward budding of cargo in early endosomes, which mature into multivesicular bodies (MVBs) that release exosomes upon fusion with the cell membrane [59, 60]. In laboratory and clinical settings, exosomes are primarily sourced from cell culture media or biofluids, including blood, ascites, and urine [61]. Commonly used cell sources include mesenchymal stem cells (MSCs) and human umbilical vein endothelial cells (HUVECs) [62–64]. Due to their relatively inert nature, these exosomes are often utilized for loading therapeutic cargo and demonstrate predictable biodistribution and bioavailability. They are also easily isolated in large quantities in laboratory settings. Other specific cell types could also be chosen as the source depending on their properties. For instance, exosomes from the bone marrow niche promote tumor dormancy and have shown anti-tumor effects in breast cancer models [65]. Additionally, exosomes from dendritic cells contain essential immune-stimulatory elements, including the simultaneous presence of MHC I and MHC II complexes, high levels of co-stimulatory molecules, and therefore could serve as a cell-free alternative for delivering neoantigens in vaccines [66]. Tumors can also serve as sources for exosomes, which can be extracted from tumor tissues or circulating tumor exosomes found in biofluids [67]. In addition, exosomes and exosome-like nanoparticles derived from animal or plant products represent potential sources that could be developed into nanomedicine carriers using similar methodologies [68–71].

Exosome characterization

Rigorous characterization of exosomes to validate exosome quality and consistency is essential for advancing exosome-based research and applications. According to Minimal information for studies of extracellular vesicles by the International Society for Extracellular Vesicles (MISEV2023), exosomes in general are characterized through the physical and biochemical properties which include sizes, protein compositions, and structural morphology [59]. Nanoparticle Tracking Analysis (NTA) is frequently utilized to assess the size distribution and concentration of exosomes in suspension by tracking the Brownian motion of particles using laser light scattering, offering insights into exosome population dynamics and heterogeneity. Similarly, Dynamic Light Scattering (DLS) assesses the size distribution of exosomes based on the fluctuation of scattered light from particles in suspension. While DLS has limitations regarding the detection of polydispersity and the influence of particle concentration, it remains a convenient method for preliminary size assessment [72]. High resolution imaging techniques such as transmission electron microscopy (TEM) are also commonly used to visualize the morphology and size of exosomes, thus distinguishing them from other types of extracellular vesicles. Exosomes are known to appear biconcave or cup-shaped under TEM. Moreover, Western blotting and enzyme-linked immunosorbent assays (ELISAs) can be used for detecting specific protein markers associated with exosomes (e.g., CD63 and CD81), helping to confirm their identity and purity. Nano-flow cytometry emerges as another useful tool for exosome characterization by assessing and quantifying multiple surface markers simultaneously. Using specific antibodies conjugated to fluorescent tags, this technique allows for the profiling of exosomal surface proteins, aiding in identifying subpopulations of exosomes based on their protein content [73] Another important method is measuring zeta potential, a critical physicochemical property that reflects the electrostatic charge on exosome surfaces. This charge influences stability, interactions, and overall function in biological systems. A stable zeta potential indicates that exosomes remain dispersed and do not aggregate during storage, preserving their bioactivity and therapeutic properties over time. Fluctuations in zeta potential have been correlated with changes in stability, shelf-life, and

efficacy metrics [74]. Thus, zeta potential serves as a vital indicator of exosomal integrity and stability. In therapeutic and clinical settings, additional characterization will include stability assessments, toxicology studies using animal models, and evaluations of batch reproducibility [30, 45].

Comprehensive characterization allows researchers to validate the identity, purity, and functional capabilities of exosomes, thereby strengthening the reliability of exosome-based research and facilitating the translation of these therapeutics from research to clinical use. It could enhance our understanding of exosome composition, particularly lipid and protein profiles, which sheds light on their mechanisms of action in various diseases. The continuous refinement of these analytical techniques could promote greater standardization and reproducibility across research studies, which is essential for scientific progress. Furthermore, multifaceted characterization techniques are critical for quality control, ensuring that exosome preparations adhere to stringent purity and quality standards required for clinical applications.

Exosome isolation

According to MISEV2023, exosome isolation methods leverage either the biophysical properties of exosomes, such as size and density, or their unique surface compositions, such as specific surface molecules [59]. The choice of isolation technique is largely dependent on downstream applications and it does not affect exosome biogenesis which is an intrinsic cellular process. Exosome isolation techniques could directly impact yield, purity/specificity, efficiency/time, and membrane integrity, which are crucial for successful cargo loading efficiency and subsequent release.

Differential ultracentrifugation utilizes a series of spins at gradually increasing speeds to initially eliminate contaminants before pelleting the exosomes. It has been considered the gold standard for exosome isolation and is preferred for functional analysis in most preclinical and clinical settings due to its ability to maximize yield, preserve membrane integrity, and straightforward operation, despite being time-consuming [75, 76]. While techniques such as ultrafiltration and size exclusion chromatography might have much shorter processing time and preserve the membrane integrity well, they are prone to blockage, contamination from non-specific proteins, and high cost of consumables [77, 78]. Precipitation methods can also yield higher amounts of exosomes and are much faster than ultracentrifugation; however, they introduce contaminating isolation reagents that remain associated with the exosomes, interfering with both loading and biological activity. Residual precipitation reagents can cause vesicle aggregation and clustering, as observed via TEM, significantly impairing functional efficacy in vitro. Research by Paolini and colleagues demonstrated that these precipitation reagents directly compromise exosome biological functions, while Gámez-Valero showed reduced cell viability when using precipitated exosomes that contained residual isolation reagents [79, 80]. Tangential flow filtration (TFF) is an emerging technique that preserves exosome integrity while achieving substantially higher yields and efficiency [81, 82]. TFF applies media parallel to the membrane rather than perpendicular (as in dead-end filtration), preventing molecule accumulation and membrane fouling. This gentle approach maintains critical surface proteins like CD47 and tetraspanins that influence circulation time and targeting ability [81, 82]. The yield difference directly impacts therapeutic potential, as more intact exosomes mean more available vehicles for drug delivery. However, challenges may arise from the clogging of pores with debris and other contaminants during prolonged and/or repeated use as well as requiring specialized and expensive equipment [83, 84]. The purity of isolated exosomes profoundly affects therapeutic outcomes, with production method being the primary determinant of purity. A comparative study showed that TFF-isolated exosomes from MSCs exhibited higher expression of CD63 (an MSC exosome marker) and negligible contamination with low-density lipoprotein cholesterol compared to ultracentrifugation-isolated exosomes [83]. This enhanced purity directly translated to improved therapeutic efficacy, with highly purified MSC exosomes increasing wound healing and angiogenic effects in human coronary artery endothelial cells [83]. Filter membrane pore size during TFF isolation also impacts yield and purity, affecting downstream loading and therapeutic applications. Comparative analysis of 300 kDa versus 500 kDa filter membranes showed that while 500 kDa filters produced lower yields, they generated exosomes with higher purity ratios [82]. This increased purity improved loading efficiency by reducing competitive binding from contaminants and enhanced therapeutic efficacy through more precise target engagement. To increase specificity, alternative techniques such as immunoprecipitation can be used [59]. However, these methods may introduce a high risk of contamination with non-exosomal materials such as proteins and polymeric materials [77].

Isolation methods also influence exosome heterogeneity, which affects both loading capacity and therapeutic targeting. Different isolation techniques select for specific exosome subpopulations with varying membrane compositions and surface protein profiles [78]. For instance, size exclusion chromatography tends to isolate purer exosomes with consistent size distributions and preserved surface markers, albeit at lower yields than

precipitation methods [80]. Additionally, precipitated exosomes exhibited inconsistent expression of surface markers, suggesting selective isolation of specific exosome subpopulations with potentially varying loading capacities [80]. Such selective isolation impacts the physiochemical properties of the exosome membrane, affecting its interaction with hydrophobic drugs during loading and subsequent fusion with target cells. Exosomes isolated using optimized methods is important for maintaining their natural tumor-homing capabilities, reducing the need for additional targeting modifications that might otherwise compromise loading efficiency [81, 82].

The influence of production parameters extends beyond the isolation method itself to the specific operational conditions within each method, highlighting the nuanced relationship between production techniques and therapeutic outcomes. These methods could fundamentally determine yield, membrane integrity, surface protein composition, purity, and heterogeneity, all of which directly influence loading capacity, drug release profiles, and ultimately therapeutic efficacy. The evidence strongly suggests that optimizing production techniques should be considered an integral part of therapeutic development rather than merely a preliminary technical step. Moreover, it is important to note that exosomes are delicate structures and are often resuspended in buffered saline solution and immediately frozen at −80 °C after isolation to prevent degradation. To preserve the yield and integrity of the exosomes, it is essential to minimize and avoid freeze–thaw cycles, as they can result in the loss and aggregation of exosomes [85].

Exosome cargo & loading

Exosomes, as endogenous intercellular messengers, can deliver a wide range of bioactive cargo throughout the body [59]. Alongside housing various therapeutic agents as cargo, exosomes also carry surface proteins that can signal or activate specific pathways in target tissues [86, 87]. The composition of exosomal cargo and surface modifications varies among cell types, enabling researchers to select exosomes enriched with desired features. Beyond using natural cargo and modifications, researchers have developed a myriad of methods for loading and modifying exosomes to contain specific therapeutic agents [88]. Numerous FDA-approved drugs, including chemotherapeutics, monoclonal antibodies and immunotherapies, have been successfully loaded into exosomes, showing varying degrees of success in preclinical mouse models. For example, doxorubicin-loaded MSC-derived exosomes enhanced tumor-selective targeting and drug release along with minimizing systemic toxicity while preserving cardiac function [16]. Similarly, paclitaxel-loaded macrophage-derived exosomes overcame multidrug resistance in pancreatic adenocarcinoma (PDAC) cells, showing higher cytotoxicity in P-glycoprotein-positive PDAC cells than free paclitaxel [62]. Furthermore, exosomes have been utilized to deliver various targeted therapies, including monoclonal antibodies like cetuximab (anti-EGFR) and small molecules such as imatinib [89, 90]. Additionally, exosomes can serve as effective delivery vehicles for immune checkpoint inhibitors like atezolizumab, durvalumab, and avelumab [91]. Despite such versatility, a key challenge remains in the efficiency of loading desired cargo into exosomes. Traditionally, researchers have leveraged the similarities between the exosome membrane and the cell membrane to employ comparable techniques for loading medicine into exosomes. However, these methods are often inefficient, resulting in less effective exosomes. This is where the innovations in biotechnology, chemical biology, genetic engineering, and regenerative medicine have come into play, offering opportunities to improve traditional methods and enhance the effectiveness of exosomes as nanomedicine delivery vehicles (Table 2).

Traditional methods of cargo loading

Traditional loading methods for exosomes generally involve two main approaches: passive co-incubation or active loading. In passive co-incubation, the technique entails incubating the desired cargo, either alone or with a transfecting agent that facilitates specific cargo delivery into cells [62]. This approach offers control over the choice of cell source for the exosomes and involves fewer processing steps for the exosomes, enabling immediate freezing for preservation of the recovery rate, as well as the functional and structural integrity of the exosomes and the nanomedicine cargo. On the other hand, the active loading method places less emphasis on the exosome source, as the intended cargo is exogenously loaded mainly through techniques such as electroporation or sonication [85, 88]. While this approach is typically more efficient, its drawback lies in the fact that loading process exposes the exosomes to harsher conditions, leading to greater exosome loss and potential damage to the cargo or loss of certain exosome integrities.

Co-incubation

There are two main types of incubation methods used to load desired cargo into exosomes. The first method involves co-incubating exosome-producing cells with the cargo, facilitating its uptake by the cells. Alternatively, these cells could be exposed to specific stimuli that trigger changes in the exosome cargo, allowing for the inclusion or exclusion of specific materials. For example, immune cell-derived exosomes could be enhanced with specific cargo following exposure to certain cytokines or

Table 2 Comparison of traditional exosome loading methods & exosome loading innovations

Loading method	Pre-isolation or post-isolation	Passive or active	Cargo	Strengths	Limitations	Cancer clinical status
CO-INCUBATION	PRE-ISOLATION	PASSIVE	SMALL MOLECULE CHEMICAL DRUGS	NO POST-ISOLATION STEPS NEEDED	LOW EFFICIENCY, RELIANT ON CERTAIN CELL TYPES	CLINICAL TRIAL
TRANSFECTION	PRE-ISOLATION	ACTIVE	NUCLEIC ACIDS	NO POST-ISOLATION STEPS NEEDED	DIFFICULT TO SCALE UP	PRECLINICAL MODEL ONLY
ELECTROPORATION	POST-ISOLATION	ACTIVE	COMPATIBLE WITH MOST CARGO	COMPATIBILITY WITH EXISTING RESEARCH INFRASTRUCTURE, EXISTING GMP MANUFACTURING	REQUIRE POST-ISOLATION INCUBATION TIMES UNDER EXOSOME-DEGRADING TEMPERATURE	CLINICAL TRIAL
SONICATION	POST-ISOLATION	ACTIVE	COMPATIBLE WITH MOST CARGO	COMPATIBILITY WITH EXISTING RESEARCH INFRASTRUCTURE	REQUIRE POST-ISOLATION INCUBATION TIMES UNDER EXOSOME-DEGRADING TEMPERATURE	PRECLINICAL MODEL ONLY
MICROFLUIDIC	PRE- & POST-ISOLATION	ACTIVE	COMPATIBLE WITH MOST CARGO	EASE OF SCALING UP, HIGHER YIELD, HIGHER LOADING EFFICIENCY	REQUIRE POST-ISOLATION INCUBATION TIMES, LOWER SPECIFICITY & SELECTIVITY	PRECLINICAL MODEL ONLY
NANOPORATION	PRE-ISOLATION	ACTIVE	COMPATIBLE WITH MOST CARGO	HIGHER YIELD, HIGHER EFFICIENCY, NO POST-ISOLATION STEPS NEEDED	DIFFICULT TO SCALE UP DUE TO NOVELTY OF THE BIOCHIP	PRECLINICAL MODEL ONLY
FUSOGENIC HYBRID	POST-ISOLATION	PASSIVE	COMPATIBLE WITH MOST CARGO	HIGHER YIELD, HIGHER EFFICIENCY, COMPATIBLE WITH EXISTING LIPOSOME TECHNIQUES	REQUIRE ADDITIONAL STEPS OF GENERATING AND LOADING LIPOSOMES, REQUIRE POST-ISOLATION INCUBATION AT EXOSOME-DEGRADING TEMPERATURES	PRECLINICAL MODEL ONLY
GENETIC ENGINEERED	PRE-ISOLATION	ACTIVE	COMPATIBLE WITH CARGO CONJUGATED WITH SORTING TAG	HIGHER SELECTIVITY & SPECIFICITY, NO POST-ISOLATION STEPS NEEDED	CARGO LIMITED DUE TO SORTING TAG CONJUGATION, REQUIRE ADDITIONAL STEPS TO GENERATE DESIRED CELL LINE	PRECLINICAL MODEL ONLY

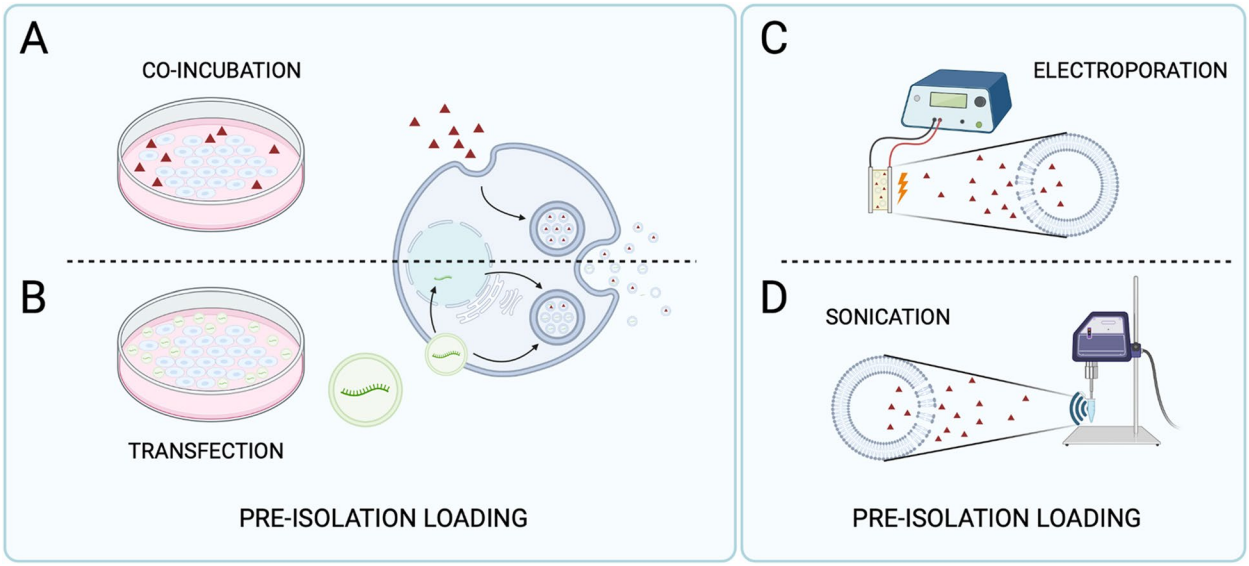

Fig. 2 Traditional Exosome Loading Methods. **A** Co-Incubation of desired cargo with the cells prior to isolation of the exosomes. This allows certain cells' natural tendencies to internalize cargos or rely on specific cargo's ability to be endocytosed into the cells. Once inside the cell, the cargos can be sorted into exosomes through various sorting pathways inside the cell. **B** Transfection of plasmids or other vectors allow the addition of specific nucleic acids into the cells prior to exosome collection. This allows the modification of the transcription and translation of specific cargos to be added or depleted from the exosomes. **C** Electroporation uses a range of voltages to create temporary openings in the membrane of the isolated exosomes. This technique is more amenable to the type of cargo loaded because the major constraint is just the size and solubility of the cargo. **D** Sonication is the use of various frequencies to create temporary openings in the exosome membrane and allow desired cargo to be loaded inside exosomes. This is similar to electroporation and is amenable to wide range of cargos with the major restrictions being their size and solubility

through co-culturing with tumor or tumor microenvironment-associated cells [92]. The second method involves incubating isolated exosomes with the desired cargo to facilitate passive incorporation. Agrawal et al. (2017) demonstrated this approach by loading milk-derived exosomes with paclitaxel, resulting in a two-fold increase in tumor growth inhibition in mouse models compared to paclitaxel alone [68]. Furthermore, modifications to nucleic acids, such as cholesterol conjugated siRNAs, have been shown to enhance the efficiency of co-incubation loading while allowing the therapeutic siRNAs to remain functional within the exosomes [93]. Although these approaches effectively preserve the cargo and exosome properties due to minimal alternations in the exosome membrane composition and integrity, one main drawback is the low efficiency of cargo loading (Fig. 2A).

Transfection

To deliver exosomes with specific cargos added or depleted, researchers can utilize transfection of the exosome-sourcing cells with siRNAs, followed by collection of the exosomes. For example, Hyung et al. (2023) demonstrated that transfecting gastric cancer patient-derived cancer (PDC) cells with siRNA targeting MET oncogene resulted in PDC-exosomes depleted of MET. The resulting allogeneic exosomes retained their functionality and were able to selectively deliver MET-silencing cargo to the tumors, leading to reduced tumor angiogenesis and invasiveness [67]. Transfection may provide an additional layer of control by enabling the genetic engineering of cargo. For instance, glycosylphosphatidylinositol (GPI) signal peptides can be used to anchor proteins, such as nanobodies, onto the surface of exosomes [94] (Fig. 2B).

Electroporation

Electroporation is a commonly used method for loading specific cargo into exosomes, taking advantage of the similarities between exosomal and cellular membranes. This approach can accommodate a wide range of cargo, as the concentration and conditions can be optimized for efficient loading. For example, Han et al. (2021) used electroporation to generate exosomes loaded with SIRT6 siRNA that impaired prostate cancer's metastatic and proliferation pathways [88]. In addition to siRNAs, drugs and other chemicals can also be loaded using electroporation. Kim et al. (2017) successfully loaded paclitaxel via this technique, while Wan et al. (2022) loaded Cas9 RNPs to target hepatocellular carcinoma [95, 96]. However, electroporation was shown to induce the formation of siRNA aggregates, reducing their efficacy and that of the exosomes [85]. It has also been reported to cause

exosome aggregation, further diminishing their effectiveness [47] (Fig. 2C).

Sonication

Another method for loading cargos into exosomes involves sonication, which utilizes different frequencies and voltages. For example, Saini et al. (2024) successfully delivered antibodies into exosomes targeting specific surface antigens of neuroendocrine prostate cancers using this technique [97]. Similarly, Du et al. (2021) employed sonication to deliver a ferroptosis inducer and a photosensitizer for chemo-photodynamic therapy in hepatocellular carcinoma mouse models [98] (Fig. 2D).

Others

In addition, researchers have utilized less popular techniques, such as freeze–thaw cycles, mechanical extrusion, and detergent-mediated loading to introduce cargo into exosomes [99].

Exosome innovations & new methods

Recent advancements in the creation and loading of exosomes aim to address the significant barriers that hinder the scaling up and clinical translation as engineered nanomedicine delivery vehicles for cancer treatment.

Microfluidics-based platforms for exosome mimetics production

Microfluidics is a promising technology for isolating and/or detecting exosomes from a variety of biological and clinical samples, including cells and ascites from cancer patients [100, 101]. This technology enables the use of small devices and minimal sample amounts for exosome collection and detection for various purposes. One significant advantage of microfluidics-based approaches in exosomes loading and selection is the ease of scalability compared to traditional methods. Recently, researchers have employed this technology to facilitate the generation, modification, and loading of exosomes and exosome-mimetic nanovesicles in a highly efficient and precise manner (Fig. 3A).

The limited production of exosomes by living cells and the complexity of collection procedures reduce their effectiveness for mass delivery to targeted cells. To address this, Jo et al. (2014) developed a microfluidics platform that pressurizes cells into nanovesicles resembling exosomes, which can function as delivery vehicles for nanomedicine [102]. Wang et al. (2024) demonstrated a platform to generate tumor-targeting exosome-mimetic nanovesicles loaded with anti-tumor nanomedicine that can penetrate the blood–brain barrier (BBB), achieving a 78% improvement in tumor retention compared with traditional methods [103]. Another approach to fabricating nanovesicles with microfluidics involves utilizing the naturally abundant and the much easier to isolate microvesicle population in EVs to create uniformly sized nanovesicles while loading therapeutic agents, such as miRNA, targeting specific tumors. Wang et al. (2021) showed that these nanovesicles achieved high loading efficiency and reduced tumor growth in preclinical mouse models [104]. In addition, Liu et al. [105]. demonstrated the use of a microfluidic sonication platform to produce exosome membrane-coated poly (lactic-co-glycolic acid) (PLGA) nanoparticles loaded with imaging agents, resulting in improved targeting of homologous tumors [105].

The use of microfluidics enables precise control over the final exosomes, allowing for specific and engineered surface modifications to enhance targeting and biodistribution. Given the many roles microfluidics technologies already play in research and in clinical settings, it is a tool that has already been well integrated in different aspects of clinical research, lowering the technical and skill barriers of the technology in clinical translation. With its scalability and the potential for optimization and pipeline development, microfluidics technology can serve as a compatible and efficient tool in preclinical and clinical settings.

Nanoporation

Nanopration, an innovative technology that employs nanofluidic devices with nanochannel electroporation, enables the efficient loading of specific cargos into exosomes with high effectiveness [106]. Despite being a relatively recent development, it has already shown success in various preclinical models and offers several advantages similar to those of microfluidic systems (Fig. 3B).

Exosome nanoporation

Nanoporation utilizes mechanical compression, fluidic shear stress to create transient nanopores in exosomes, enabling more efficient loading of desired cargo compared to microfluidics [107–109]. Similar to microfluidic devices, the entire process is conducted in specific buffers and the loaded exosomes can be promptly collected and frozen to prevent loss and degradation. The workflow involves loading exosomes obtained from prior steps into exosome nanoporation devices to load specific cargos. This technique allows for the use of exosomes sourced from different cell types for organotropic targeting or specific pharmacokinetic properties. This added control enables fine-tuning in the delivery of nanomedicine while increasing cargo loading efficiency.

The nanoporation platform developed by Hao et al. (2021) creates transient nanopores approximately 4.9 nm

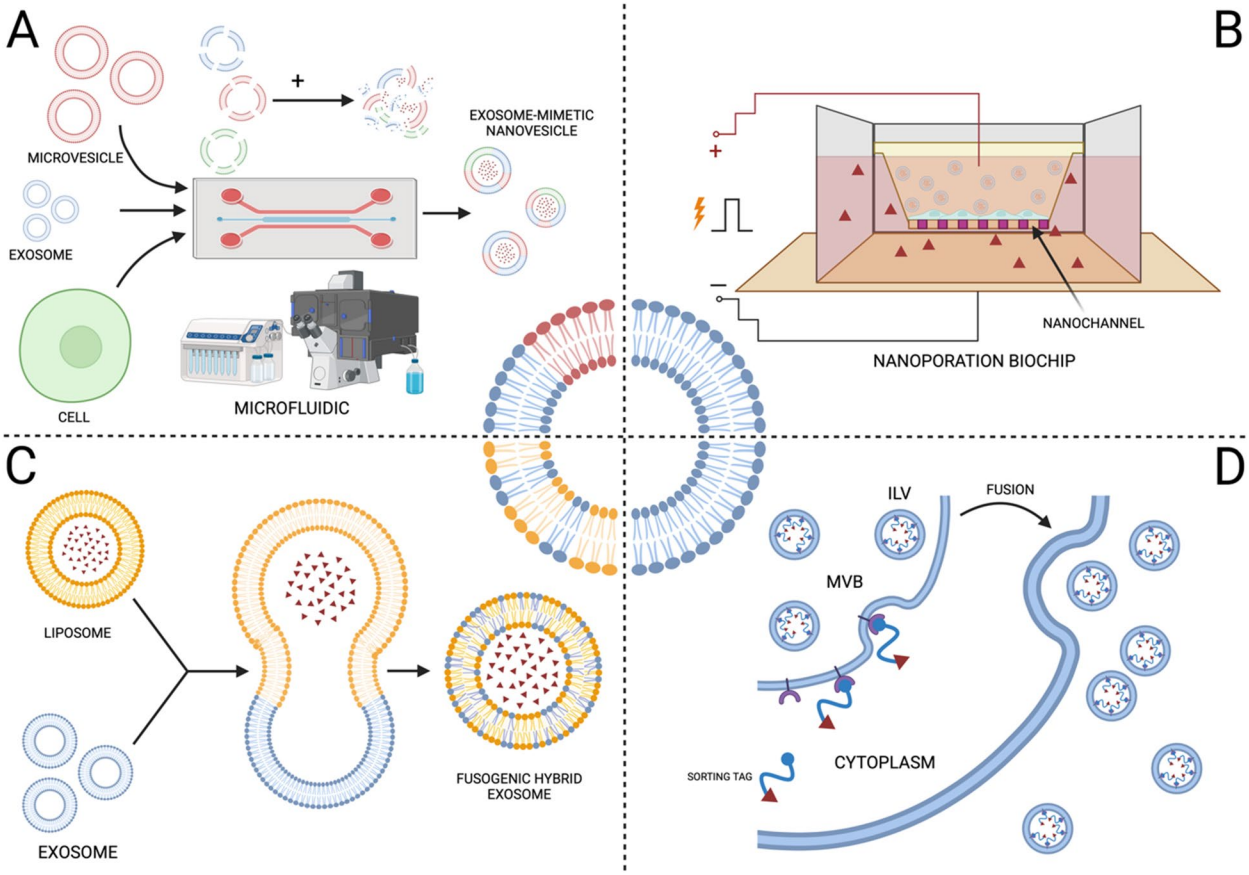

Fig. 3 Innovations & New Methods of Exosome Loading. **A** Microfluidics-mediated mechanical synthesis of exosome-like nanovesicles from a variety of sources ranging from cells, microvesicles, and exosomes. In the process of synthesis, desired cargos are encapsulated into the exosome-like nanovesicles. **B** Schematic of a nanoporation biochip. Nanochannels in the biochip use focused electric fields to load desired cargo onto cells or exosomes directly. **C** Fusogenic hybrids rely on chemically-induced fusion of liposomes with exosomes to load cargo into the resulting hybrid. **D** Engineered tags onto exosome biosynthetic machineries to load desired cargos into the exosomes in MVB. Genetically engineered tags can load a wide range of cargos depending on the tag. The resulting exosomes can be collected once MVB fuse with the cell membrane. Red triangles represent the cargos being loaded

in size in exosome membranes, allowing for the passive and diffusive loading of cargo smaller than this size [106]. The loading efficiency is influenced by the sample flow rate, suggesting that optimization of this parameter can enhance loading efficiency and streamline workflows for high-throughput cargo loading, with the primary limitation being the quantity of exosomes that can be prepared.

Cellular nanoporation

In addition to exosomes, nanoporation can be applied directly to the cells from which exosomes are derived. These cells are cultured above nanochannels in the biochip, allowing for cargo delivery that results in the production of exosomes containing the delivered cargos [110]. This technique demonstrates higher efficiency than conventional electroporation methods, as it eliminates intermediate steps between exosome sourcing and loading cargos. Compared to traditional electroporation and other loading strategies, the biochip device can yield up to 50-fold increase in exosome production and a 10^3-fold increase in exosomal mRNA transcripts [111]. Moreover, the platform is compatible with various cell types, allowing flexibility in the choice of sourcing cells. The technique has demonstrated success in preclinical mouse models, showcasing long biological half-lives, minimal cytotoxicity, and effective tumor targeting to inhibit growth and development. Cellular nanoporation techniques also achieve higher concentration of genetic cargo, such as mRNA and miRNA, inside exosomes, while allowing for the loading of large and intact mRNAs with loading efficiencies ranging from 2,000 to 10,000-fold higher than traditional methods [111]. Furthermore, research by You et al. (2023) demonstrated a tenfold increase in exosome production per cell using the cellular nanoporation biochip compared to conventional electroporation methods [112]. This enhancement in

exosome yield can lead to highest overall efficiency in loading endogenous intact mRNA from specific cells into exosomes without requiring modifications to the cells.

Cellular nanoporation utilizes nanochannels to gently create nanopores in the cell membrane with focused electric fields, facilitating accelerated delivery of genetic material into the cultured cells on the biochip [113]. The technique offers flexibility and compatibility with existing therapeutic workflows and administration routes, as demonstrated in preclinical animal models of acute lung injuries [114]. Another enhancement in cellular nanoporation technology is the Cellular Nanoporation and Exosome Assessment Device (CEAD) developed by Sheng et al. (2022) [109]. The CEAD platform has achieved capture efficiencies of 75%−90% and transfection efficiencies of 90−100% in the exosomes produced, which were loaded with specific cargo [109]. This demonstrates the potential of cellular nanoporation techniques for efficient cargo loading and assessment in exosomes.

The biochip nature of cellular nanoporation technology, akin to microfluidics, offers scalability in production. Coupled with the observed enhancement in total exosome release and mRNA transcript packaging, this indicates strong potential for future clinical translation. Despite its promising aspects, the system currently has a limited selection of compatible cargo. However, as the technology advances, it is likely that a broader range of cargo will become compatible, leading to significant improvements.

Fusogenic liposome-exosome hybrids

Artificial exosomes or fusogenic hybrids are created by combining liposomes with isolated exosomes, aiming to encapsulate cargo within the liposomes while incorporating the desirable properties of exosomes into the resulting hybrid structure (Fig. 3C). Piffoux et al. (2018) presented a method for fusing exosomes with liposomes to achieve improved control over the cargo loading, extended biological half-lives, enhanced penetration of biological barriers, reduced immunogenicity, and enhanced natural targeting capabilities similar to native exosomes [115]. This fusogenic hybrid model leverages the membrane fusion process mediated by chemicals such as PEG, which has been demonstrated to facilitate membrane fusion between cells, exosomes, and liposomes [116]. The liposomes used were composed of phosphatidylcholine and phosphatidylethanolamine, maintaining the lipid composition of exosomes post-fusion to preserve natural exosome properties [115]. Piffoux et al. showcased that for the anti-tumor drug mTHPC, the loading efficiency achieved with fusogenic hybrids was 90%, a substantial improvement compared to the 3% loading efficiency achieved with traditional exosome loading techniques [115].

Fusogenic hybrids have demonstrated promising applications in preclinical studies. When researchers combined liposomes loaded with anti-tumor drugs with exosomes, the fusogenic hybrids when administered resulted in a significant systemic anti-tumor response, improved immune infiltration of tumors, and reduced tumor recurrence in mouse models [117]. Lin et al. (2018) showed that a fusogenic hybrid could deliver CRISPR/Cas9 for in vivo gene manipulation, while Xie et al. (2024) used a hybrid with tissue-homing exosomes loaded with siRNA cargo for targeted delivery to specific tissues [118, 119]. One advantage of fusogenic hybrids is their partial reliance on liposome delivery, a well-established method in nanomedicine with over five decades of history and extensive clinical use [120]. This approach can overcome common liposome drawbacks, enhancing efficiency and safety by achieving exosome-like qualities such as low immunogenicity and superior pharmacokinetics.

The fusogenic hybrid technique can be optimized by adjusting various fusion conditions to achieve high efficiency. Mukherjee et al. (2021) demonstrated that delivering MCF-7 siRNA to breast cancer cells using fusogenic hybrids led to twofold and fourfold increases in efficiency compared to liposomes and exosomes, respectively [121]. Piffoux et al. (2018) showed that with optimization, up to 60% of the soluble cargo in liposomes could be transferred to the exosomes, leveraging the ease of loading cargos into liposomes [116]. Selecting the appropriate source of exosomes also contributes to optimization, as the fusogenic hybrids can retain many original properties of the exosomes, including targeting capabilities [119, 122]. Given that liposomes have been approved as nanocarriers for various FDA-approved drugs such as Onivyde, Marqibo, Doxil, the fusogenic hybrid technique could potentially be implemented rapidly, provided it demonstrates a similar safety profile [123]. Overall, while the fusogenic hybrid approach shows promise in enhancing cargo delivery and targeting capabilities, addressing challenges related to production efficiency and scalability will be critical for its widespread application in clinical settings [120]. However, concerns associated with their toxicity and safety profile persist due to their synthetic nature and may inherit challenges from liposomes, which necessitates more extensive testing compared to naturally sourced or endogenous exosomes. Additionally, the larger size of fusogenic hybrids could hinder their ability to cross certain biological barriers, limiting their effectiveness in targeting tissues.

Other exosome hybrids

While fusogenic exosome-liposome hybrids are widely studied, there is growing interest in combining exosomes with other nanomaterials to harness complementary advantages. Exosome-polymer hybrids, for instance, merge the natural biocompatibility and targeting capabilities of exosomes with the customizable physicochemical properties of synthetic polymers. These hybrids could be created by functionalizing exosome surfaces with biocompatible polymers using methods such as atom transfer radical polymerization or DNA-tethered block copolymer attachment [124]. This polymer coating significantly improves exosome stability during storage and in biological environments, protects against enzymatic degradation, and can extend blood circulation time up to fourfold compared to unmodified exosomes, all while maintaining their bioactivity and targeting abilities. Beyond polymers, exosomes can also form hybrids with metallic nanoparticles, such as iron oxide, to create theranostic platforms that combine imaging and targeted drug delivery functionalities. These hybrids were produced by incubating the parental cells with iron oxide nanoparticles, which were subsequently incorporated into the secreted exosomes [125]. These hybrids have been shown to induce ferrotopsis with immunotherapeutic effects against pathological angiogenesis, provide MRI contrast, avoid toxicity associated with bare metallic nanoparticles, and enhance tumor accumulation. Overall, these innovations highlight the unique potential of exosome hybrids to overcome the limitations of traditional delivery platforms, offering reduced immunogenicity, improved targeting, and multifunctionality.

Genetic engineered sorting

Researchers have leveraged their understanding of exosome loading mechanisms to develop techniques for selectively loading tagged or modified cargos into cells. By employing key components of the ESCRT pathways, proteins with exosome-sorting ability, and established exosomal markers, they have developed methods to enrich specific cargos within exosomes [126, 127]. This approach involves loading prior to exosome isolation, which minimizes loss or damage to exosomes. Unlike techniques such as electroporation, which can expose exosomes to freeze–thaw cycles and destabilizing temperatures, this method effectively preserves their structural integrity. Furthermore, the establishment of a stable cell line that can be propagated and expanded for scalability is often required, potentially enhancing loading efficiency and production output. However, due to the genetic engineering constraints, this method is only suitable for cargos that can be naturally produced within cells, thereby excluding small molecule drugs (Fig. 3D).

Small molecular tag

WW-Tag Based Sorting. Sterzenbach et al. (2017) demonstrated a novel approach known as WW-tag based sorting, where proteins tagged with a WW tag are selectively loaded into exosomes using an evolutionarily conserved L-domain pathway as a loading switch mechanism [128]. This process involves the interaction between an L-domain recognized by the WW-tag and a sorting switch protein, ensuing the specific loading of tagged protein into exosomes [128]. By genetically modifying cells to express the desired tag on proteins, researchers successfully achieved the selective binding of these proteins into exosomes. The resulting exosome were functional and the WW-tagged cargo exhibited functionality after injection into mouse models.

RNA Signal Motif. The differences between a cell's endogenous RNA profiles and those of its exosomes suggest the existence of active sorting mechanisms. Oka et al. (2023) identified specific RNA motifs, including one located in truncated 3' end of the RAB13 3' UTR, that play an active role in the sorting process [129]. By leveraging these RNA motifs, it becomes possible to selectively load RNAs bearing the specific tag into exosomes with high precision and efficiency [129].

Marker/scaffold protein tag

RUSH System. Zhang et al. (2023) utilized a technique called retention using selective hook (RUSH) to enable the specific loading of Cas9 proteins into exosomes for targeted modification of recipient cells [130]. Originally developed by Boncompain et al. (2012) as a means of synchronizing secretory protein traffic within cells, the RUSH system was adapted to tether proteins of interest indirectly to exosomal marker proteins, such as CD63, facilitating their selective loading into exosomes [130, 131].

EXPLOR System. The Exosome for Protein Loading via Optically Reversible protein–protein interactions (EXPLORs) platform is an optogenetically engineered method for efficiently and specifically sorting endogenous cargo into exosomes within cells before exosome isolation. This technique involves creating a reversible protein–protein interaction between the exosome marker CD9 and a cargo protein tagged with a photoreactive mCherry-CRY2 protein, enabling transient docking with the exosome biogenesis pathway [132]. The EXPLOR system has demonstrated success in loading therapeutic proteins and delivering them in various preclinical animal models for conditions such as alcohol-induced liver injury, acute kidney injury, and sepsis-associated organ damage [133–135]. Moreover, the EXPLOR method of loading and generating exosomes has achieved clinically relevant GMP-grade standards [136].

High Sorting Scaffold Protein. Zhang et al. (2023) explored variations in cargo sorting by tethering desired cargo to exosomal scaffold proteins such as TSPAN2 and TSPAN3 [130]. Lipidation of proteins has been shown to enhance their sorting into exosomes. Researchers demonstrated that by tagging cargo with protein sequences modified with lipid moieties, they could improve the sorting of fused cargo into exosomes for a set of structurally diverse transmembrane and peripheral membrane proteins [137].

EXOtic Devices. The EXOtic devices workflow involves designing specific exosome producer cells using synthetic biological approaches to enhance exosome production and improve endogenous mRNA delivery and loading. This precise method enables the selective loading of therapeutic mRNAs into exosomes at significantly higher concentrations compared to other similar techniques, and has demonstrated clinical relevance by successfully targeting Parkinson's disease in preclinical mouse models [138]. These genetically engineered exosomes allow for tagging or tethering specific cargo within the exosome biogenesis pathways, leading to greater control and efficient enrichment of desired cargo. This approach helps prevent misfolding or excessive degradation of recombinant proteins, which can occur with exogenously loaded cargo [132]. Moreover, it offers precise control over cargo selection, minimizing complications and processing after isolation. Since the cargo is already within the exosomes, it can be frozen immediately, reducing exposure to temperature fluctuations and re-concentration steps. This results in higher yields and better preserved functional and structural integrity. Additionally, because the genetic pathway is present in all cells, researchers can choose various cell types for exosome production.

Exosome pharmacokinetics & pharmacodynamics

Exosomes stand out from conventional nanocarriers due to their unique characteristics that provide enhanced targeting capabilities and the potential for modifications to redirect their biodistributions. Ongoing research focusing on exosome targeting and homing within naturally occurring EV pathways aims to identify key factors involved in homing, improved retention, and evasion of clearance pathways. These studies hold promise for future applications and re-engineering efforts to achieve desired specificity in exosome biodistribution and pharmacokinetics.

Biodistribution & retention

Exosomes, unlike liposomes and other non-exosome nanoparticles that are synthetic or chemical/metallic in nature, have a significantly lower risk of eliciting immune reactions. Additionally, exosomes can be sourced from safe and scalable sources like milk and plants [68]. Exosomes do not typically require extensive chemical (e.g. altering surface charges) or biological modifications (e.g. adding immune evading factors) to their surface to ensure non-toxicity [138, 139]. The biodistribution of exosomes in the body can vary between different cell types, but they generally have the ability to reach various parts of the body, crossing barriers that may be challenging or impossible for traditional nanoparticles. Exosomes can travel through the bloodstream, lymph fluids, and even cerebrospinal fluids [59, 140]. When administered systemically, exosomes mainly accumulate in the liver, lungs, kidneys, and spleen, with varying retention in tumors depending on the tumor type [59]. Intratumoral injection of exosomes may enhance their retention within the tumor tissues compared to other administration routes [141]. The biodistribution of exosomes remains relatively consistent even after processing and cargo loading, indicating the selection of exosome source can influence their biodistribution properties.

Exosomes in the body are mainly cleared through the liver by macrophages via the RES [142, 143]. Exosomes that are modified with PEGylation or expressing CD47 can significantly enhance their circulation and ability to target tumors [41–45, 144, 145]. Altering the lipid compositions of exosomes or exosome-mimetics can potentially hinder the rapid clearance by the RES, leading to longer circulation times and improved targeting of tissues. Interestingly, exosomes derived from bovine, murine, or porcine milk have demonstrated the ability to cross species-barrier and transport a range of cargo including miRNA and proteins. These exosomes can bypass traditional biodistribution and clearance pathways, accumulating in the brain as well as in the liver and spleen [68, 71] (Fig. 4).

Targeting

Some tissues' exosomes naturally exhibit homing abilities towards their own tissue type, even after being modified or loaded ex vivo. This characteristic can be exploited by researchers to isolate exosomes from specific tissues they wish to target [93]. Exosomes derived from immune cells are commonly used in both preclinical and clinical cancer research due to their inherent immune homing capabilities and targeting abilities. Dendritic cell-derived exosomes, as mentioned earlier, possess tumor homing capabilities and can serve as effective delivery vehicles for chemotherapeutic agents such as doxorubicin to target specific tumors [146]. Macrophage-derived exosomes have also demonstrated immunotropic and tumor homing capabilities under certain conditions. Wang et al. (2021) showed that through chemical modification, macrophage-derived exosomes could selectively accumulate

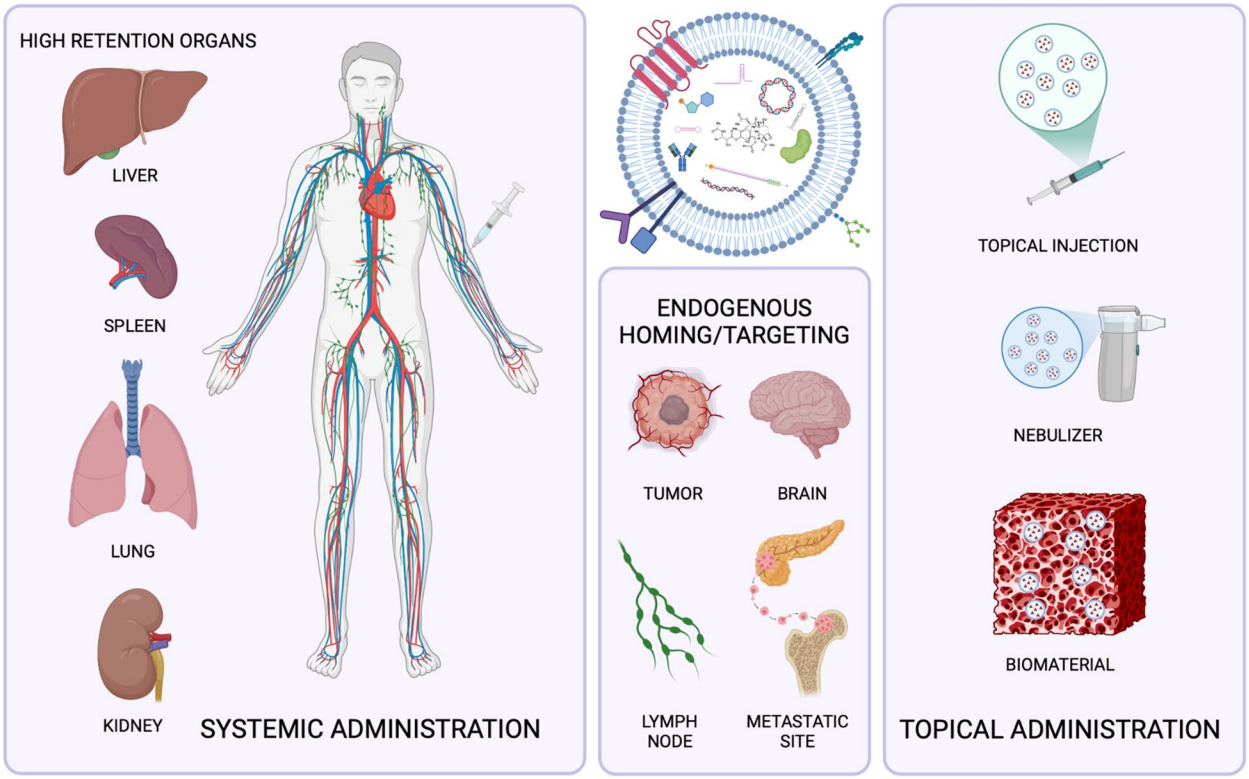

Fig. 4 Exosome Biodistribution & Targeting. Exosomes in the body through systemic administration will mainly be retained in the liver, spleen, lungs, and kidneys. Different sources of exosomes will demonstrate different endogenous homing, organotropism/tissue-targeting as well as preferential retention. In addition to topic injections, other topical administration techniques involve nebulizers to target the central nervous system and biomaterials implantation for longer and sustained release

in lymph nodes and tumors, thereby exerting anti-tumor activities [147] (Fig. 4).

Certain cancers produce organotropic exosomes that exhibit preferential tissue targeting in where the cancer metastasizes and forms secondary tumors. This organotropism is partially guided by surface integrins on the exosomes, and modifying these surface integrins can redirect the exosomes in different target tissues. For example, exosomal integrin β4 has been shown to possess lung tropism, when it was depleted from the exosomes, the modified exosomes demonstrated a threefold decrease in lung retention compared with control [40]. In addition, the specific cargo carried within tumor-derived exosomes can also influence their organotropic behavior. For instance, the cell migration-inducing and hyaluronan-binding protein (CEMIP) is elevated in tumor cells and tumor-derived exosomes, contributing to preferential metastasis to the brain. The uptake of exosomal CEMIP by brain endothelial and microglial cells promotes brain vascular remodeling and metastasis. Notably, depleting CEMIP from exosomes reduces brain metastasis without affecting primary tumor growth [138] (Fig. 4). To achieve more precise tissue or cell-specific targeting, surface modifications can be used.

Surface modifications

Exosomes, like traditional nanoparticles, can be surface-modified to enhance efficiency and stability. Classical techniques, such as attaching proteins, aptamers, and radioisotopes, aim to improve biodistribution and targeting. Among emerging strategies, glycosylation stands out as particularly promising. Exosomes, similar to cells, naturally exhibit surface glycosylation on their proteins and lipids, which can be engineered to enhance targeting capabilities, stability, and biodistribution.

Classical surface modifications of exosomes

Classical surface modifications enhance exosome functionality by improving their biodistribution, targeting, and labeling capabilities. General approaches involve engineering surface proteins, aptamers, or chemical/radioisotopes by means of genetic engineering, covalent or non-covalent interactions to achieve precise therapeutic outcomes.

Protein modifications Surface protein modification is the most common strategy, primarily achieved by genetic engineering. For example, CD47 is expressed on exosomes to inhibit macrophage phagocytosis by binding SIRPα receptors, extending circulation time and enhancing tumor accumulation [148]. Similarly, neuron-targeting RVG peptides, derived from rabies virus glycoprotein, enable exosomes to cross the BBB by interacting with nicotinic acetylcholine receptors on neurons and endothelial cells, facilitating siRNA delivery for neurodegenerative diseases like Alzheimer's [149]. EGFR-targeting GE11 peptides, which bind EGFR without activating mitogenic signaling, allow exosomes to deliver let-7a miRNA to breast cancer cells, significantly reducing tumor volume compared to unmodified exosomes [44].

Protein engineering enables precise control over exosome interactions with specific tissues. The iRGD peptide, a tumor-penetrating peptide targeting integrin αvβ3/β5 overexpressed in tumors, enhances exosome delivery to cancer cells. For instance, iRGD-modified exosomes loaded with KRAS siRNA suppressed tumor growth in murine models, demonstrating their potential for treating genetically driven cancers [45]. Exosomes can also be functionalized with antibodies via chemical reactions such as 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide/N-hydroxy succinimide (EDC/NHS) coupling, azide–alkyne cycloaddition (a canonical type of click chemistry), or thiol-maleimide addition [150]. Alternatively, Wiklander et al. developed a platform to produce antibody-displaying exosomes for targeted delivery. By transducing producer cells with a construct expressing an Fc-binding domain, these exosomes can be decorated with various antibodies or Fc-fused proteins, loaded with therapeutic cargo [151].

Aptamer modifications Aptamers, which are single-stranded DNA/RNA molecules, offer high specificity and low immunogenicity, making them ideal for exosome functionalization [88, 152]. For example, functionalizing doxorubicin-loaded exosomes with AS1411, a nucleolin-targeting aptamer, directs these exosomes to colorectal cancer cells in vivo, reducing off-target toxicity [153]. New techniques such as hydrophobic diacyllipid-tailed aptamers or RNA nanotechnology (e.g., cholesterol-anchored aptamers) further enable controlled ligand display on exosome membranes [154, 155].

Chemical/radioisotope modifications Radioisotope modifications enhance exosome tracking and localized therapy using advanced nuclear medicine techniques like SPECT and PET. Radionuclides can be incorporated directly into the exosome membrane or through stabilizing chelators. For SPECT imaging, radioiodines are ideal for long-term tracking, while Technetium-99 m ($^{99\ m}$Tc) and Indium-111 (^{111}In) are widely used due to their availability, low cost, and favorable properties despite a shorter half-life [156]. In PET imaging, Copper-64 (^{64}Cu) allows real-time biodistribution studies of exosomes. Recent research has shown the effective use of PEGylated exosomes with bifunctional chelators for ^{64}Cu radiolabeling [157]. Overall, radioisotope modifications significantly improve exosome tracking and therapeutic capabilities, though challenges radiostability and toxicity remain.

Glycan modifications Glycan modifications have emerged as a powerful functionalization strategy for exosomes, leveraging their naturally occurring surface glycans that play critical roles in cellular recognition and immune evasion. By engineering these glycans, researchers can introduce functional groups or ligands onto the exosome surface, enabling precise modifications without compromising exosome integrity. One key approach is enzymatic glycoengineering, where a sialyltransferase enzyme transfers chemically modified sialic acid residues—such as azide-tagged N-acetylneuraminic acid—onto glycans [158]. The azide groups introduced through this process serve as"molecular handles"for click chemistry, allowing for the attachment of various probes (like fluorophores), targeting ligands (such as transferrin), or therapeutic agents [159]. Microfluidic platforms could further enhance this process, allowing multi-step glycan modifications with improved efficiency compared to traditional methods [160, 161]. Additionally, glycan motifs or peptides can be fused to the extracellular domains of exosomal surface proteins, ensuring their presentation and facilitating specific interactions with target cells [159]. Bioorthogonal metabolic glycoengineering offers another approach, where parent cells are incubated with unnatural sugars, resulting in the endogenous addition of custom glycans that enhance exosome stability and delivery efficiency [162].

Glycan modifications also protect exosomal surface proteins from proteolytic degradation and can facilitate lysosomal escape, further improving delivery outcomes [163]. Interestingly, glycan removal of exosomes can redirect their biodistribution; for instance, O-deglycosylation has been shown to enhance exosome accumulation in distant organs like the lungs, likely by reducing nonspecific uptake by proximal tissues or macrophages [164]. Despite these advantages, challenges remain, such as achieving specificity in glycosylation patterns and avoiding unintended loss of function in certain surface ligands. As glycan biology advances, new targets and mechanisms

will likely be identified, paving the way for even more precise and effective exosome-based therapeutics.

Routes of delivery

The administration of therapeutic exosomes leverages their natural pharmacokinetics and biodistribution to enhance clinical outcomes. Exosomes can be suspended in buffer solutions and administered through various standard injection methods, with the route of administration significantly influencing their biodistribution. For instance, exosomes targeting the brain can be delivered intranasally via a non-invasive nebulizer, enabling their circulation to the brain while bypassing clearance pathways in the bloodstream [104, 165]. Similarly, intraperitoneal injections are ideal for targeting specific peritoneal organs, similar to the delivery of chemotherapy for ovarian cancer [166]. Importantly, studies have demonstrated that repeated administration and modification of exosomes do not increase immunogenicity or toxicity, making them suitable for long-term therapeutic applications [167]. In addition to direct injection methods, biomaterials such as 3D hydrogels offer innovative approaches for exosome delivery. These biomaterials are biodegradable, biocompatible, and non-toxic, making them ideal for sustained nanomedicine release. Hydrogels can be loaded with exosomes and other therapeutic cargo to provide controlled and localized delivery with extended biological half-lives and enhanced therapeutic effects [83, 146, 167]. By implanting hydrogels strategically or orthotopically, researchers can achieve slow and localized release of exosomes at optimal sites with minimal off-target effects, improving anti-tumor efficacy [168]. For example, Li et al. (2023) demonstrated success in targeting tumor-associated macrophages in ovarian cancer and triple-negative breast cancer models using hydrogels supplemented with modified exosomes [169]. Furthermore, advancements in bioink technology for bioprinting could enable the creation of customized products loaded with exosomes for tailored therapeutic applications [83]. While still relatively new in oncology, injectable biomaterials loaded with therapeutic exosomes have shown promise in regenerative medicine, such as near injury sites in the heart or spinal cord [167, 168]. By utilizing the natural targeting and homing properties of exosomes or engineering cells with known targeting capabilities, researchers can achieve greater control over delivery routes and therapeutic efficacy. These strategies hold potential for overcoming challenges related to off-target effects while maximizing the clinical impact of exosome-based nanomedicine delivery systems.

Current status of exosome in clinical translations

Numerous clinical trials are investigating exosomes, primarily as diagnostic and prognostic biomarkers in cancer and other conditions. While most focus on liquid biopsy and biomarker applications, a small but growing number are exploring their use as nanomedicine delivery vehicles (Table 3). Exosomes in these trials are produced in FDA-approved GMP-certified facilities to ensure safety and suitability for clinical testing [15, 170, 171]. They are classified into three types: unmodified, minimally modified, and engineered. Unmodified exosomes are directly isolated and used for their natural cargo and biodistribution. Minimally modified exosomes come from modified sources to enrich specific cargos, like tumor antigens, and can be combined with other agents for synergistic effects. Engineered exosomes undergo design optimization at every stage, including source selection, surface modifications, cargo loading, and administration routes, to achieve optimal therapeutic outcomes.

Exosomes in cancer immunotherapy

Dexosomes, which are exosomes from dendritic cells, have demonstrated considerable potential as cell-free anticancer vaccines in two phase I studies and one phase II study involving patients with advanced-stage cancers [172, 173]. These vaccines are manufactured from dendritic cells obtained from patients through a single leukapheresis procedure. These autologous dexosomes were minimally modified by loading with the MAGE tumor antigens via co-incubation. The manufacturing process follows GMP standards, with quality control measures that include verifying the expression of key tetraspanin proteins such as CD63, CD81, CD82 and the overexpression of HLA-DR (MHC class II) [174]. The successful loading of tumor-associated antigens onto dexosomes was confirmed by functional assays with antigen-specific T cell clones and by pulsing dexosomes with or without HLA-A2-positive dendritic cells. In the phase II trial, the production protocol was refined to enrich dexosome batches for tetraspanins and HLA-DR, without the acid elution of natural MHC I-bound epitopes used in phase I. This adjustment was based on in vitro evidence showing that high-affinity therapeutic peptides could compete with or replace endogenous epitopes on the dexosome surface, as demonstrated with MART1-specific cytotoxic T cell clones [175]. These clinical and manufacturing advances underscore the potential of dexosomes as scalable, precisely engineered cancer therapuetics.

In the non-small cell lung cancer (NSCLC) phase I trial, weekly administration of dexosomes to HLA-A2 + individuals resulted in systemic MAGE-specific immune reactivity in some cases, leading to modest T-cell activation and increased natural killer (NK) cell activity. Several

Table 3 Cancer interventional clinical trial involving exosomes as a delivery vehicle

Interventional clinical trial	Exosome formulation	Source of exosome	Cargo	Loading method	Isolation method	Route of administration	Timing/dosage	Cancer type	Phase	Status
Trial of a Vaccination With Tumor Antigen-loaded Dendritic Cell-derived Exosomes (CSET 1437) [NCT01159288]	MINIMALLY MODIFIED	DENDRITIC CELL	TUMOR ANTIGEN	CO-INCUBATION	ULTRACENTRIFUGATION	INTRADERMAL INJECTION	Induction phase of once a week injection for 4 weeks; Continuation phase of an injection every 2-weeks for 6-weeks	NON-SMALL CELL LUNG CANCER	II	COMPLETED
A phase I study of dexosome immunotherapy in patients with advanced non-small cell lung cancer [172]	MINIMALLY MODIFIED	DENDRITIC CELL	TUMOR ANTIGEN	CO-INCUBATION	ULTRACENTRIFUGATION	SUBCUTANEOUS & INTRADERMAL INJECTIONS	Weekly injection for 4-weeks	NON-SMALL CELL LUNG CANCER	I	COMPLETED
Phase I clinical trial of autologous ascites-derived exosomes combined with GM-CSF for colorectal cancer [176]	MINIMALLY MODIFIED	ASCITES	TUMOR ANTIGEN	ENDOGENOUS CARGO	ULTRACENTRIFUGATION	SUBCUTANEOUS INJECTION	Weekly injection with GM-CSF for 4-weeks	COLORECTAL CANCER	I	COMPLETED
Vaccination of metastatic melanoma patients with autologous dendritic cell (DC) derived-exosomes: results of the first phase I clinical trial [173]	MINIMALLY MODIFIED	DENDRITIC CELL	TUMOR ANTIGEN	CO-INCUBATION	ULTRACENTRIFUGATION	SUBCUTANEOUS & INTRADERMAL INJECTIONS	Weekly injection for 4-weeks	METASTATIC MELANOMA	I	COMPLETED
Study Investigating the Ability of Plant Exosomes to Deliver Curcumin to Normal and Colon Cancer Tissue [NCT01294072]	UNMODIFIED	PLANT EXTRACT	CURCUMIN & ENDOGENOUS CARGO	NOT APPLICABLE	NOT APPLICABLE	ORAL	Daily administration	COLORECTAL CANCER	NOT APPLICABLE	RECRUITING

Table 3 (continued)

Interventional clinical trial	Exosome formulation	Source of exosome	Cargo	Loading method	Isolation method	Route of administration	Timing/dosage	Cancer type	Phase	Status
Edible Plant Exosome Ability to Prevent Oral Mucositis Associated With Chemoradiation Treatment of Head and Neck Cancer [NCT01668849]	UNMODIFIED	PLANT EXTRACT	ENDOGENOUS CARGO	NOT APPLICABLE	NOT APPLICABLE	ORAL	Daily administration for 35 days	HEAD & NECK CANCER	I	COMPLETED
IExosomes in Treating Participants with Metastatic Pancreas Cancer with KrasG12D Mutation [NCT03608631]	ENGINEERED	MESENCHYMAL STEM CELL	ANTI-KRAS G12D siRNA	ELECTROPORATION	ULTRACENTRIFUGATION	INTRAVENOUS INJECTION	Injection over 15–20 minutes on days 1, 4, and 10 of trial, repeat for total of 4 courses of treatments	METASTATIC PANCREATIC DUCTAL ADENOCARCINOMA	I	ACTIVE, NOT RECRUITING
UCMSC-Exo for Chemotherapy-induced Myelosuppression in Acute Myeloid Leukemia [NCT06245746]	UNMODIFIED	UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELL	ENDOGENOUS CARGO	NOT APPLICABLE	UNKNOWN	INTRAVENOUS INJECTION	Single time infusion with 3 escalation presets	ACUTE MYELOID LEUKEMIA	I	RECRUITING

patients experienced disease stabilization, with some maintaining stability for over 12 months [176]. Similarly, the melanoma trial observed partial immune responses, although peripheral T-cell activation remained limited [173]. Dexosomes promoted NK cell proliferation and cytotoxicity through mechanisms involving NKG2D ligands and IL15Rα, consistent with preclinical models showing NK-mediated tumor control. Building on these results, the follow-up phase II NSCLC trial employed second-generation dexosomes derived from IFNγ-matured dendritic cells (DCs), which upregulated immunostimulatory markers such as MHC II and costimulatory molecules. These dexosomes were administered as maintenance therapy following chemotherapy, aiming to extend progression-free survival (PFS). Although the primary endpoint of 50% progression-free survival (PFS) at 4 months was not met, enhanced NKp30-mediated NK function (linked to dexosomal BAG6 expression) was associated with longer PFS in some patients. Remarkably, one patient achieved sufficient disease stabilization to qualify for tumor resection and adjuvant radiotherapy. However, T-cell responses were still lacking, even with the addition of cyclophosphamide to suppress regulatory T cells. These trials underscore the ability of dexosomes to activate innate immunity via NK cells while highlighting the difficulties in generating robust adaptive T-cell responses. The variations in NK activation pathways—NKG2D in phase I melanoma trials versus BAG6-NKp30 in phase II NSCLC—indicate context-dependent mechanisms. While dexosomes hold promise as cell-free vaccines, further optimization of antigen presentation and combination strategies is crucial to fully realize their therapeutic potential.

In another phase I trial involving colorectal cancer patients, unmodified exosomes were isolated from malignant ascites using sucrose/deuterium oxide gradient ultracentrifugation [176]. Their morphology and protein components were confirmed through electron microscopy and Western blot analysis. The study found that combination therapy using ascites-derived exosomes with granulocyte–macrophage colony-stimulating factor (GM-CSF) induced more robust anti-tumor immune responses than the exosomes alone, suggesting that this combination could be a feasible and safe alternative for immunotherapy in advanced colorectal cancer.

Unmodified stem cell and plant-derived exosomes

Patients with acute myeloid leukemia often face severe myelosuppression from chemotherapy, leading to complications like infections, bleeding, and impaired organ function. These complications can result in dose reductions and increased treatment-related mortality, highlighting the urgent need for early recovery strategies. Umbilical cord-derived mesenchymal stem cell (UCMSC) exosomes can promote the repair and regeneration of essential bone marrow cells, making them a promising option for recovery. In a phase I trial (NCT06245746), patients with acute myeloid leukemia undergoing consolidation chemotherapy are being recruited to receive intravenous infusions of these exosomes to evaluate their effectiveness in accelerating recovery from myelosuppression.

Plant-derived exosomes present a biocompatible alternative for drug delivery. Curcumin is an anti-inflammatory agent known to have poor bioavailability. In an exploratory trial (NCT01294072), curcumin-loaded fruit-derived exosomes will be tested for targeted delivery to colon cancer tissues. This clinical trial aims to characterize the effects of these exosomes on immune modulation, cellular metabolism, and phospholipid profiles in normal and malignant colon cells from patients undergoing surgery for newly diagnosed colon cancer.

Engineered exosomes for targeted therapy

Advances in exosome engineering have enabled precise therapeutic delivery. An ongoing phase I trial is investigating the intravenous delivery of siRNA-loaded MSC-derived exosomes targeting KRAS G12D mutations in metastatic PDAC patients who failed multiple lines of therapy (NCT03608631) [45]. Clinical GMP-grade exosomes were obtained from the culture supernatant of human bone marrow MSCs. The conditioned media from these cells, grown in a bioreactor, were processed through filtration and centrifugation [170]. The resulting exosomes were characterized using NTA and flow cytometry, followed by electroporation with siRNA. Results showed that these engineered exosomes were well-tolerated, with no reported dose-limiting toxicity, and some patients showed stable disease responses [30]. The maximum tolerated infusion was not reached, even at the highest dose, and downregulation of KRASG12D DNA along with increased infiltration of CD8+ T cells were observed in patient samples [30]. This trial highlights the potential of exosomes to deliver gene-editing tools with high specificity.

The above clinical trials investigating exosomes as delivery vehicles in cancer therapy represent a promising frontier in nanomedicine. With most studies currently in phase I, the encouraging results regarding safety, tolerability, and early efficacy are laying a strong foundation for future phase II trials. Advances in exosome engineering are enhancing their loading capacity, targeting precision, and production scalability, potentially leading to a broader range of clinical applications in cancer treatment.

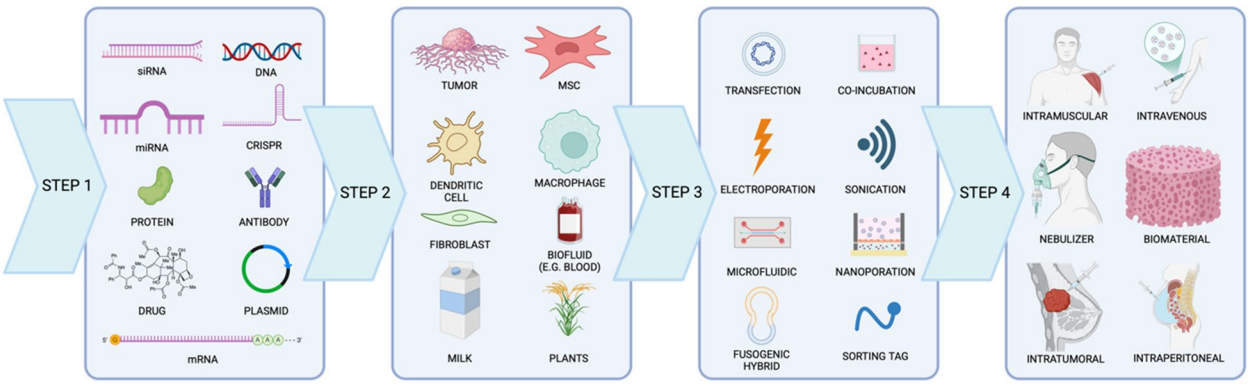

Fig. 5 Designing Exosome as Ideal Nanomedicine Delivery Vehicle. Step 1 involves selection of desired cargo ranging from nucleic acids to small molecule drugs. Step 2 involves the selection of exosome source to imbue the resulting exosomes with specific targeting/homing properties or surface modifications. Step 3 involves the loading technique which range from traditional approaches to the innovative new methods depending on the desired properties of the resulting nanomedicine-loaded exosomes. Step 4 involves the selection of administration route which depends on the anatomical location and the type of the tumor

Conclusion & future prospects

Nanomedicine for cancer treatment faces persistent challenges in delivery, particularly in achieving precise targeting, ensuring safety, and optimizing efficiency. While loading efficiency is generally sufficient with traditional nanoparticles, critical issues remain regarding safety and targeting. These include avoiding immune responses, retaining therapeutic agents in target tissues, crossing biological barriers, and facilitating internalization into target cells [31, 177, 178]. Such limitations have hindered the clinical efficacy of traditional nanoparticles. In contrast, exosomes, which are naturally occurring EVs, offer distinct advantages in addressing these challenges. Exosomes are gaining attention as promising nanomedicine delivery vehicles due to their favorable preclinical outcomes and advancements in exosome-related technologies. However, their development still faces challenges related to loading efficiency and scaling up production to meet clinical demand. Innovative interdisciplinary approaches have emerged to improve cargo loading for diverse therapeutic agents. These advancements are being validated in preclinical models. Another challenge is biodistribution and targeting specificity, which researchers are addressing through optimized cargo-loading methods and careful selection of exosome sources that leverage their natural homing properties [71, 83, 138]. While clinical trials involving exosomes as cancer nanomedicine delivery vehicles remain in early phases, promising preclinical results suggest that more interventional trials will be initiated in the near future.

In cancer immunotherapy, similar challenges arise with nanomedicine delivery systems offering improved safety and efficacy compared to traditional anti-tumor treatments. Exosomes are compatible with existing FDA-approved immunotherapies and can enhance their delivery to tumor tissues. For instance, engineered platelet-derived exosome hybrid liposomes have demonstrated enhanced breast cancer immunotherapy outcomes in preclinical models [117]. Similarly, mRNA immunotherapies targeting cancer have been successfully loaded into exosomes using electroporation techniques, resulting in improved tumor retention and infiltration while overcoming resistance mechanisms [179–181]. Furthermore, exosomes themselves are being developed as novel immunotherapeutic agents—such as dendritic cell-derived or tumor-derived exosome-based vaccines—to target tumors effectively [92, 182, 183].

The inherent variability among tumors and patients has driven the development of precision medicine strategies to optimize therapies based on specific molecular signatures. While traditional lipid and metallic nanoparticles have been utilized in precision nanomedicine development, exosomes offer superior adaptability for personalized therapies [184–186]. Their tunable nature allows precise modifications for targeting specific tumors through surface engineering or by serving as carriers for personalized nanomedicine [187–189]. These properties position exosomes as promising candidates for addressing the complexity of tumor heterogeneity while improving upon the capabilities of traditional nanoparticles. The future of cancer nanomedicine delivery using exosomes is envisioned to follow a structured approach encompassing four key steps: (1) selecting the therapeutic cargo (e.g., siRNA, mRNA, plasmid DNA, soluble drugs); (2) choosing the optimal source of exosomes based on factors such as tumor homing abilities or organotropic properties; (3) determining the best loading mechanism suited to the cargo type (e.g., fusogenic hybrids for chemical drugs

or cellular nanoporation for genetic materials); and (4) selecting the most effective administration route (e.g., intravenous injection or nebulizer delivery). Additional steps may include personalized modifications to optimize efficacy while minimizing toxicity. This systematic framework aims to refine research pipelines and accelerate clinical translation (Fig. 5). As ongoing research continues to uncover new insights into exosome biology and innovative techniques mature, the clinical translation of exosomes as nanomedicine delivery platforms is poised to revolutionize cancer treatment by addressing long-standing challenges in targeting, safety, and efficiency.

Abbreviations

BBB	Blood-brain barrier
CEAD	Cellular nanoporation and Exosome Assessment Device
CEMIP	Cell migration-inducing and hyaluronan-binding protein
CNT	Carbon nanotube
COVID-19	Coronavirus 19
CRISPR	Clustered regularly interspaced palindromic repeats
DNA	Deoxyribonucleic acid
EPR	Enhanced permeability and retention
ESCRT	Endosomal sorting complex required for transport
EV	Extracellular vesicle
EXPLOR	Exosome for Protein Loading via Optically Reversible protein–protein interactions
FDA	Food and Drug Administration
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GMP	Good manufacturing practice
GPI	Glycosylphosphatidylinositol
HUVEC	Human umbilical vein endothelial cell
iRGD	Internalizing RGD
ILV	Intraluminal vesicle
ISEV	International Society for Extracellular Vesicles
KRAS	Kirsten rat sarcoma virus
LNP	Lipid nanoparticle
MCF-7	Michigan Cancer Foundation-7
miRNA	MicroRNA
mRNA	Messenger RNA
MSC	Mesenchymal stem cell
MSN	Mesoporous silica nanoparticle
mTHPC	meta-tetra(hydroxyphenyl)chlorine
MVB	Multivesicular body
NSCLC	Non-small cell lung cancer
PDAC	Pancreatic ductal adenocarcinoma
PDC	Patient-derived cancer
PEG	Polyethylene glycol
PFS	Progression-free survival
PLGA	Poly(lactic-co-glycolic acid)
RES	Reticuloendothelial system
RNA	Ribonucleic acid
RNP	Ribonucleoprotein
ROS	Reactive oxygen species
RUSH	Retention using selective hook
siRNA	Small interfering RNA
TEM	Transmission electron microscopy
TSPAN2	Tetraspanin-2
TSPAN3	Tetraspanin-3
UCMSC	Umbilical cord mesenchymal stem cell
UTR	Untranslated region

Acknowledgements
Not applicable.

Authors' contributions
JJJL drafted the manuscript and prepared all figures and tables. DL reviewed the manuscript and provided comments. SKYT reviewed and edited the manuscript. ASTW reviewed the manuscript, provided supervision, and secured funding. All authors read and approved the final manuscript.

Funding
This work was supported by the Research Grant Council General Research Fund 17103523 and Senior Research Fellow Scheme SRFS2223-7S05 to A. S. T. Wong, and the funding support from "Laboratory for Synthetic Chemistry and Chemical Biology" under the Health@InnoHK Program launched by Innovation and Technology Commission, HKSAR.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 31 March 2025 Accepted: 28 May 2025
Published online: 07 June 2025

References

1. Hou X, Zaks T, Langer R, Dong Y. Lipid nanoparticles for mRNA delivery. Nat Rev Mater. 2021;6(12):1078–94.
2. Huang X, Kong N, Zhang X, Cao Y, Langer R, Tao W. The landscape of mRNA nanomedicine. Nat Med. 2022;11:2273–87.
3. Gjetting T, Arildsen NS, Christensen CL, Poulsen TT, Roth JA, Handlos VN, Poulsen HS. In vitro and in vivo effects of polyethylene glycol (PEG)-modified lipid in DOTAP/cholesterol-mediated gene transfection. Int J Nanomedicine. 2010;5:371–83.
4. Ibrahim M, Ramadan E, Elsadek NE, Emam SE, Shimizu T, Ando H, Ishima Y, Elgarhy OH, et al. Polyethylene glycol (PEG): The nature, immunogenicity, and role in the hypersensitivity of PEGylated products. J Control Release. 2022;351:215–30.
5. Knop K, Hoogenboom R, Fischer D, Schubert US. Poly(ethylene glycol) in drug delivery: pros and cons as well as potential alternatives. Angew Chem Int Ed Engl. 2010;49(36):6288–308.
6. Suzuki T, Suzuki Y, Hihara T, Kubara K, Kondo K, Hyodo K, Yamazaki K, Ishida T, et al. PEG shedding-rate-dependent blood clearance of PEGylated lipid nanoparticles in mice: Faster PEG shedding attenuates anti-PEG IgM production. Int J Pharm. 2020;588: 119792.
7. Lin C, Jans A, Wolters JC, Mohamed MR, Van der Vorst EPC, Trautwein C, Bartneck M. Targeting ligand independent tropism of siRNA-LNP by small molecules for directed therapy of liver or myeloid immune cells. Adv Healthc Mater. 2024;13(26): e2202670.
8. Zhu S, Tang Y, Lin C, Liu XY, Lin Y. Recent advances in patterning natural polymers: From nanofabrication techniques to applications. Small Methods. 2021;5(3): e2001060.
9. Ghezzi M, Pescina S, Padula C, Santi P, Del Favero E, Cantù L, Nicoli S. Polymeric micelles in drug delivery: An insight of the techniques for their characterization and assessment in biorelevant conditions. J Control Release. 2021;332:312–36.
10. Li T, Shi S, Goel S, Shen X, Xie X, Chen Z, Zhang H, Li S, et al. Recent advancements in mesoporous silica nanoparticles towards therapeutic applications for cancer. Acta Biomater. 2019;89:1–13.
11. Hajji S, Younes I, Ghorbel-Bellaaj O, Hajji R, Rinaudo M, Nasri M, Jellouli K. Structural differences between chitin and chitosan extracted from three different marine sources. Int J Biol Macromol. 2014;65:298–306.
12. Li J, Wang J, Chen Z. Emerging role of exosomes in cancer therapy: progress and challenges. Mol Cancer. 2025;24(1):13.
13. Mashouri L, Yousefi H, Aref AR, Ahadi AM, Molaei F, Alahari SK. Exosomes: composition, biogenesis, and mechanisms in cancer metastasis and drug resistance. Mol Cancer. 2019;18(1):75.

14. Liu M, Wang Y, Zhang Y, Hu D, Tang L, Zhou B, Yang L. Landscape of small nucleic acid therapeutics: moving from the bench to the clinic as next-generation medicines. Signal Transduct Target Ther. 2025;10(1):73.
15. Ahn SH, Ryu SW, Choi H, You S, Park J, Choi C. Manufacturing Therapeutic Exosomes: from Bench to Industry. Mol Cells. 2022;45(5):284–90.
16. Cai W, He D. Bone marrow mesenchymal stem cell-derived exosomes improve cancer drug delivery in human cell lines and a mouse osteosarcoma model. Front Oncol. 2024;14:1482087.
17. Tenchov R, Bird R, Curtze AE, Zhou Q. Lipid nanoparticles — From liposomes to mRNA vaccine delivery, a landscape of research diversity and advancement. ACS Nano. 2021;15(11):16982–7015.
18. Inglut CT, Sorrin AJ, Kuruppu T, Vig S, Cicalo J, Ahmad H, Huang HC. Immunological and toxicological considerations for the design of liposomes. Nanomaterials (Basel). 2020;10(2):190.
19. Johnston HJ, Hutchison G, Christensen FM, Peters S, Hankin S, Stone V. A review of the in vivo and in vitro toxicity of silver and gold particulates: particle attributes and biological mechanisms responsible for the observed toxicity. Crit Rev Toxicol. 2010;40(4):328–46.
20. Petrarca C, Clemente E, Amato V, Pedata P, Sabbioni E, Bernardini G, Iavicoli I, Cortese S, et al. Engineered metal based nanoparticles and innate immunity. Clin Mol Allergy. 2015;13(1):13.
21. Norouzi M, Yathindranath V, Thliveris JA, Kopec BM, Siahaan TJ, Miller DW. Doxorubicin-loaded iron oxide nanoparticles for glioblastoma therapy: a combinational approach for enhanced delivery of nanoparticles. Sci Rep. 2020;10(1):11292.
22. Mohammadpour R, Yazdimamaghani M, Cheney DL, Jedrzkiewicz J, Ghandehari H. Subchronic toxicity of silica nanoparticles as a function of size and porosity. J Control Release. 2019;304:216–32.
23. Kobayashi N, Izumi H, Morimoto Y. Review of toxicity studies of carbon nanotubes. J Occup Health. 2017;59(5):394–407.
24. Meng J, Yang M, Jia F, Kong H, Zhang W, Wang C, Xing J, Xie S, Xu H. Subcutaneous injection of water-soluble multi-walled carbon nanotubes in tumor-bearing mice boosts the host immune activity. Nanotechnology. 2010;21(14): 145104.
25. Morimoto Y, Horie M, Kobayashi N, Shinohara N, Shimada M. Inhalation toxicity assessment of carbon-based nanoparticles. Acc Chem Res. 2013;46(3):770–81.
26. Abulikemu A, Zhao X, Xu H, Li Y, Ma R, Yao Q, Wang J, Sun Z, et al. Silica nanoparticles aggravated the metabolic associated fatty liver disease through disturbed amino acid and lipid metabolisms-mediated oxidative stress. Redox Biol. 2023;59: 102569.
27. Jeppesen DK, Fenix AM, Franklin JL, Higginbotham JN, Zhang Q, Zimmerman LJ, Liebler DC, Ping J, et al. Reassessment of exosome composition. Cell. 2019;177(2):428-445.e18.
28. Prunevieille A, Babiker-Mohamed MH, Aslami C, Gonzalez-Nolasco B, Mooney N, Benichou G. T cell antigenicity and immunogenicity of allogeneic exosomes. Am J Transplant. 2021;21(7):2583–9.
29. Kou M, Huang L, Yang J, Chiang Z, Chen S, Liu J, Guo L, Zhang X, et al. Mesenchymal stem cell-derived extracellular vesicles for immunomodulation and regeneration: a next generation therapeutic tool? Cell Death Dis. 2022;13(7):580.
30. LeBleu VS, Smaglo BG, Mahadevan KK, Kirtley ML, McAndrews KM, Mendt M, Yang S, Maldonado AS, et al. KRAS G12D-specific targeting with engineered exosomes reprograms the immune microenvironment to enable efficacy of immune checkpoint therapy in PDAC patients. Preprint. medRxiv. 2025;2025.03.03.25322827. Published 2025 Mar 6. https://doi.org/10.1101/2025.03.03.25322827.
31. Johnsen KB, Gudbergsson JM, Skov MN, Pilgaard L, Moos T, Duroux M. A comprehensive overview of exosomes as drug delivery vehicles - endogenous nanocarriers for targeted cancer therapy. Biochim Biophys Acta. 2014;1846(1):75–87.
32. Wilhelm S, Tavares A, Dai Q, et al. Analysis of nanoparticle delivery to tumours. Nat Rev Mater. 2016;1(5):16014.
33. Danhier F. To exploit the tumor microenvironment: Since the EPR effect fails in the clinic, what is the future of nanomedicine? J Control Release. 2016;244(Pt A):108–21.
34. Richards DA, Maruani A, Chudasama V. Antibody fragments as nanoparticle targeting ligands: a step in the right direction. Chem Sci. 2017;8(1):63–77.
35. Mamaeva V, Rosenholm JM, Bate-Eya LT, Bergman L, Peuhu E, Duchanoy A, Fortelius LE, Landor S, et al. Mesoporous silica nanoparticles as drug delivery systems for targeted inhibition of Notch signaling in cancer. Mol Ther. 2011;19(8):1538–46.
36. Miao L, Newby JM, Lin CM, Zhang L, Xu F, Kim WY, Forest MG, Lai SK, et al. The Binding Site Barrier Elicited by Tumor-associated fibroblasts interferes disposition of nanoparticles in stroma-vessel type tumors. ACS Nano. 2016;10(10):9243–58.
37. Hennig R, Pollinger K, Veser A, Breunig M, Goepferich A. Nanoparticle multivalency counterbalances the ligand affinity loss upon PEGylation. J Control Release. 2014;194:20–7.
38. Fidan Y, Muçaj S, Timur SS, Gürsoy RN. Recent advances in liposome-based targeted cancer therapy. J Liposome Res. 2024;34(2):316–34.
39. Escamilla-Rivera V, Solorio-Rodríguez A, Uribe-Ramírez M, Lozano O, Lucas S, Chagolla-López A, Winkler R, De Vizcaya-Ruiz A. Plasma protein adsorption on Fe_3O_4-PEG nanoparticles activates the complement system and induces an inflammatory response. Int J Nanomedicine. 2019;14:2055–67.
40. Hoshino A, Costa-Silva B, Shen TL, Rodrigues G, Hashimoto A, Tesic Mark M, Molina H, Kohsaka S, et al. Tumour exosome integrins determine organotropic metastasis. Nature. 2015;527(7578):329–35.
41. Suk JS, Xu Q, Kim N, Hanes J, Ensign LM. PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. Adv Drug Deliv Rev. 2016;99(Pt A):28–51.
42. Ishida T, Harada M, Wang XY, Ichihara M, Irimura K, Kiwada H. Accelerated blood clearance of PEGylated liposomes following preceding liposome injection: effects of lipid dose and PEG surface-density and chain length of the first-dose liposomes. J Control Release. 2005;105(3):305–17.
43. 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.
44. Ohno S, Takanashi M, Sudo K, Ueda S, Ishikawa A, Matsuyama N, Fujita K, Mizutani T, et al. Systemically injected exosomes targeted to EGFR deliver antitumor microRNA to breast cancer cells. Mol Ther. 2013;21(1):185–91.
45. Kamerkar S, LeBleu VS, Sugimoto H, Yang S, Ruivo CF, Melo SA, Lee JJ, Kalluri R. Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer. Nature. 2017;546(7659):498–503.
46. Briuglia ML, Rotella C, McFarlane A, Lamprou DA. Influence of cholesterol on liposome stability and on in vitro drug release. Drug Deliv Transl Res. 2015;5(3):231–42.
47. Kuttner C, Mayer M, Dulle M, Moscoso A, López-Romero JM, Förster S, Fery A, Pérez-Juste J, Contreras-Cáceres R. Seeded growth synthesis of gold nanotriangles: Size control, SAXS analysis, and SERS performance. ACS Appl Mater Interfaces. 2018;10(13):11152–63.
48. Wu Y, Zhu K, Zhang X, Du W, Song J, Yang H. Emerging plasmonic nanoparticles and their assemblies for cancer radiotherapy. Adv Drug Deliv Rev. 2023;194: 114710.
49. Zhu Y, Bai Y, He J, Qiu X. Advances in the stimuli-responsive mesoporous silica nanoparticles as drug delivery system nanotechnology for controlled release and cancer therapy. 3 Biotech. 2023;13(8):274.
50. Popowski KD, Moatti A, Scull G, Silkstone D, Lutz H, López de Juan Abad B, George A, Belcher E, et al. Inhalable dry powder mRNA vaccines based on extracellular vesicles. Matter. 2022;5(9):2960–2974.
51. Wang D, Bai Y, Cheng G, Shen S, Xiao G, Ma D, Zhao G, Chen W, et al. Exosome-drug conjugates delivery: a promising strategy for ameliorating the pharmacokinetic profile of artesunate. Front Bioeng Biotechnol. 2024;12:1437787.
52. Semple SC, Akinc A, Chen J, Sandhu AP, Mui BL, Cho CK, Sah DW, Stebbing D, et al. Rational design of cationic lipids for siRNA delivery. Nat Biotechnol. 2010;28(2):172–6.
53. Poland CA, Duffin R, Kinloch I, Maynard A, Wallace WA, Seaton A, Stone V, Brown S, et al. Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. Nat Nanotechnol. 2008;3(7):423–8.
54. Nabitabar M, Shaterian M, Danafar H, Enhessari M. Multi-wall carbon Nanotube surface-based functional nanoparticles for stimuli-responsive dual pharmaceutical compound delivery. Sci Rep. 2024;14(1):12073.
55. Cui J, Wang X, Li J, Zhu A, Du Y, Zeng W, Guo Y, Di L, Wang R. Immune exosomes loading selfassembled nanomicelles traverse the blood-brain

barrier for chemo-immunotherapy against glioblastoma. ACS Nano. 2023;17(2):1464–84.
56. Alhasan AH, Patel PC, Choi CH, Mirkin CA. Exosome encased spherical nucleic acid gold nanoparticle conjugates as potent microRNA regulation agents. Small. 2014;10(1):186–92.
57. Chan MH, Chang ZX, Huang CF, Lee LJ, Liu RS, Hsiao M. Integrated therapy platform of exosomal system: hybrid inorganic/organic nanoparticles with exosomes for cancer treatment. Nanoscale Horiz. 2022;7(4):352–67.
58. Bobo D, Robinson KJ, Islam J, Thurecht KJ, Corrie SR. Nanoparticle-based medicines: A review of FDA-approved materials and clinical trials to date. Pharm Res. 2016;33(10):2373–87.
59. Welsh JA, Goberdhan DCI, O'Driscoll L, Buzas EI, Blenkiron C, Bussolati B, Cai H, Di Vizio D, et al. Minimal information for studies of extracellular vesicles (MISEV2023): From basic to advanced approaches. J Extracell Vesicles. 2024;(2):e12404. [Erratum in: J Extracell Vesicles. 2024;(5):e12451].
60. Lee YJ, Shin KJ, Jang HJ, Ryu JS, Lee CY, Yoon JH, Seo JK, Park S, et al. GPR143 controls ESCRT-dependent exosome biogenesis and promotes cancer metastasis. Dev Cell. 2023;58(4):320-334.e8.
61. Han QF, Li WJ, Hu KS, Gao J, Zhai WL, Yang JH, Zhang SJ. Exosome biogenesis: machinery, regulation, and therapeutic implications in cancer. Mol Cancer. 2022;21(1):207.
62. Pascucci L, Coccè V, Bonomi A, Ami D, Ceccarelli P, Ciusani E, Viganò L, Locatelli A, et al. Paclitaxel is incorporated by mesenchymal stromal cells and released in exosomes that inhibit in vitro tumor growth: a new approach for drug delivery. J Control Release. 2014;192:262–70.
63. Rosenberger L, Ezquer M, Lillo-Vera F, Pedraza PL, Ortúzar MI, González PL, Figueroa-Valdés AI, Cuenca J, et al, Alcayaga-Miranda F. Stem cell exosomes inhibit angiogenesis and tumor growth of oral squamous cell carcinoma. Sci Rep. 2019;9(1):663.
64. Yoon J, Lee SK, Park A, Lee J, Jung I, Song KB, Choi EJ, Kim S, et al. Exosome from IFN-γ-primed induced pluripotent stem cell-derived mesenchymal stem cells improved skin inflammation and barrier function. Int J Mol Sci. 2023;24(14):11635.
65. Ono M, Kosaka N, Tominaga N, Yoshioka Y, Takeshita F, Takahashi RU, Yoshida M, Tsuda H, et al. Exosomes from bone marrow mesenchymal stem cells contain a microRNA that promotes dormancy in metastatic breast cancer cells. Sci Signal. 2014;7(332):ra63.
66. Zuo B, Zhang Y, Zhao K, Wu L, Qi H, Yang R, Gao X, Geng M, et al. Universal immunotherapeutic strategy for hepatocellular carcinoma with exosome vaccines that engage adaptive and innate immune responses. J Hematol Oncol. 2022;15(1):46.
67. Hyung S, Ko J, Heo YJ, Blum SM, Kim ST, Park SH, Park JO, Kang WK, et al. Patient-derived exosomes facilitate therapeutic targeting of oncogenic MET in advanced gastric cancer. Sci Adv. 2023;9(47):eadk1098.
68. Agrawal AK, Aqil F, Jeyabalan J, Spencer WA, Beck J, Gachuki BW, Alhakeem SS, Oben K, et al. Milk-derived exosomes for oral delivery of paclitaxel. Nanomedicine. 2017;13(5):1627–36.
69. Dad HA, Gu TW, Zhu AQ, Huang LQ, Peng LH. Plant exosome-like nanovesicles: Emerging therapeutics and drug delivery nanoplatforms. Mol Ther. 2021;29(1):13–31.
70. Kim J, Li S, Zhang S, Wang J. Plant-derived exosome-like nanoparticles and their therapeutic activities. Asian J Pharm Sci. 2022;17(1):53–69.
71. Manca S, Upadhyaya B, Mutai E, Desaulniers AT, Cederberg RA, White BR, Zempleni J. Milk exosomes are bioavailable and distinct microRNA cargos have unique tissue distribution patterns. Sci Rep. 2018;8(1):11321.
72. Wu S, Zhao Y, Zhang Z, Zuo C, Wu H, Liu Y. The advances and applications of characterization technique for exosomes: From dynamic light scattering to super-resolution imaging technology. Photonics. 2024;11(2):101.
73. Kobayashi H, Shiba T, Yoshida T, Bolidong D, Kato K, Sato Y, Mochizuki M, Seto T, et al. Precise analysis of single small extracellular vesicles using flow cytometry. Sci Rep. 2024;14(1):7465.
74. Midekessa G, Godakumara K, Ord J, Viil J, Lättekivi F, Dissanayake K, Kopanchuk S, Rinken A, et al. Zeta potential of extracellular vesicles: Toward understanding the attributes that determine colloidal stability. ACS Omega. 2020;5(27):16701–10.
75. Théry C, Amigorena S, Raposo G, Clayton A. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. Curr Protoc Cell Biol. 2006;30(1):3.22.1–3.22.29. https://doi.org/10.1002/0471143030.cb0322s30.
76. Livshits MA, Khomyakova E, Evtushenko EG, Lazarev VN, Kulemin NA, Semina SE, Generozov EV, Govorun VM. Isolation of exosomes by differential centrifugation: Theoretical analysis of a commonly used protocol. Sci Rep. 2015;5:17319.
77. Li P, Kaslan M, Lee SH, Yao J, Gao Z. Progress in exosome isolation techniques. Theranostics. 2017;7(3):789–804.
78. Lobb RJ, Becker M, Wen SW, Wong CS, Wiegmans AP, Leimgruber A, Möller A. Optimized exosome isolation protocol for cell culture supernatant and human plasma. J Extracell Vesicles. 2015;4:27031.
79. Paolini L, Zendrini A, Di Noto G, Busatto S, Lottini E, Radeghieri A, Dossi A, Caneschi A, et al. Residual matrix from different separation techniques impacts exosome biological activity. Sci Rep. 2016;6:23550.
80. Gámez-Valero A, Monguió-Tortajada M, Carreras-Planella L, Franquesa MI, Beyer K, Borràs FE. Size-Exclusion Chromatography-based isolation minimally alters Extracellular Vesicles' characteristics compared to precipitating agents. Sci Rep. 2016;6:33641.
81. Busatto S, Vilanilam G, Ticer T, Lin WL, Dickson DW, Shapiro S, Bergese P, Wolfram J. Tangential flow filtration for highly efficient concentration of extracellular vesicles from large volumes of fluid. Cells. 2018;7(12):273.
82. Kim JY, Rhim WK, Yoo YI, Kim DS, Ko KW, Heo Y, Park CG, Han DK. Defined MSC exosome with high yield and purity to improve regenerative activity. J Tissue Eng. 2021;12:20417314211008624.
83. Amondarain M, Gallego I, Puras G, Saenz-Del-Burgo L, Luzzani C, Pedraz JL. The role of microfluidics and 3D-bioprinting in the future of exosome therapy. Trends Biotechnol. 2023;41(11):1343–59.
84. Hassanpour Tamrin S, Sanati Nezhad A, Sen A. Label-free isolation of exosomes using microfluidic technologies. ACS Nano. 2021;15(11):17047–79.
85. Kooijmans SAA, Stremersch S, Braeckmans K, de Smedt SC, Hendrix A, Wood MJA, Schiffelers RM, Raemdonck K, et al. Electroporation-induced siRNA precipitation obscures the efficiency of siRNA loading into extracellular vesicles. J Control Release. 2013;172(1):229–38.
86. Lin S, Zhou S, Yuan T. The, "sugar-coated bullets" of cancer: Tumor-derived exosome surface glycosylation from basic knowledge to applications. Clin Transl Med. 2020;10(6): e204.
87. Shimoda A, Sawada SI, Sasaki Y, Akiyoshi K. Exosome surface glycans reflect osteogenic differentiation of mesenchymal stem cells: Profiling by an evanescent field fluorescence-assisted lectin array system. Sci Rep. 2019 8;9(1):11497.
88. Han Q, Xie QR, Li F, et al. Targeted inhibition of SIRT6 via engineered exosomes impairs tumorigenesis and metastasis in prostate cancer. Theranostics. 2021;11(13):6526–41.
89. Chu L, Sun Y, Zhao Y, Wang A, Sun Y, Duan X, Li N, Xia H, et al. Exosome-mediated delivery platform of biomacromolecules into the brain: Cetuximab in combination with doxorubicin for glioblastoma therapy. Int J Pharm. 2024;660: 124262.
90. Bellavia D, Raimondo S, Calabrese G, Forte S, Cristaldi M, Patinella A, Memeo L, Manno M, Raccosta S, Diana P, Cirrincione G, Giavaresi G, Monteleone F, Fontana S, De Leo G, Alessandro R. Interleukin 3- receptor targeted exosomes inhibit *in vitro* and *in vivo* chronic myelogenous leukemia cell growth. Theranostics. 2017;7(5):1333–45.
91. Zhang H, Wang S, Sun M, et al. Exosomes as smart drug delivery vehicles for cancer immunotherapy. Front Immunol. 2023;13:1093607.
92. Li J, Li J, Peng Y, Du Y, Yang Z, Qi X. Dendritic cell derived exosomes loaded neoantigens for personalized cancer immunotherapies. J Control Release. 2023;353:423–33.
93. Haraszti RA, Miller R, Didiot MC, Biscans A, Alterman JF, Hassler MR, Roux L, Echeverria D, et al. Optimized cholesterol-siRNA chemistry improves productive loading onto extracellular vesicles. Mol Ther. 2018;26(8):1973–82.
94. Kooijmans SA, Aleza CG, Roffler SR, van Solinge WW, Vader P, Schiffelers RM. Display of GPI-anchored anti-EGFR nanobodies on extracellular vesicles promotes tumour cell targeting. J Extracell Vesicles. 2016;5:31053.
95. Kim MS, Haney MJ, Zhao Y, Mahajan V, Deygen I, Klyachko NL, Inskoe E, Piroyan A, et al. Development of exosome-encapsulated paclitaxel to overcome MDR in cancer cells. Nanomedicine. 2016;12(3):655–64.
96. Wan T, Zhong J, Pan Q, Zhou T, Ping Y, Liu X. Exosome-mediated delivery of Cas9 ribonucleoprotein complexes for tissue-specific gene therapy of liver diseases. Sci Adv. 2022;8(37):eabp9435.

97. Saini S, Sreekumar A, Nathani S, Asante DM, Simmons MN. A novel exosome based therapeutic intervention against neuroendocrine prostate cancer. Sci Rep. 2024;14(1):2816.
98. Du J, Wan Z, Wang C, Lu F, Wei M, Wang D, Hao Q. Designer exosomes for targeted and efficient ferroptosis induction in cancer via chemo-photodynamic therapy. Theranostics. 2021;11(17):8185–96.
99. Haney MJ, Klyachko NL, Zhao Y, Gupta R, Plotnikova EG, He Z, Patel T, Piroyan A, et al. Exosomes as drug delivery vehicles for Parkinson's disease therapy. J Control Release. 2015;207:18–30. [Erratum in: J Control Release. 2021;339:232–234].
100. Li Z, Liu C, Cheng Y, Li Y, Deng J, Bai L, Qin L, Mei H, et al. Cascaded microfluidic circuits for pulsatile filtration of extracellular vesicles from whole blood for early cancer diagnosis. Sci Adv. 2023;9(16):eade2819.
101. Naquin TD, Canning AJ, Gu Y, Chen J, Naquin CM, Xia J, Lu B, Yang S, et al. Acoustic separation and concentration of exosomes for nucleotide detection: ASCENDx. Sci Adv. 2024;10(10):eadm8597.
102. Jo W, Jeong D, Kim J, Cho S, Jang SC, Han C, Kang JY, Gho YS, et al. Microfluidic fabrication of cell-derived nanovesicles as endogenous RNA carriers. Lab Chip. 2014;14(7):1261–9.
103. Wang J, Ma X, Wu Z, Cui B, Zou C, Zhang P, Yao S. Microfluidics-prepared ultra-small biomimetic nanovesicles for brain tumor targeting. Adv Healthc Mater. 2024;13(5): e2302302.
104. Wang K, Kumar US, Sadeghipour N, Massoud TF, Paulmurugan R. A microfluidics-based scalable approach to generate extracellular vesicles with enhanced therapeutic microRNA loading for intranasal delivery to mouse glioblastomas. ACS Nano. 2021;15(11):18327–46.
105. Liu C, Zhang W, Li Y, Chang J, Tian F, Zhao F, Ma Y, Sun J. Microfluidic sonication to assemble exosome membrane-coated nanoparticles for immune evasion-mediated targeting. Nano Lett. 2019;19(11):7836–44.
106. Hao R, Yu Z, Du J, Hu S, Yuan C, Guo H, Zhang Y, Yang H. A high-throughput nanofluidic device for exosome nanoporation to develop cargo delivery vehicles. Small. 2021;17(35): e2102150.
107. Dalmay C, Villemejane J, Joubert V, Silve A, Arnaud-Cormos D, Français O, Mir LM, Leveque P, et al. A microfluidic biochip for the nanoporation of living cells. Biosens Bioelectron. 2011;26(12):4649–55.
108. Hosseini II, Liu Z, Capaldi X, AbdelFatah T, Montermini L, Rak J, Reisner W, Mahshid S. Nanofluidics for simultaneous size and charge profiling of extracellular vesicles. Nano Lett. 2021;21(12):4895–902.
109. Sheng Y, Huang Z, Zhang T, Qian F, Zhu Y, Dong Z, Zhang Q, Lei Q, et al. Living Cell Nanoporation and Exosomal RNA analysis platform for real-time assessment of cellular therapies. J Am Chem Soc. 2022;144(21):9443–50.
110. Boukany PE, Morss A, Liao WC, Henslee B, Jung H, Zhang X, Yu B, Wang X, et al. Nanochannel electroporation delivers precise amounts of biomolecules into living cells. Nat Nanotechnol. 2011;6(11):747–54.
111. Yang Z, Shi J, Xie J, Wang Y, Sun J, Liu T, Zhao Y, Zhao X, et al. Large-scale generation of functional mRNA-encapsulating exosomes via cellular nanoporation. Nat Biomed Eng. 2020;4(1):69–83. [Erratum in: Nat Biomed Eng. 2021;5(8):944–945].
112. You Y, Tian Y, Yang Z, Shi J, Kwak KJ, Tong Y, Estania AP, Cao J, et al. Intradermally delivered mRNA-encapsulating extracellular vesicles for collagen-replacement therapy. Nat Biomed Eng. 2023;7(7):887–900.
113. Gallego-Perez D, Pal D, Ghatak S, Malkoc V, Higuita-Castro N, Gnyawali S, Chang L, Liao WC, et al. Topical tissue nano-transfection mediates non-viral stroma reprogramming and rescue. Nat Nanotechnol. 2017;12(10):974–9.
114. Chen SY, Chen YL, Li PC, Cheng TS, Chu YS, Shen YS, Chen HT, Tsai WN, et al. Engineered extracellular vesicles carrying let-7a-5p for alleviating inflammation in acute lung injury. J Biomed Sci. 2024;31(1):30.
115. Lentz BR, Lee JK. Poly(ethylene glycol) (PEG)-mediated fusion between pure lipid bilayers: a mechanism in common with viral fusion and secretory vesicle release? Mol Membr Biol. 1999;16(4):279–96.
116. Piffoux M, Silva AKA, Wilhelm C, Gazeau F, Tareste D. Modification of extracellular vesicles by fusion with liposomes for the design of personalized biogenic drug delivery systems. ACS Nano. 2018;12(7):6830–42.
117. Ning S, Zhang X, Suo M, Lyu M, Pan Y, Jiang Y, et al. Platelet-derived exosomes hybrid liposomes facilitate uninterrupted singlet oxygen generation to enhance breast cancer immunotherapy. Cell Reports Physical Science. 2023;4(7): 101505.
118. Lin Y, Wu J, Gu W, Huang Y, Tong Z, Huang L, Tan J. Exosome-liposome hybrid nanoparticles deliver CRISPR/Cas9 system in MSCs. Adv Sci (Weinh). 2018;5(4):1700611.
119. Xie M, Wu Y, Zhang Y, Lu R, Zhai Z, Huang Y, Wang F, Xin C, et al. Membrane fusion-mediated loading of therapeutic siRNA into exosome for tissue-specific application. Adv Mater. 2024;36(33): e2403935.
120. Mukherjee A, Bisht B, Dutta S, Paul MK. Current advances in the use of exosomes, liposomes, and bioengineered hybrid nanovesicles in cancer detection and therapy. Acta Pharmacol Sin. 2022;43(11):2759–76.
121. Mukherjee D, Paul D, Sarker S, Hasan MN, Ghosh R, Prasad SE, Vemula PK, Das R, et al. Polyethylene glycol-mediated fusion of extracellular vesicles with cationic liposomes for the design of hybrid delivery systems. ACS Appl Bio Mater. 2021;4(12):8259–66.
122. Evers MJW, van de Wakker SI, de Groot EM, de Jong OG, Gitz-François JJJ, Seinen CS, Sluijter JPG, Schiffelers RM, et al. Functional siRNA delivery by extracellular vesicle-liposome hybrid nanoparticles. Adv Healthc Mater. 2022;11(5): e2101202.
123. Zhang P, Xiao Y, Sun X, Lin X, Koo S, Yaremenko AV, Qin D, Kong N, Farokhzad OC, Tao W. Cancer nanomedicine toward clinical translation: Obstacles, opportunities, and future prospects. Med. 2023;4(3):147–67.
124. Lathwal S, Yerneni SS, Boye S, Muza UL, Takahashi S, Sugimoto N, Lederer A, Das SR, et al. Engineering exosome polymer hybrids by atom transfer radical polymerization. Proc Natl Acad Sci U S A. 2021;118(2):e2020241118.
125. Zhang H, Mao Y, Nie Z, Li Q, Wang M, Cai C, Hao W, Shen X, et al. Iron oxide nanoparticles engineered macrophage-derived exosomes for targeted pathological angiogenesis therapy. ACS Nano. 2024;18(10):7644–55.
126. Dixson AC, Dawson TR, Di Vizio D, Weaver AM. Context-specific regulation of extracellular vesicle biogenesis and cargo selection. Nat Rev Mol Cell Biol. 2023;24(7):454–76.
127. Zheng W, Rädler J, Sork H, Niu Z, Roudi S, Bost JP, Görgens A, Zhao Y, et al. Identification of scaffold proteins for improved endogenous engineering of extracellular vesicles. Nat Commun. 2023;14(1):4734.
128. Sterzenbach U, Putz U, Low LH, Silke J, Tan SS, Howitt J. Engineered exosomes as vehicles for biologically active proteins. Mol Ther. 2017;25(6):1269–78.
129. Oka Y, Tanaka K, Kawasaki Y. A novel sorting signal for RNA packaging into small extracellular vesicles. Sci Rep. 2023;13(1):17436.
130. Zhang C, Schekman R. Syncytin-mediated open-ended membrane tubular connections facilitate the intercellular transfer of cargos including Cas9 protein. Elife. 2023;12: e84391.
131. Boncompain G, Divoux S, Gareil N, de Forges H, Lescure A, Latreche L, Mercanti V, Jollivet F, et al. Synchronization of secretory protein traffic in populations of cells. Nat Methods. 2012;9(5):493–8.
132. Yim N, Ryu SW, Choi K, Lee KR, Lee S, Choi H, Kim J, Shaker MR, et al. Exosome engineering for efficient intracellular delivery of soluble proteins using optically reversible protein-protein interaction module. Nat Commun. 2016;7:12277.
133. Choi H, Kim Y, Mirzaaghasi A, Heo J, Kim YN, Shin JH, Kim S, Kim NH, et al. Exosome-based delivery of super-repressor IκBα relieves sepsis-associated organ damage and mortality. Sci Adv. 2020;6(15):eaaz6980.
134. Kim S, Lee SA, Yoon H, Kim MY, Yoo JK, Ahn SH, Park CH, Park J, et al. Exosome-based delivery of super-repressor IκBα ameliorates kidney ischemia-reperfusion injury. Kidney Int. 2021;100(3):570–84.
135. Kim HH, Shim YR, Choi SE, Falana TE, Yoo JK, Ahn SH, Park M, Seo H, et al. Exosome-based delivery of super-repressor IκBα alleviates alcohol-associated liver injury in mice. Pharmaceutics. 2023;15(2):636.
136. Choi H, Kim MY, Kim DH, Yun H, Oh BK, Kim SB, Song IH, Park HS, et al. Quantitative biodistribution and pharmacokinetics study of GMP-grade exosomes labeled with ^{89}Zr radioisotope in mice and rats. Pharmaceutics. 2022;14(6):1118.
137. Peruzzi JA, Gunnels TF, Edelstein HI, Lu P, Baker D, Leonard JN, Kamat NP. Enhancing extracellular vesicle cargo loading and functional delivery by engineering protein-lipid interactions. Nat Commun. 2024;15(1):5618.
138. Obiedallah MM, Mironov MA, Belyaev DV, Ene A, Vakhrusheva DV, Krasnoborova SY, Bershitsky SY, Shchepkin DV, et al. Optimization, characterization, and cytotoxicity studies of novel anti-tubercular agent-loaded liposomal vesicles. Sci Rep. 2024;14(1):524.

139. Zhang Y, Li N, Suh H, Irvine DJ. Nanoparticle anchoring targets immune agonists to tumors enabling anti-cancer immunity without systemic toxicity. Nat Commun. 2018;9(1):6.
140. Rodrigues G, Hoshino A, Kenific CM, Matei IR, Steiner L, Freitas D, Kim HS, Oxley PR, et al. Tumour exosomal CEMIP protein promotes cancer cell colonization in brain metastasis. Nat Cell Biol. 2019;21(11):1403–12.
141. Smyth T, Kullberg M, Malik N, Smith-Jones P, Graner MW, Anchordoquy TJ. Biodistribution and delivery efficiency of unmodified tumor-derived exosomes. J Control Release. 2015;199:145–55.
142. Imai T, Takahashi Y, Nishikawa M, Kato K, Morishita M, Yamashita T, Matsumoto A, Charoenviriyakul C, et al. Macrophage-dependent clearance of systemically administered B16BL6-derived exosomes from the blood circulation in mice. J Extracell Vesicles. 2015;4:26238.
143. Zhang YN, Poon W, Tavares AJ, McGilvray ID, Chan WCW. Nanoparticle-liver interactions: Cellular uptake and hepatobiliary elimination. J Control Release. 2016;240:332–48.
144. Baek S, Jeon M, Jung HN, Lee W, Hwang JE, Lee JS, Choi Y, Im HJ. M1 Macrophage-derived exosome-mimetic nanovesicles with an enhanced cancer targeting ability. ACS Appl Bio Mater. 2022;5(6):2862–9.
145. Zheng G, Ma HW, Xiang GH, He GL, Cai HC, Dai ZH, Chen YL, Lin Y, et al. Bone-targeting delivery of platelet lysate exosomes ameliorates glucocorticoid-induced osteoporosis by enhancing bone-vessel coupling. J Nanobiotechnology. 2022;20(1):220.
146. Tian Y, Li S, Song J, Ji T, Zhu M, Anderson GJ, Wei J, Nie G. A doxorubicin delivery platform using engineered natural membrane vesicle exosomes for targeted tumor therapy. Biomaterials. 2014;35(7):2383–90.
147. Wang S, Li F, Ye T, Wang J, Lyu C, Qing S, Ding Z, Gao X, et al. Macrophage-tumor chimeric exosomes accumulate in lymph node and tumor to activate the immune response and the tumor microenvironment. Sci Transl Med. 2021;13(615):eabb6981.
148. Li L, He D, Guo Q, Zhang Z, Ru D, Wang L, Gong K, Liu F, et al. Exosome-liposome hybrid nanoparticle codelivery of TP and miR497 conspicuously overcomes chemoresistant ovarian cancer. J Nanobiotechnology. 2022;20(1):50.
149. Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJ. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nat Biotechnol. 2011;29(4):341–5.
150. Wang C, Li N, Li Y, Hou S, Zhang W, Meng Z, Wang S, Jia Q, et al. Engineering a HEK-293T exosome-based delivery platform for efficient tumor-targeting chemotherapy/internal irradiation combination therapy. J Nanobiotechnology. 2022;20(1):247.
151. Wiklander OPB, Mamand DR, Mohammad DK, Zheng W, Jawad Wiklander R, Sych T, Zickler AM, Liang X, et al. Antibody-displaying extracellular vesicles for targeted cancer therapy. Nat Biomed Eng. 2024;8(11):1453–68.
152. Ishiguro K, Yan IK, Lewis-Tuffin L, Patel T. Targeting liver cancer stem cells using engineered biological nanoparticles for the treatment of hepatocellular cancer. Hepatol Commun. 2020;4(2):298–313.
153. Hosseini NF, Amini R, Ramezani M, Saidijam M, Hashemi SM, Najafi R. AS1411 aptamer-functionalized exosomes in the targeted delivery of doxorubicin in fighting colorectal cancer. Biomed Pharmacother. 2022;155: 113690.
154. Pishavar E, Yazdian-Robati R, Abnous K, Hashemi M, Ebrahimian M, Feizpour R, Salmasi Z, Taghdisi SM. Aptamer-functionalized mesenchymal stem cells-derived exosomes for targeted delivery of SN38 to colon cancer cells. Iran J Basic Med Sci. 2023;26(4):388–94.
155. Salunkhe S, Dheeraj, Basak M, Chitkara D, Mittal A. Surface functionalization of exosomes for target-specific delivery and in vivo imaging & tracking: Strategies and significance. J Control Release. 2020;326:599–614.
156. Shi S, Li T, Wen X, Wu SY, Xiong C, Zhao J, Lincha VR, Chow DS, et al. Copper-64 labeled PEGylated exosomes for *in vivo* positron emission tomography and enhanced tumor retention. Bioconjug Chem. 2019;30(10):2675–83.
157. Varki A. Biological roles of glycans. Glycobiology. 2017;27(1):3–49.
158. Moons SJ, Adema GJ, Derks MT, Boltje TJ, Büll C. Sialic acid glycoengineering using N-acetylmannosamine and sialic acid analogs. Glycobiology. 2019;29(6):433–45.
159. Smyth T, Petrova K, Payton NM, Persaud I, Redzic JS, Graner MW, Smith-Jones P, Anchordoquy TJ. Surface functionalization of exosomes using click chemistry. Bioconjug Chem. 2014;25(10):1777–84.
160. Zhou X, Jaiswal M, Shi J, Guo J, Kundu S, Guo Z, Zeng Y. Efficient enzymatic glycan engineering of extracellular vesicles using nanomaterial-interfaced microfluidics. ACS Appl Mater Interfaces. 2025;17(1):2689–700.
161. Schmidt EN, Lamprinaki D, McCord KA, Joe M, Sojitra M, Waldow A, Nguyen J, Monyror J, et al. Siglec-6 mediates the uptake of extracellular vesicles through a noncanonical glycolipid binding pocket. Nat Commun. 2023;14(1):2327.
162. Song S, Shim MK, Lim S, Moon Y, Yang S, Kim J, Hong Y, Yoon HY, et al. In situ one-step fluorescence labeling strategy of exosomes via bioorthogonal click chemistry for real-time exosome tracking *in vitro* and *in vivo*. Bioconjug Chem. 2020;31(5):1562–74.
163. Hung ME, Leonard JN. Stabilization of exosome-targeting peptides via engineered glycosylation. J Biol Chem. 2015;290(13):8166–72.
164. Shimoda A, Tahara Y, Sawada SI, Sasaki Y, Akiyoshi K. Glycan profiling analysis using evanescent-field fluorescence-assisted lectin array: Importance of sugar recognition for cellular uptake of exosomes from mesenchymal stem cells. Biochem Biophys Res Commun. 2017;491(3):701–7.
165. Zhuang X, Xiang X, Grizzle W, Sun D, Zhang S, Axtell RC, Ju S, Mu J, et al. Treatment of brain inflammatory diseases by delivering exosome encapsulated anti-inflammatory drugs from the nasal region to the brain. Mol Ther. 2011;19(10):1769–79.
166. Armstrong DK, Bundy B, Wenzel L, Huang HQ, Baergen R, Lele S, Copeland LJ, Walker JL, et al; Gynecologic Oncology Group. Intraperitoneal cisplatin and paclitaxel in ovarian cancer. N Engl J Med. 2006;354(1):34–43.
167. 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.
168. Li L, Zhang Y, Mu J, Chen J, Zhang C, Cao H, Gao J. Transplantation of human mesenchymal stem-cell-derived exosomes immobilized in an adhesive hydrogel for effective treatment of spinal cord injury. Nano Lett. 2020;20(6):4298–305.
169. Li Q, Song Q, Zhao Z, Lin Y, Cheng Y, Karin N, Luan Y. Genetically engineered artificial exosome-constructed hydrogel for ovarian cancer therapy. ACS Nano. 2023;17(11):10376–92.
170. Mendt M, Kamerkar S, Sugimoto H, McAndrews KM, Wu CC, Gagea M, Yang S, Blanko EVR, et al. Generation and testing of clinical-grade exosomes for pancreatic cancer. JCI Insight. 2018;3(8): e99263.
171. Tang M, Chen Y, Li B, Sugimoto H, Yang S, Yang C, LeBleu VS, McAndrews KM, et al. Therapeutic targeting of STAT3 with small interference RNAs and antisense oligonucleotides embedded exosomes in liver fibrosis. FASEB J. 2021;35(5): e21557.
172. Morse MA, Garst J, Osada T, Khan S, Hobeika A, Clay TM, Valente N, Shreeniwas R, et al. A phase I study of dexosome immunotherapy in patients with advanced non-small cell lung cancer. J Transl Med. 2005;3(1):9.
173. Escudier B, Dorval T, Chaput N, André F, Caby MP, Novault S, Flament C, Leboulaire C, et al. Vaccination of metastatic melanoma patients with autologous dendritic cell (DC) derived-exosomes: results of thefirst phase I clinical trial. J Transl Med. 2005;3(1):10.
174. Nikfarjam S, Rezaie J, Kashanchi F, Jafari R. Dexosomes as a cell-free vaccine for cancer immunotherapy. J Exp Clin Cancer Res. 2020;39(1):258.
175. Viaud S, Ploix S, Lapierre V, Théry C, Commere PH, Tramalloni D, Gorrichon K, Virault-Rocroy P, et al. Updated technology to produce highly immunogenic dendritic cell-derived exosomes of clinical grade: a critical role of interferon-γ. J Immunother. 2011;34(1):65–75.
176. Dai S, Wei D, Wu Z, Zhou X, Wei X, Huang H, Li G. Phase I clinical trial of autologous ascites-derived exosomes combined with GM-CSF for colorectal cancer. Mol Ther. 2008;16(4):782–90.
177. Kimiz-Gebologlu I, Oncel SS. Exosomes: Large-scale production, isolation, drug loading efficiency, and biodistribution and uptake. J Control Release. 2022;347:533–43.
178. Vader P, Mol EA, Pasterkamp G, Schiffelers RM. Extracellular vesicles for drug delivery. Adv Drug Deliv Rev. 2016;106(Pt A):148–56.

179. Dong S, Liu X, Bi Y, Wang Y, Antony A, Lee D, Huntoon K, Jeong S, et al. Adaptive design of mRNA-loaded extracellular vesicles for targeted immunotherapy of cancer. Nat Commun. 2023;14(1):6610.
180. Fan D, Cao Y, Cao M, Wang Y, Cao Y, Gong T. Nanomedicine in cancer therapy. Signal Transduct Target Ther. 2023;8(1):293.
181. Zhang W, Ngo L, Tsao SC, Liu D, Wang Y. Engineered cancer-derived small extracellular vesicle-liposome hybrid delivery system for targeted treatment of breast cancer. ACS Appl Mater Interfaces. 2023;15(13):16420–33.
182. Huang L, Rong Y, Tang X, Yi K, Qi P, Hou J, Liu W, He Y, et al. Engineered exosomes as an *in situ* DC-primed vaccine to boost antitumor immunity in breast cancer. Mol Cancer. 2022;21(1):45.
183. Morrissey SM, Zhang F, Ding C, Montoya-Durango DE, Hu X, Yang C, Wang Z, Yuan F, et al. Tumor-derived exosomes drive immunosuppressive macrophages in a pre-metastatic niche through glycolytic dominant metabolic reprogramming. Cell Metab. 2021;33(10):2040-2058.e10.
184. Guo Y, Wu Z, Shen S, Guo R, Wang J, Wang W, Zhao K, Kuang M, et al. Nanomedicines reveal how PBOV1 promotes hepatocellular carcinoma for effective gene therapy. Nat Commun. 2018;9(1):3430.
185. Khoobchandani M, Khan A, Katti KK, Thipe VC, Al-Yasiri AY, MohanDoss DKD, Nicholl MB, Lugão AB, et al. Green nanotechnology of MGF-AuNPs for immunomodulatory intervention in prostate cancer therapy. Sci Rep. 2021;11(1):16797.
186. Wang Y, Zhou SK, Wang Y, Lu ZD, Zhang Y, Xu CF, Wang J. Engineering tumor-specific gene nanomedicine to recruit and activate T cells for enhanced immunotherapy. Nat Commun. 2023;14(1):1993.
187. Ho D, Wang CH, Chow EK. Nanodiamonds: The intersection of nanotechnology, drug development, and personalized medicine. Sci Adv. 2015;1(7): e1500439.
188. Tian X, Angioletti-Uberti S, Battaglia G. On the design of precision nanomedicines. Sci Adv. 2020;6(4):eaat0919.
189. von Roemeling C, Jiang W, Chan CK, Weissman IL, Kim BYS. Breaking down the barriers to precision cancer nanomedicine. Trends Biotechnol. 2017;35(2):159–71.

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