

Exosomes as Emerging Nanocarriers for Targeted Cancer Therapy

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Abstract: Exosomes are nanoscale lipid bilayer vesicles secreted by eukaryotic cells into biological fluids. As an important subtype of extracellular vesicles, they can mediate intercellular material exchange and signal transmission by carrying bioactive substances such as proteins, nucleic acids, and lipids, and participate in the maintenance of physiological homeostasis and the regulation of pathophysiological processes in the body. These biological characteristics make exosomes less likely to be recognized and cleared by the immune system after entering the human body, nor do they cause obvious immune rejection reactions, laying the foundation for their use as delivery carriers. Through engineering techniques, they can be modified, and nucleic acids, small molecule drugs, and other substances can be precisely encapsulated inside exosomes through artificial intervention, forming “exosome-treatment payload” complexes. These complexes can take advantage of the properties of exosomes to significantly enhance their permeability in human tissues and cells, easily cross biological barriers, and promote the enrichment of treatment payloads in specific sites such as tumor tissues, thereby effectively optimizing treatment outcomes. However, the current application of exosomes is still limited by low separation and purification efficiency, high preparation costs, and easy damage to vesicle structure, and the clinical transformation process needs to be accelerated. This review focuses on the latest progress in the research of exosome-targeted delivery platforms, combines existing exosome drug loading technologies, and analyzes the clinical application potential and core challenges of this delivery system in cancer treatment, aiming to provide research directions for the development and clinical transformation of exosome-mediated anti-tumor targeted therapy strategies.

Keywords: exosome, drug delivery system, cargo loading, cancer

Introduction

Cancer, as a significant global public health issue, maintains persistently high incidence and mortality rates.¹ Although traditional cancer treatments (such as surgical resection, chemotherapy, and radiotherapy) are widely applied, they are often constrained by the complex physiological barriers of the human body, resulting in low drug bioavailability and inevitably causing severe toxic side effects in peripheral healthy tissues. To overcome these obstacles, drug delivery systems (DDS) refer to systems that comprehensively regulate the spatial, temporal, and dosing distribution of drugs or molecules within the organism, which have undergone rapid development over the past few decades. However, conventional synthetic delivery systems (including viral vectors, liposomes, and polymer nanoparticles) still face inherent limitations in clinical translation, such as high immunogenicity, short in vivo circulation half-life, and rapid clearance by the mononuclear phagocyte system (MPS).



Against this backdrop, exosomes (Exosomes) as an emerging natural delivery vehicle have garnered increasing attention. Since their formal nomenclature in 1985, exosomes have been redefined from initially being considered carriers of “cellular waste” to pivotal mediators regulating intercellular communication and remodeling the tumor microenvironment.² As a significant component of extracellular vesicles (EVs), exosomes are widely present in various body fluids and naturally carry biomacromolecules such as proteins, nucleic acids, and lipids.³ With advancing research on exosomes, natural exosomes can be engineered into “engineered exosomes” through surface modification or content loading, thereby enhancing their targeting specificity and therapeutic efficacy, which opens new avenues for exosome applications.^{4–6} Compared to synthetic liposomes or viral vectors, exosomes exhibit unparalleled biological advantages: exceptional biocompatibility, minimal immunogenicity, and the ability to naturally traverse stringent biological barriers (eg., the blood-brain barrier). This enables more precise drug delivery to target cells, significantly improving the efficacy of tumor therapy. Consequently, utilizing exosomes as carriers for chemotherapeutic drug delivery may emerge as an effective anti-tumor strategy.^{7,8}

Despite the increasing research on engineered exosomes, this field still faces a significant clinical translation gap. Current review literature predominantly focuses on describing the fundamental biogenesis of exosomes or their natural roles in the tumor microenvironment, while lacking in-depth and critical discussions on how to systematically overcome clinical application barriers through engineering approaches (such as severe off-target effects *in vivo*, heterogeneity in large-scale production, and long-term safety evaluation). To address this literature gap, this review systematically summarizes the latest advancements of exosomes as emerging nanocarriers for targeted cancer therapy. This article not only provides a detailed summary of advanced engineering strategies to enhance exosome targeting efficacy and drug loading (including macromolecular nucleic acids and gene-editing tools), but also highlights their advantages and disadvantages compared to other delivery systems, as well as their applications and challenges in clinical translation. The aim is to offer comprehensive theoretical support and forward-looking guidance for the clinical design and interdisciplinary translation of next-generation intelligent exosome-based drugs.

Exosome Composition, Biogenesis and Function

Exosomes are ubiquitous in biological fluids such as blood, saliva, urine, and cerebrospinal fluid (CSF).⁹ Depending on their compositional modifications, exosomes fall into two primary categories: naturally occurring and engineered variants. Natural exosomes are further differentiated based on their origin, distinguishing between animal- and plant-derived sources. Among animal-derived exosomes, subtypes include those shed by healthy tissues and those secreted by tumor cells.

Exosomes originate from the endosomal system and typically measure 30–150 nanometers in diameter. Their biogenesis commences with the invagination of the plasma membrane, forming early sorting endosomes (ESE).¹⁰ These early endosomes mature into late sorting endosomes (LSE) through the orchestration of the endosomal sorting complexes required for transport (ESCRT) machinery and associated regulatory proteins. Cargo trafficking between the Golgi apparatus and inward budding events generates multivesicular bodies (MVBs). MVBs may undergo two fates: fusion with lysosomes or autophagosomes for degradation, or merger with the plasma membrane to release extracellular vesicles as exosomes^{11,12} (Figure 1). The biogenesis of exosomes is not a unidirectional linear process, but rather a highly complex and finely regulated mechanism governed by multiple pathways with dynamic competitive and synergistic interactions. Currently, it is primarily classified into ESCRT-dependent and ESCRT-independent pathways, which are also the fundamental causes of exosomal cargo heterogeneity.¹³ In the ESCRT-dependent pathway, ESCRT-0, -I, -II, and -III complexes precisely sort specific protein cargoes (such as growth factor receptors) by recognizing ubiquitination tags and mediate their entry into multivesicular bodies (MVBs). The expression levels of HRS and TSG101 significantly influence the proteomic profiles and vesicle sizes of secreted exosomes, while ALIX not only assists in membrane cleavage but also directly determines the loading efficiency of specific transmembrane proteins such as MHC class II molecules. In contrast, the ESCRT-independent pathway primarily relies on lipid rafts (eg., ceramides, cholesterol) and four transmembrane proteins (eg., CD63, CD81, CD9). The ceramide production mediated by neutral sphingomyelinase (nSMase) and the lipid-mediated pathway driven by Rab31 GTPase both belong to this category. Notably, Rab31 GTPase specifically sorts cargo carrying epidermal growth factor receptor (EGFR) into CD63-positive

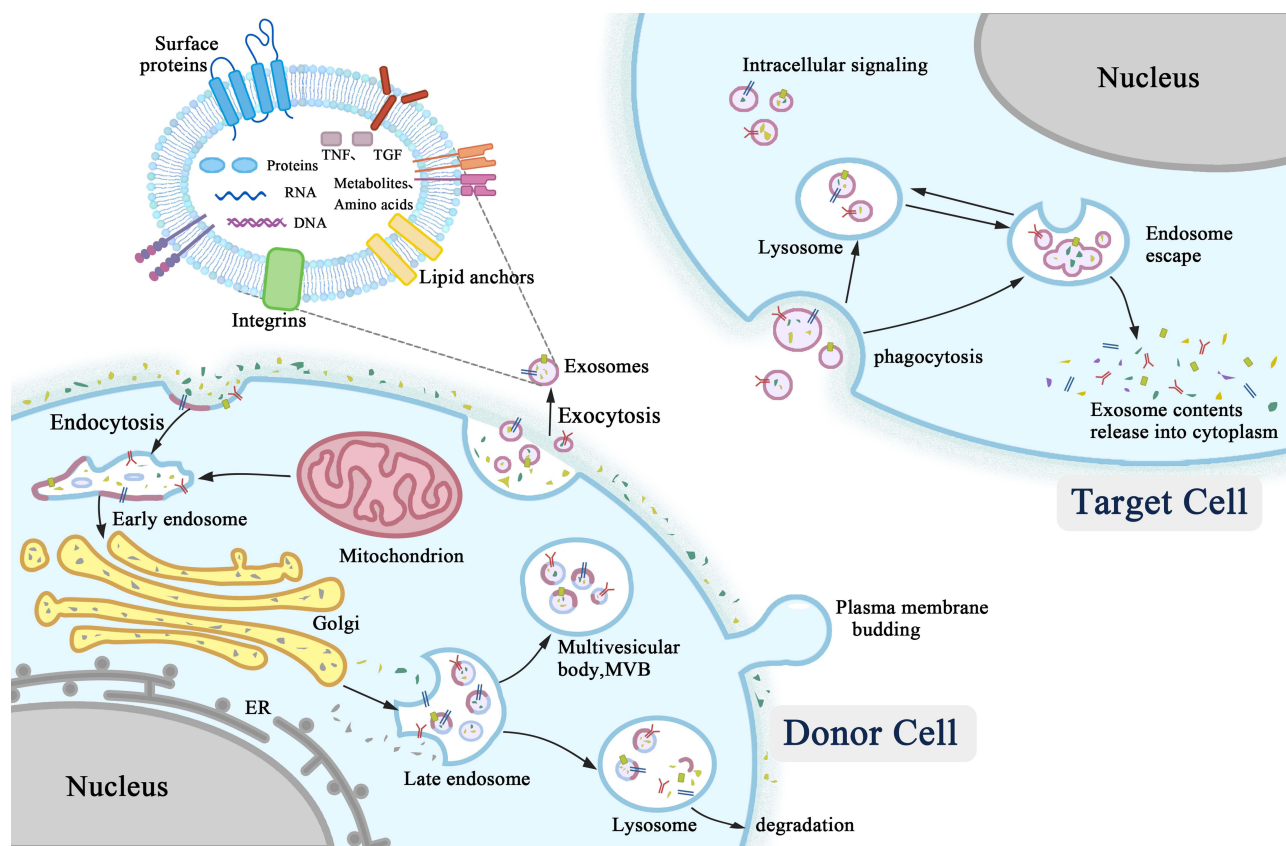


Figure 1 Biogenesis of Exosomes. Exosomes originate from the endosomal system, beginning with the inward budding of the plasma membrane to form early sorting endosomes (ESEs). As ESEs mature into late sorting endosomes, they exchange cargo with the Golgi apparatus and subsequently undergo inward budding to generate intraluminal vesicles (ILVs), giving rise to multivesicular bodies (MVBs). During this stage, exosomal contents are selectively packaged. MVBs can either fuse with lysosomes or autophagosomes for degradation, or fuse with the plasma membrane to release the enclosed ILVs as exosomes into the extracellular space. Exosomes carry a wide array of bioactive molecules, including proteins and nucleic acids. Tetraspanins such as CD9, CD63, and CD81, which are enriched on the exosomal membrane, are commonly used as canonical markers for exosomes.

exosomal subpopulations, whereas exosomes generated via the nSMase-dependent pathway are often enriched with specific lipids and RNA.¹⁴ It is noteworthy that previous studies often treated exosomes as homogeneous entities, masking functional differences among subpopulations mediated by distinct biological pathways. In reality, cells dynamically adjust the weighting of these two pathways in response to external stress conditions (eg., hypoxia, immune stress), secreting exosome subpopulations with high molecular heterogeneity and targeted specificity to meet diverse communication needs. This suggests that viewing exosomes as a single homogeneous population in future research and clinical applications presents significant limitations.

The internalization mechanism of cells fundamentally determines the targeting efficiency and pharmacokinetic distribution of exosomes or nanocarriers *in vivo*.¹⁵ Among these, the endocytosis mediated by clathrin is the main pathway for receptor-ligand interactions.¹⁶ In highly heterogeneous malignant tumors (eg., triple-negative breast cancer, TNBC), surface-engineered exosomes (eg., targeting specific integrins or peptides) rely heavily on the CME pathway to enter target cells. The clinical value of this mechanism lies in its exceptional targeting specificity: it not only significantly enhances drug accumulation in complex tumor microenvironments but also precisely blocks metastasis-promoting communication between tumor cells and surrounding stromal cells (eg., lymphatic endothelial cells).^{17–19} Therefore, optimizing CME-based uptake strategies is a critical prerequisite for reducing systemic off-target toxicity and improving therapeutic outcomes such as inhibiting lymphatic metastasis.²⁰ Unlike the high specificity of CME, phagocytosis provides a non-specific, bulk internalization pathway.²¹ Many oncogenic mutations (eg., Ras-driven tumors) significantly upregulate phagocytic activity to uptake extracellular macromolecular nutrients. Exosomes and biomimetic nanocarriers

can effectively exploit this metabolic vulnerability of tumor cells to facilitate uptake.^{22,23} The high permeability of phagocytic vesicles not only exponentially increases the absolute uptake of intracellular drugs but also holds decisive clinical significance for enhancing the penetration efficiency of macromolecular chemotherapeutic agents into dense tumor tissues and overcoming drug resistance.^{24,25}

The ability of exosomes to achieve efficient “endosomal escape” after being internalized by recipient cells, thereby releasing their carried nucleic acids or proteins into the cytoplasm to function, is a critical stage in exosome-mediated intercellular signaling. In early research on nanomedicine and engineered exosomes (eg., cationically modified exosomes, PEI-modified exosomes), the “proton sponge effect” was frequently used to explain the endosomal escape process. This mechanism posits that cationically modified exosomes absorb a large number of protons in the acidic endosomal environment, prompting chloride influx and ultimately leading to endosomal rupture through a sudden increase in osmotic pressure. However, the actual contribution and reproducibility of this theory *in vivo* have been highly controversial: substantial *in vivo* evidence indicates that relying solely on cation-mediated endosomal osmotic imbalance not only results in extremely low escape efficiency (typically <1%) but also readily induces severe irreversible cytotoxicity. Moreover, the complex buffering systems *in vivo* often prevent the proton sponge effect from reaching the threshold required for endosomal rupture, leaving most exosomal cargo trapped in lysosomes for degradation.²⁶ Recent *in vivo* fluorescence tracing and membrane biophysical studies further confirm that the buffering capacity of endosomes under physiological conditions is insufficient to generate an osmotic gradient large enough to cause membrane rupture.²⁷ In contrast, the efficient intracellular delivery mechanism of natural exosomes and the more rational escape pathways of engineered exosomes rely more on specific “membrane fusion” or direct membrane destabilization caused by localized charge interactions. Under the micro-acidic environment of late endosomes (pH 5.0–6.0), specific lipid conformations on the exosomal membrane undergo changes, or fusion proteins on its surface (such as viral-like envelope protein fragments) are activated, promoting direct fusion of the exosomal membrane with the endosomal boundary membrane. The reverse fusion process within multivesicular bodies (MVBs) is also a key physiological pathway for exosomes to evade lysosomal degradation and release their functional contents.^{28,29} Membrane fusion enables the carrier to directly release its contents into the cytoplasm of target cells, completely circumventing the capture by the endosome-lysosome system and avoiding the inactivation of a large number of drugs before they can exert their therapeutic effects.^{30–32} For therapeutic strategies that require direct action on intracellular targets or the nucleus, membrane fusion can enhance the absolute bioavailability of active substances by hundreds or thousands of times, making it the most ideal internalization strategy for achieving efficient immune microenvironment remodeling and improving long-term patient survival outcomes.^{33,34} Zhuo Y et al demonstrated that increasing the cholesterol content of the exosomal membrane promoted vesicle deformation and expanded membrane contact areas, significantly enhancing siRNA delivery into recipient cells.³⁰ Kumari P et al further demonstrated that CD14—a member of the Transporters Associated with Antigen Processing and Inflammation (TAXI) protein family—functions as a critical mediator in lipopolysaccharide (LPS) uptake. Their findings suggested that LPS bound to exosomes fused more efficiently with endosomal membranes compared to free LPS, highlighting the unique role of exosomes in immune signal transduction.³⁵

The biological importance of exosomes is rooted not only in their unique structure and composition but also in their functional versatility. Accumulating evidence indicates that exosomes, by transporting DNA, RNA, proteins, and metabolites, regulate cellular processes and disease progression.^{36,37} (Table 1). Advances in high-throughput sequencing and mass spectrometry have greatly expanded our understanding of exosome classification and functional diversity.

In cancer biology, exosomes accelerate tumor malignancy by remodeling the extracellular matrix (ECM) and driving tumor growth and dissemination.⁴⁸ Moreover, these vesicles show promise as diagnostic tools for tracking tumor progression and enabling early clinical detection.¹¹ Non-coding RNAs (eg., miRNAs, circRNAs) within exosomes exhibit exceptional stability in body fluids such as blood and urine due to protection by the lipid bilayer, making them highly promising biomarkers for liquid biopsy. Their diagnostic advantages have been confirmed in multiple large-scale clinical quantitative studies.⁴⁹ In clinical applications for specific cancer types, different exosomal ncRNA combinations demonstrate superior diagnostic performance: For the early diagnosis of non-small cell lung cancer (NSCLC) and hepatocellular carcinoma (HCC), a clinical diagnostic model based on the combined detection of multiple exosomal miRNAs (eg., miR-1268b, miR-6075, miR-21) achieves a sensitivity of 95%–97.7% and specificity of 94.7%–99%, with

Table 1 Various Transport Substances of Exosome

Type	Cargo	Target Cancer	Biological Effect
RNA	miR-1827	Colon cancer	Tumor growth inhibition ³⁸
	miR-183-5p	Breast cancer	Tumor growth inhibition ³⁹
	miR-145-5p	Pancreatic ductal adenocarcinoma	Tumor cell proliferation and invasion inhibition ⁴⁰
	Cas9 mRNA	Breast cancer	Tumor growth inhibition ⁴¹
	CircABCB10	Colon cancer	Tumor growth inhibition ⁴²
Proteins	NF- κ B	Prostate cancer	Tumor growth inhibition ⁴³
	CIITA	Lymphoma	Promote T cell response ⁴⁴
Compounds	Doxorubicin	Breast cancer	Tumor growth inhibition ⁴⁵
	Paclitaxel	Breast cancer	Tumor growth inhibition ⁴⁶
	Curcumin	Glioblastoma	Tumor growth inhibition ⁴⁷

the area under the receiver operating characteristic curve (AUC) remaining stable between 0.95 and 0.99, enabling precise screening for early-stage (Stage I) malignancies.⁵⁰ In breast cancer diagnosis, the combination of miR-21 and miR-1246 in plasma exosomes demonstrates moderately high diagnostic efficacy, with an AUC of 0.73.⁵¹ Furthermore, circular RNAs (circRNAs) are emerging as next-generation diagnostic targets due to their exceptional nuclease resistance conferred by their closed-loop structure. Similarly, studies on exosomal circRNAs in blood and cerebrospinal fluid (CSF) have provided novel insights into the underlying mechanisms of neuropsychiatric disorders.⁵² These findings validate their practical translational value in precision medicine. Additionally, since the surface-specific antigens secreted by cells can reflect the properties of donor cells, researchers have identified multiple protein biomarkers in exosomes. These biomarkers not only facilitate the identification and isolation of exosomes but also hold promise as novel targets for disease diagnosis and treatment. These research achievements not only provide new perspectives for exosome studies but also lay a solid foundation for their application in disease diagnosis and therapy.

Isolation and Characterization of Exosomes

In exosome research, overcoming technical challenges remains a crucial task. Efficient, accurate isolation, purification, and identification of these nanoscale vesicles continues to be a significant hurdle. Exosome separation methods are typically based on properties like density, size, and antigenicity. However, the yield, purity, and physicochemical traits of isolated exosomes differ based on the chosen separation technique.⁵³ Conventional approaches—including ultracentrifugation, density gradient separation, have been widely used to eliminate protein contaminants and cellular debris. Meanwhile, researchers are actively exploring novel technologies such as microfluidics, immunoaffinity capture, and nano-flow cytometry, which have demonstrated promising potential in recent years.^{54,55} To more intuitively demonstrate the advantages and disadvantages of the aforementioned separation techniques, we conducted a systematic comparative evaluation of the core performance metrics of different methods, detailing the key performance indicators and pros and cons of current mainstream exosome separation methods (Table 2). As previously mentioned, the high heterogeneity of exosomes and the limitations of extraction techniques remain significant technical challenges in current research. Traditional methods (such as ultracentrifugation, ultrafiltration, and precipitation) are widely applied but often involve trade-offs in terms of purity, recovery rate, or vesicle integrity. In contrast, emerging technologies (such as microfluidic chips and the EXODUS system) exhibit great potential for automated, high-throughput, and large-scale clinical applications in exosome separation.

Following isolation and purification, due to the intrinsic heterogeneity of exosomes, detailed characterization and quantification through multiple approaches are necessary. These characterization methods are broadly categorized into two types: external (morphology and size analysis) and internal (membrane proteins, lipids, etc). Common techniques include transmission electron microscopy (TEM), nanoparticle tracking analysis (NTA), Western blotting of exosomal surface markers, tunable resistive pulse sensing (TRPS), and atomic force microscopy (AFM).^{65–67} These technologies provide comprehensive information on exosome morphology, size distribution, purity, concentration, and overall biophysical properties, which are critically important for advancing therapeutic and diagnostic applications, especially

Table 2 Isolation Method of Exosome

Isolation Method	Yield	Purity	Scalability	Cost/Time	Main Features
UC	Moderate	Low	Low	Requires expensive equipment and time-consuming	Gold standard for isolation, Prone to lipoprotein contamination and aggregation, high speed may damage vesicle membranes. ^{56,57}
Ultrafiltration	Low	Moderate	Moderate	Reduced the time of operation	Improves integrity, Easily adsorb onto the filter membrane. ⁵⁸
SEC	Moderate	High	Low	Costly and time-consuming	Separates based on size, Excellent purity. Potentially damage membranes. ⁵⁹
Precipitation	Low	Low	High	Easy to operate	Low cost, suitable for large-batch processing. Susceptible to free protein contamination. ⁵⁶
Immunoaffinity	Low	High	Low	High purity but stringent storage requirements	Based on antigen-antibody interaction, unsuitable for large-scale isolation, May generate interfering proteins. ⁶⁰
Microfluidics	High	High	High	Faster, requires less sample and reagent	High efficiency. Requires specially designed chips and microfluidic platforms, still under development. ^{61,62}
Exodus	High	High	High	High efficiency and automation	Suitable for large-scale clinical and research use. Relies on dedicated ultrafast separation equipment. High initial instrument cost. ^{63,64}

in oncology.^{68,69} In practice, the purity, quantity, and physicochemical properties of exosomes may vary depending on the separation conditions. Therefore, it is essential to comprehensively consider specific requirements and select appropriate separation and characterization methods.

Traditional Isolation Techniques

Ultracentrifugation is widely regarded as the benchmark method for exosome purification, encompassing both differential and density gradient-based protocols. Differential centrifugation achieves particle separation by manipulating centrifugal force and sedimentation time, while density gradient centrifugation leverages media to establish a density gradient for enhanced separation. Nonetheless, ultracentrifugation may cause exosome aggregation, contamination with lipoproteins, and potential membrane damage due to high-speed forces. Ultrafiltration utilizes size-selective membranes to fractionate exosomes from impurities, effectively excluding non-vesicular contaminants. Compared to ultracentrifugation, ultrafiltration offers improved purity, integrity, and shorter processing time; however, membrane adsorption can reduce exosome yield, and pore-induced platelet rupture remains a concern. SEC partitions particles according to size, allowing larger vesicles like exosomes to elute earlier while smaller molecules penetrate the pores. Despite its advantages, SEC can be time-consuming, costly, and may damage vesicle membranes. To overcome these constraints, researchers have engineered multiple commercial kits tailored to address these challenges. Polymer-based precipitation, typically utilizing polyethylene glycol (PEG) or salts to neutralize surface charges, aggregates exosomes for subsequent centrifugation-based recovery. While simple and scalable, precipitation methods often suffer from co-isolation of soluble proteins, resulting in lower purity and potential false positives.

Specific Affinity Techniques

Immunoaffinity capture relies on the binding of specific antibodies to exosome membrane proteins, providing exceptionally high specificity. Nevertheless, this approach requires stringent storage conditions, unsuitability for large-scale isolation, and the potential generation of interfering proteins.

Emerging High-Throughput Technologies

Microfluidics offer a high-throughput approach to exosome isolation, utilizing chips that separate vesicles based on size, density, and other properties. When biological fluids flow through microfluidic devices, microvortices enhance exosome interactions, while probes selectively capture vesicles, achieving >70% separation efficiency without compromising downstream analysis. Microfluidic technology serves as an emerging high-throughput approach that integrates principles such as electrophoresis, acoustics, or 3D porous structures, achieving separation efficiencies exceeding 70%. It offers advantages of being faster and more cost-effective with lower sample requirements, while also allowing exosomes to be stored in their native form, thereby retaining biological activity. Compared to traditional methods, microfluidic platforms are faster, more cost-effective, require smaller sample volumes, and better preserve exosome bioactivity.

Additionally, the EXODUS platform, an ultra-fast separation system, enables automatic, label-free exosome purification, demonstrating high efficiency and rapidity for large-scale clinical exosome enrichment from biofluids (eg., urine and plasma). Zhu Q et al utilized EXODUS to isolate urinary exosomes for lipidomic profiling of NAFLD and NASH patients, revealing potential biomarkers via machine learning analysis. EXODUS-based plasma exosome profiling has been applied to monitor dynamic treatment responses in esophageal squamous cell carcinoma. Overall, EXODUS combines efficiency, speed, and automation, making it well-suited for clinical and research-scale applications.

Advanced Characterization Technologies

Following isolation, advanced technologies such as Nano-Flow Cytometry and the ExoView system provide detailed, single-particle level characterization to address exosome heterogeneity. Nano-Flow Cytometry, utilizing light scattering and fluorescence, offers multidimensional analysis of size distribution, concentration, and surface markers detection (eg., the specific marker PODXL in stem cell-derived exosomes).^{70–72} The automated fluorescence analysis system ExoView, based on single-particle interferometric reflectance imaging sensor (SP-IRIS) technology, simultaneously performs counting, protein phenotyping, and cargo detection of exosome subpopulations in complex samples (eg., cerebrospinal fluid and plasma), providing a highly precise biomarker analysis tool for diagnostics in fields such as neurodegenerative diseases.^{73,74} In addition, recent advancements in nanomaterial-peptide platforms have introduced novel strategies for exosome characterization. For instance, a smart sensing system integrating graphene oxide (GO) and a CD63-binding peptide (CP05) has been developed for the highly sensitive, dual-modal quantitative detection of tumor-derived exosomes in serum, significantly improving diagnostic accuracy.⁷⁵

Preparation of Engineered Exosomes

Drug Loading

Genetic editing or chemical approaches can be employed to modify exosomes, thereby conferring novel functionalities upon these extracellular vesicles, increasing their potential in biomedical applications. To efficiently load drugs into exosomes, various techniques have been developed, including genetic engineering, chemical methods, physical techniques, and microfluidic systems. These methods allow drugs to be incorporated into exosomes without losing their biological activity.

The exosomal membrane structure serves a dual role: it shields encapsulated drugs from enzymatic hydrolysis and concurrently evades immune recognition and clearance, thereby conferring exceptional biocompatibility and stability to these extracellular vesicles.^{76,77} Leveraging their amphipathic nature, exosomes facilitate the targeted delivery of diverse therapeutic agents to recipient cells, rendering them highly effective carriers for drug delivery systems. Two primary strategies are employed for drug loading: pre-secretory (incorporation during exosome biogenesis) and post-secretory (loading into mature exosomes). Pre-secretory loading involves incubating the drug with cells before isolating the exosomes, allowing the cells to load the drug naturally into the exosomes.⁷⁸ This method supports continuous production and avoids membrane degradation. Post-secretory loading involves adding the drug to isolated exosomes. Common methodologies for modifying exosomes include electroporation, repetitive freezing-thawing cycles, sonication, extrusion and surfactant disruption. Post-secretory loading is more efficient and gives better control over drug encapsulation, making it suitable for different drug delivery needs (Figure 2).

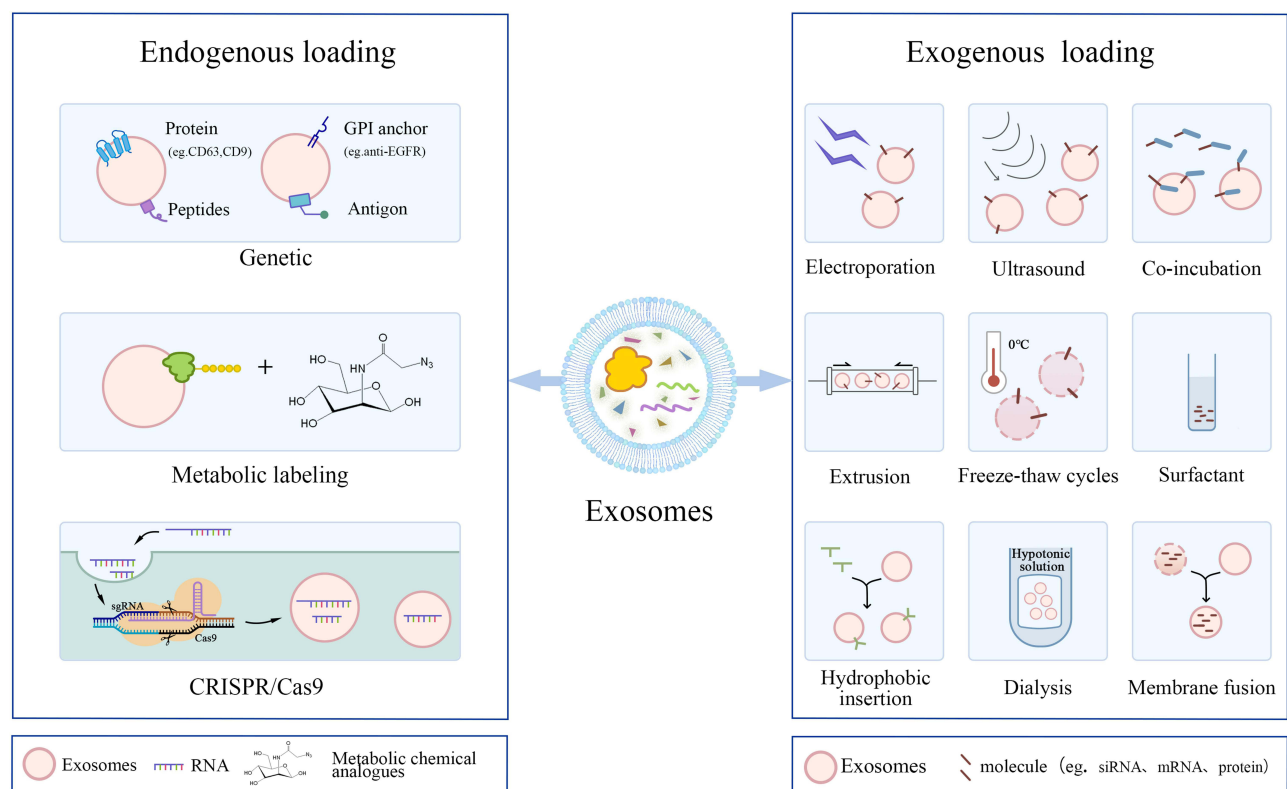


Figure 2 Drug Loading Methods for Exosomes. Drug loading into exosomes is generally categorized into two main approaches: pre-secretion loading and post-secretion loading. (Left) Pre-secretion loading involves introducing the drug into donor cells before exosome secretion, leveraging the cells' endogenous mechanisms to incorporate the therapeutic agents into the exosomes. Common strategies include co-incubation of drugs with cells, gene transfection, and metabolic labeling et al (Right) Post-secretion loading is performed after exosomes have been isolated from the cells. Drugs are directly introduced into the purified exosomes using techniques such as electroporation, sonication, extrusion, freeze–thaw cycles, treatment with surfactants, hydrophobic insertion, and dialysis et al. These methods are adaptable to accommodate various drug delivery requirements.

Co-Incubation

Recent research highlights that co-culturing hydrophobic drugs (eg., doxorubicin and paclitaxel) with parental cells facilitates their vesicular secretion via exosomes, thereby triggering cytotoxicity and suppressing tumor progression.⁷⁹ For instance, He et al engineered NK cells to express interleukin-15 (IL-15), generating IL-15-enriched exosomes. Compared to unmodified NK-EVs, these cytokine-loaded vesicles exhibited superior tumor-targeting efficacy, resulting in elevated apoptosis rates among cancer cells.⁸⁰ Pascucci et al incubated mesenchymal stem cells (MSC) with PTX before exosome isolation, resulting in significantly improved PTX delivery to cancer cells, enhancing cytotoxicity and apoptosis.⁸¹ The co-incubation method is simple, cost-effective, and works well for drugs that can easily pass through cell membranes. However, its efficacy may be reduced for other drugs, and the toxicity of the drug during the incubation process could have adverse effects.⁸²

Transfection

Transfection is a technique for delivering therapeutic DNA, RNA, or proteins into parental cells, followed by the overexpression of these compounds in exosomes.⁸³ For instance, to determine whether exosomes derived from adipose mesenchymal stem cells (AMSCs) could serve as effective vectors for delivering miR-199a-3p to enhance the chemotherapeutic efficacy of hepatocellular carcinoma, AMSCs were modified with miR-199a-3p via LV-199a infection (AMSC-199a). qRT-PCR validation confirmed a significant increase in miR-199a-3p expression in the treated cells (9.8 ± 1.1 -fold), demonstrating that exosomes can directly deliver therapeutic agents to tumor cells.⁸⁴ The transfection method offers advantages such as high reproducibility and operational simplicity, but it has low transfection efficiency and is highly dependent on cell viability.

Electroporation

Electroporation enhances the permeability of exosome membranes by applying an electric field, thereby loading hydrophilic small molecules such as amino acids and peptides. Yan et al utilized electroporation to encapsulate miR-31-5p in exosomes and quantified miR-31-5p levels from each sample. The results demonstrated that free miR-31-5p was almost completely degraded, whereas over 60% of the miR-31-5p loaded in exosomes remained stable. This indicates that exosome-mediated miRNA delivery offers multiple advantages compared to direct miRNA delivery, including enhanced cellular uptake, resistance to miRNA enzymatic degradation, and improved miRNA bioavailability.⁸⁵ However, electroporation may compromise the integrity of exosomes, thereby reducing their loading capacity.

Sonication

Translational studies demonstrate that ultrasound probes create transient pores on the exosome membrane, allowing hydrophilic small molecules to enter the exosomes. Xiang X et al loaded siRNA into exosomes via ultrasound treatment and calculated the encapsulation rate by the weight ratio of siRNA to loaded exosomes, achieving a drug loading efficiency as high as 24%.⁸⁶ Yuan R et al loaded siHSP47 (EXO-siHSP47) into exosomes and improved the loading efficiency of siHSP47 in exosomes using ultrasound microfluidic methods, reaching 31.1%.⁸⁷ Compared with methods such as electroporation, ultrasound treatment typically achieves higher drug loading efficiency. Kim MS et al measured the amount of paclitaxel loaded into exosomes using high-performance liquid chromatography (HPLC), with the highest loading efficiency of 28.3% achieved by ultrasound treatment, while incubation and electroporation yielded loading efficiencies of 1.4% and 5.3%, respectively.⁸

Membrane Fusion

Membrane fusion is the process by which two lipid bilayers approach and merge to form a stable structure. The drug is dissolved or dispersed in a buffer system and mixed with the exosome suspension. The mixture is then subjected to multiple extrusions through a lipid bilayer extruder, during which the complementary DNA strands from each vesicle are compressed together, triggering vesicle docking and membrane fusion to produce therapeutic exosomes. Xie et al fused siRNA-loaded liposomes targeting NFKBIZ with exosomes derived from corneal epithelial cells (CEC) to treat dry eye disease (DED), effectively delivering siRNA to corneal cells.⁸⁸

Load Efficiency and Cargo Stability

Evaluating the encapsulation efficiency and cargo stability of exosomes is a crucial step in ensuring the effectiveness of therapeutic delivery systems. By quantitatively analyzing drug loading and stability, researchers can optimize the design of these platforms.

Regarding drug loading efficiency, post-secretion physical disruption methods (such as sonication, extrusion, and electroporation) generally exhibit significantly higher yields compared to passive incubation. As previously noted, sonication and microfluidic extrusion can boost the loading rates of hydrophobic or hydrophilic drugs to 28.3% and nearly 20%, respectively. However, electroporation may compromise the structural integrity of exosomes when loading certain macromolecular drugs, paradoxically reducing the final encapsulation efficiency. Pre-secretion loading strategies (eg., co-incubation), while highly convenient and cost-effective, typically yield extremely low loading efficiencies for most non-hydrophobic drugs due to the natural permeability constraints of cell membranes.

In terms of cargo and membrane stability, payload integrity relies on preserving the macromolecular conformation, biological activity, and therapeutic function under physiological conditions. In this regard, mild pre-secretory techniques (like co-incubation and transfection) are superior. Because they harness the natural vesicular secretion mechanisms, they maximally preserve the integrity of the exosomal lipid bilayer and the bioactivity of surface-targeting proteins. Conversely, while sonication and extrusion offer high loading rates, the intense mechanical shear forces, thermal effects, and electric field interference can cause irreversible membrane damage or lipid reorganization. Such structural instability not only accelerates premature drug leakage during *in vivo* circulation but may also trigger the abnormal aggregation and degradation of nucleic acids or protein macromolecules, ultimately compromising the controlled-release capabilities of the system.

As for the transition to industrial-scale production, co-incubation methods demonstrate exceptional scalability for large-batch processing, much like precipitation techniques. Electroporation also shows favorable batch-processing potential through high-throughput systems. Sonication, however, exhibits poor scalability for large-scale clinical-grade production due to equipment limitations, risks of localized overheating, and significant batch-to-batch variability. Notably, emerging microfluidic extrusion systems are increasingly viewed as the most promising solution for balancing high loading efficiency with outstanding scalability, thanks to their capacity for continuous, stable, and automated fluid control. Cargo release kinetics quantify the fraction of the drug liberated from exosomes over time, reflecting the loading capacity and sustained-release performance. Standard evaluation techniques include UV-Vis absorbance spectroscopy, fluorescent labeling, high-performance liquid chromatography (HPLC), and Tinopal assays. For instance, Chen et al engineered a graphene oxide nanocomposite and validated a 73% sustained cargo retention using UV-Vis spectrophotometry.⁸⁹ Similarly, Sun et al encapsulated curcumin and utilized HPLC to correlate retention time with antioxidant efficacy.⁹⁰ Additionally, the retention of exosome payloads is heavily regulated by multiple factors, including the hydrophobicity of drug and the exosome storage buffer.^{91,92}

Ultimately, the selection of an exosome loading strategy requires a careful balance between encapsulation efficiency, structural preservation, and industrial scalability. Future advancements will likely rely on hybrid approaches or advanced microfluidic technologies to achieve the optimal combination of high therapeutic payload and robust clinical translation.

Construction of Delivery Systems

Exosomes demonstrate remarkable potential as therapeutic nanocarriers, leveraging their intrinsic biocompatibility and targeted payload translocation mechanisms to enhance bioavailability and mitigate off-target effects. First, exosomes are derived from the body itself, providing high biocompatibility, which reduces immunogenicity and toxicity during the drug delivery process. This characteristic makes exosomes highly promising for drug delivery applications. Second, exosomes have a distinct membrane structure that can effectively encapsulate drug molecules, reducing degradation and loss, thus improving drug bioavailability. Nevertheless, the transduction efficacy of exosomes is regulated by both the parent cells and the recipient cells, and the yield varies significantly depending on the source of the exosomes. Natural exosomes also have the drawback of poor targeting ability. To enhance drug delivery efficiency and minimize side effects, researchers are focusing on developing exosome delivery systems with high targeting specificity. By designing specific targeting molecules and functionalizing the surface of exosomes with targeting ligands, active agents achieve site-specific accumulation in cells or tissues via exosomal carriers, thereby augmenting the therapeutic efficacy of exosome-mediated drug delivery systems (Figure 3).

Targeted Modification

The biodistribution of natural exosomes after intravenous injection exhibits high localization, with over 80% of systemically administered exosomes being rapidly cleared by the mononuclear phagocyte system (MPS), primarily accumulating in the liver, spleen, and lungs. This significant off-target uptake not only substantially reduces the effective dose delivered to the tumor site but may also induce potential hepatosplenotoxicity. The principle of targeted therapy is to precisely act on diseased cells or tissues through specific drugs or carriers, thereby minimizing damage to normal cells. Surface ligand engineering is a key strategy to overcome this natural distribution barrier. By employing methods such as chemical modification, physical techniques, or gene editing, specific targeting ligands—including antibodies, peptides, or other biomolecules—can be introduced on the outer layer of exosomes. These ligands direct exosomes to cells expressing the corresponding receptors, enabling precise targeting and delivery to specific cells.^{93–95} For example, the EGFR-targeting GE11 peptide can be conjugated to exosomes, enabling the selective delivery of tumor-suppressive miRNAs to EGFR-overexpressing breast carcinomas.⁹⁶ Koh et al modified exosome surfaces with SIRP- α , designing exosomes with SIRP- α variants that interfere with the CD47-SIRP- α interaction between cancer cells and bone marrow-derived macrophages (BMDM), enhancing the macrophage-guided phagocytosis of tumor cells.⁹⁷ Alternatively, ligands can be used to anchor proteins in cells, which are then incorporated into secreted exosomes. This targeted delivery reduces side effects on non-target cells and enhances therapeutic efficacy. For instance, Liu Y et al modified exosomes with a neuron-specific RVG peptide, which binds to acetylcholine receptors on neurons, enabling the brain-specific delivery of MOR

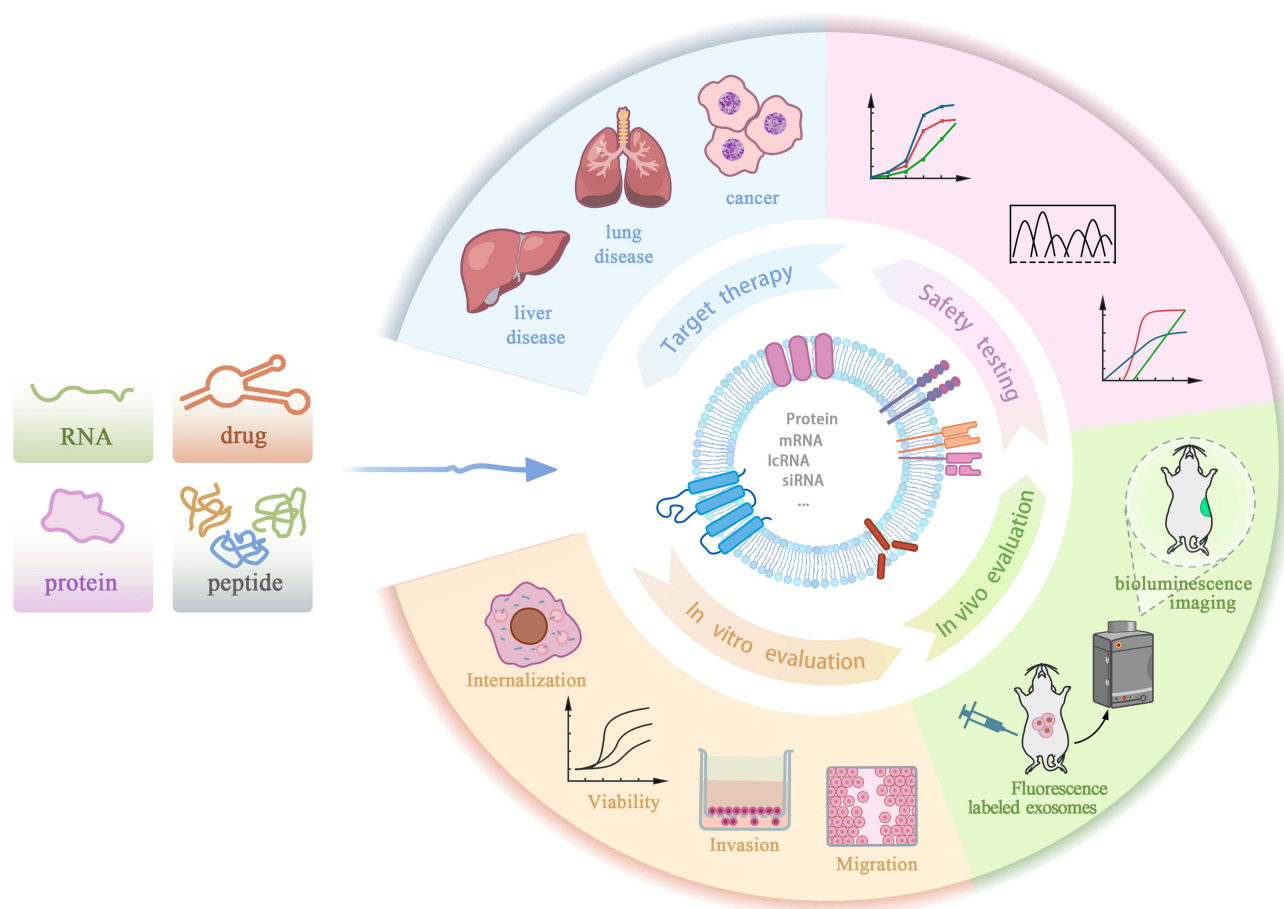


Figure 3 Validation and Application Workflow of Drug-Loaded Exosomes. Evaluation of the therapeutic efficacy and safety of engineered exosomes via in vitro, in vivo, and clinical analyses. ① In Vitro Evaluation: Exosome-mediated drug delivery efficiency is evaluated by measuring intracellular drug accumulation and assessing cell viability or functional changes post-treatment. ② In Vivo Evaluation: Disease models in mice are used to study the biodistribution and therapeutic efficacy of drug-loaded exosomes in a physiological setting. ③ Safety Testing: The safety of engineered exosomes is assessed by examining drug loading efficiency, release kinetics, and potential cytotoxic or systemic adverse effects. ④ Targeted Therapy: Engineered exosomes are utilized for targeted treatment of specific diseases, customized to the unique pathological features and therapeutic needs of each condition.

siRNA, effectively inhibiting morphine relapse by downregulating MOR expression.⁹⁸ Aptamers, short oligonucleotides that specifically recognize target molecules, were previously conjugated to cholesterol moieties on exosome surfaces. A bivalent RNA aptamer targeting PSMA enhanced PSMA-mediated endocytosis in prostate cancer cells, facilitating exosome uptake and promoting antitumor efficacy.⁹⁹ Pi et al modified exosome surfaces with RNA ligands non-covalently attached to folic acid or cholesterol, altering the surface properties of exosomes to more effectively deliver siRNA and miRNA to tumor regions, thereby enhancing anti-tumor effects.¹⁰⁰ Antibody fragments targeting unique cell epitopes are another common approach for exosome-based targeting. Wiklander OPB et al successfully modified exosome surfaces to incorporate Fc-specific antibodies, enabling Fc-EVs to target PD-L1-expressing cancer cells. When loaded with doxorubicin, these exosomes reduced tumor burden and extended survival in melanoma mouse models.¹⁰¹

Integrin-targeting peptides (eg., iRGD) or nanobodies targeting the epidermal growth factor receptor (EGFR) are modified onto the surface of exosomes. This surface engineering not only significantly enhances the specific uptake of exosomes in tumor parenchymal cells through receptor-ligand-mediated endocytosis, but more importantly, it competitively reduces nonspecific cell recognition by altering the surface charge and steric hindrance of exosomes.¹⁰² Therefore, ligand modification is crucial for reshaping global biodistribution and reducing off-target toxicity.^{103,104} Furthermore, when introducing targeting ligands, the potential impact of these exogenous modifications on immune system recognition is often overlooked. The physicochemical properties of surface ligands (eg., hydrophilicity, hydrophobicity, charge

distribution) may be recognized by serum opsonins (such as complement proteins or immunoglobulins), thereby accelerating phagocytic clearance.¹⁰⁵ To achieve active targeting while evading immune recognition, a dual surface engineering strategy has been developed. For example, tumor-targeting ligands are not only modified on the exosome surface but also co-expressed with the CD47 molecule. CD47, as a potent “don’t eat me” signal, binds to the SIRP α receptor on macrophage surfaces, activates SHP-1/2 phosphatase to emit inhibitory signals, and directly blocks phagocytosis.¹⁰⁶ This strategy effectively prolongs the circulation half-life of target-expressed exosomes in the bloodstream, providing sufficient time windows for their penetration of vascular barriers into the tumor microenvironment. However, it must be noted that if ligand modification density is excessively high or cross-linking agents are immunogenic, acquired immune responses may still be induced. Therefore, future designs of target-expressed exosomes must strike an precise balance between “maximizing targeting affinity” and “minimizing immunogenicity”.¹⁰⁷

Engineering Strategies: Targeted Efficacy, and Safety of Exosome Delivery

With ongoing advancements in biotechnology, various strategies for engineering exosomes have emerged. A major frontier in this domain is the incorporation of macromolecular nucleic acids and genome editing tools. Among them, gene editing techniques, particularly the CRISPR-Cas9 system, allow researchers to edit specific genes, enabling the manipulation of exosome composition, size, and function to meet specific therapeutic needs. However, loading large molecular cargos (such as Cas9 proteins, large mRNA transcripts, or plasmid DNA) presents significant challenges. Conventional physical encapsulation methods often suffer from low loading efficiency, exosome aggregation, or structural disruption of the lipid bilayer due to the sheer size of these macromolecules.¹⁰⁸

To overcome these barriers, emerging membrane permeabilization and endogenous loading techniques have been rapidly developed. Advanced membrane permeabilization methods, such as microfluidic-assisted gentle electroporation or the use of mild chemical permeabilizing agents (eg., saponin), can transiently destabilize the exosomal membrane, facilitating the entry of large genome-editing tools while preserving vesicle integrity and cargo bioactivity.¹⁰⁹ Alternatively, endogenous loading provides a highly efficient biological solution. By genetically engineering producer cells to co-express specific sorting motifs (such as viral RNA-binding proteins) or by fusing Cas9 to exosome-associated tetraspanins (eg., CD63), macromolecules can be actively recruited into vesicles during biogenesis. Consequently, Cas9 proteins are selectively isolated and incorporated into exosomes, forming complexes with sgRNA.^{110,111} These engineered sgRNA-Cas9-loaded exosomes provide a reliable and effective delivery method that addresses the limitations of traditional viral or lipid-based vectors.¹¹¹⁻¹¹³ Kim et al showed that CRISPR/Cas9-engineered exosomes triggered apoptosis in ovarian cancer cells via PARP-1 suppression, highlighting the therapeutic potential of exosome-based CRISPR delivery. Emerging evidence also demonstrates that CRISPR/Cas9-engineered exosomes targeting PARP-1 markedly augmented the cisplatin responsiveness of SKOV3 ovarian cancer cells and suppressed tumor growth in murine xenograft models.¹¹⁴

Given the potent capabilities of these engineered exosomes—especially those carrying genome-editing tools or nanomaterials—evaluating their targeted efficacy and safety profile is a critical step to ensure their clinical applicability. The therapeutic potential and safety profiles of exosome-based delivery platforms must be systematically assessed via *in vitro* cellular assays and *in vivo* animal models. Jiang L et al engineered exosomes co-loaded with triptolide (TPL) and TRAIL for melanoma-targeted therapy, which effectively inhibited tumor growth while mitigating TPL-induced systemic toxicity, exhibiting excellent biocompatibility.¹¹⁵ Xie et al developed a machine learning surface-enhanced Raman scattering (SERS) platform to quantify HER2 expression dynamics in breast malignancy cells, enabling longitudinal monitoring of drug-loaded exosome efficacy.¹¹⁶ This technology captures temporal changes in cellular viability, supporting data-driven therapeutic decisions. Ultimately, as the development and clinical translation of engineered exosomes accelerate, ethical frameworks must be rigorously applied to safeguard participant welfare.

Comparison to Other Delivery Systems

Although engineered exosomes demonstrate unprecedented advantages in precision delivery, an objective side-by-side comparison of their pros and cons with widely applied traditional carriers (eg., liposomes, viral vectors, and polymers)

can more clearly define their unique niche and current challenges in modern nanomedicine. Viral vectors exhibit high transfection efficiency and long-term gene expression capabilities. However, their clinical application is severely hampered by critical limitations, including high immunogenicity, limited cargo capacity, and the inherent risk of insertional mutagenesis. Synthetic nanocarriers such as liposomes and polymeric nanoparticles represent the most clinically advanced and commercially viable delivery systems. Liposomes, as one of the earliest lipid-based nanoparticle systems, effectively prevent drug dilution, degradation, or inactivation in the bloodstream by encapsulating the drugs. Similarly, polymeric nanoparticles (eg., PLGA) offer highly tunable properties and controlled drug release kinetics. Synthetic carriers, offer significant advantages from a manufacturing perspective, such as highly scalable production, precise control over physicochemical properties, and standardized drug loading capacities. Nevertheless, synthetic drug delivery approaches still encounter multiple hurdles, including inadequate specific targeting, harmful effects from the carriers, the ability to trigger immune responses, and restricted therapeutic effectiveness. For instance, the cationic lipids utilized in LNPs and the acidic degradation byproducts of polymeric materials can induce distinct cellular toxicity and elicit strong innate immune responses. Furthermore, synthetic nanoparticles are prone to rapid clearance by the mononuclear phagocyte system (MPS) and struggle to passively penetrate formidable biological barriers, such as the blood-brain barrier (BBB), without complex and often unstable surface modifications. In conclusion, the clinical application of lipid-based carriers remains challenging, due to issues like low bioavailability, toxicity, rapid clearance from the bloodstream, and the potential for immune responses.

In sharp contrast, exosomes—as naturally occurring vesicular carriers—completely circumvent the risk of mutagenesis and exhibit significantly lower immunogenicity, offering a much safer alternative for systemic administration. Compared to these synthetic counterparts, exosomes offer enhanced biocompatibility and lower immunogenicity. Exosomes, which stem from biological systems, inherently possess enhanced biocompatibility, profound ability to cross the BBB, and highly specific cellular tropism driven by their natural surface proteins (eg., tetraspanins and integrins). Once their delivery mission is accomplished, exosomes are naturally metabolized and eliminated from the body, thereby minimizing long-term accumulation toxicity. Moreover, because of their tiny size, they can effortlessly pass through blood vessel walls and the extracellular matrix. When compared with other delivery systems, exosomes trigger only minor immune reactions. They are also deemed to have the least tumorigenic potential. These characteristics make exosomes a highly promising option for targeted drug delivery.

However, despite these unique biological advantages, the exosome delivery system is not without significant limitations. First, unlike the highly reproducible and massive synthesis of LNPs, exosome production from cell cultures suffers from profoundly low yields, making clinical-scale manufacturing economically and technically daunting. Second, exosomes exhibit significant molecular and structural heterogeneity, heavily depending on the producer cell type and its physiological state. Third, while synthetic carriers easily encapsulate large therapeutic payloads during bottom-up self-assembly, introducing exogenous cargoes into pre-formed exosomes via physical or chemical methods (eg., electroporation) is remarkably inefficient and often compromises vesicle integrity. Finally, the lack of universally standardized, high-yield, and high-purity isolation techniques remains a formidable bottleneck, significantly impeding the translation of engineered exosomes from laboratory concepts to robust clinical therapeutics. Overcoming these manufacturing and standardization bottlenecks remains a critical hurdle before exosomes can fully outcompete mature synthetic delivery platforms in clinical settings (Table 3).

Applications in Cancer Therapy

Exosomes have shown tremendous potential in disease applications, particularly in cancer precision therapy and immunotherapy. They exhibit high heterogeneity in size and molecular composition, making them highly valuable for various applications (Figure 4).

Exosome-Based Therapeutic Delivery

Delivery systems centered around exosomes have showcased considerable promise within the realm of cancer treatment. These systems can encapsulate potent anticancer drugs and be administered through multiple routes, directly targeting cancer cells for specific killing while minimizing damage to normal tissues. Common administration methods contain

Table 3 Comparison of Drug Delivery Systems

Delivery System	Advantages	Disadvantages
Viral Vectors	Extremely high transfection efficiency Stable and long-term gene expression Natural ability to infect cells	High immunogenicity and potential toxicity Risk of insertional mutagenesis Limited payload capacity Complex and costly scale-up production. ¹¹⁷
Liposomes	High biocompatibility and biodegradability Easy to synthesize and scale up Capable of loading both hydrophilic and hydrophobic drugs	Rapid clearance by the mononuclear phagocyte system Lack of inherent targeting capability Potential cytotoxicity of cationic lipids. ^{118,119}
Polymeric Nanoparticles	Highly tunable structural properties Excellent controlled/sustained release profiles Structural stability	Potential cytotoxicity (eg., PEI) Slow degradation may lead to in vivo accumulation. ¹²⁰
Exosomes	Biocompatibility and low immunogenicity Natural biological targeting (homing effect) Ability to cross biological barriers Protected by natural lipid bilayer (prevents payload degradation)	Low extraction yield and high isolation cost Lack of standardized purification and quality control protocols Inherent heterogeneity. ^{121–124}

intravenous infusion, subcutaneous shot, intraperitoneal administration, intratumoral infusion, nasal delivery, and oral administration.^{125–131} All these routes have been proven effective for delivering exosomal drug carriers to target tissues. However, despite the achievements of exosome-based delivery systems in preclinical models, the direct application of exosomes in clinical cancer treatment still faces severe challenges, with the most critical bottleneck being their poor in vivo targeted delivery efficiency and significant off-target effects.^{132,133} Firstly, the actual delivery efficiency of exosomes is severely constrained at the intracellular level by “endosomal entrapment”. Studies have shown that the

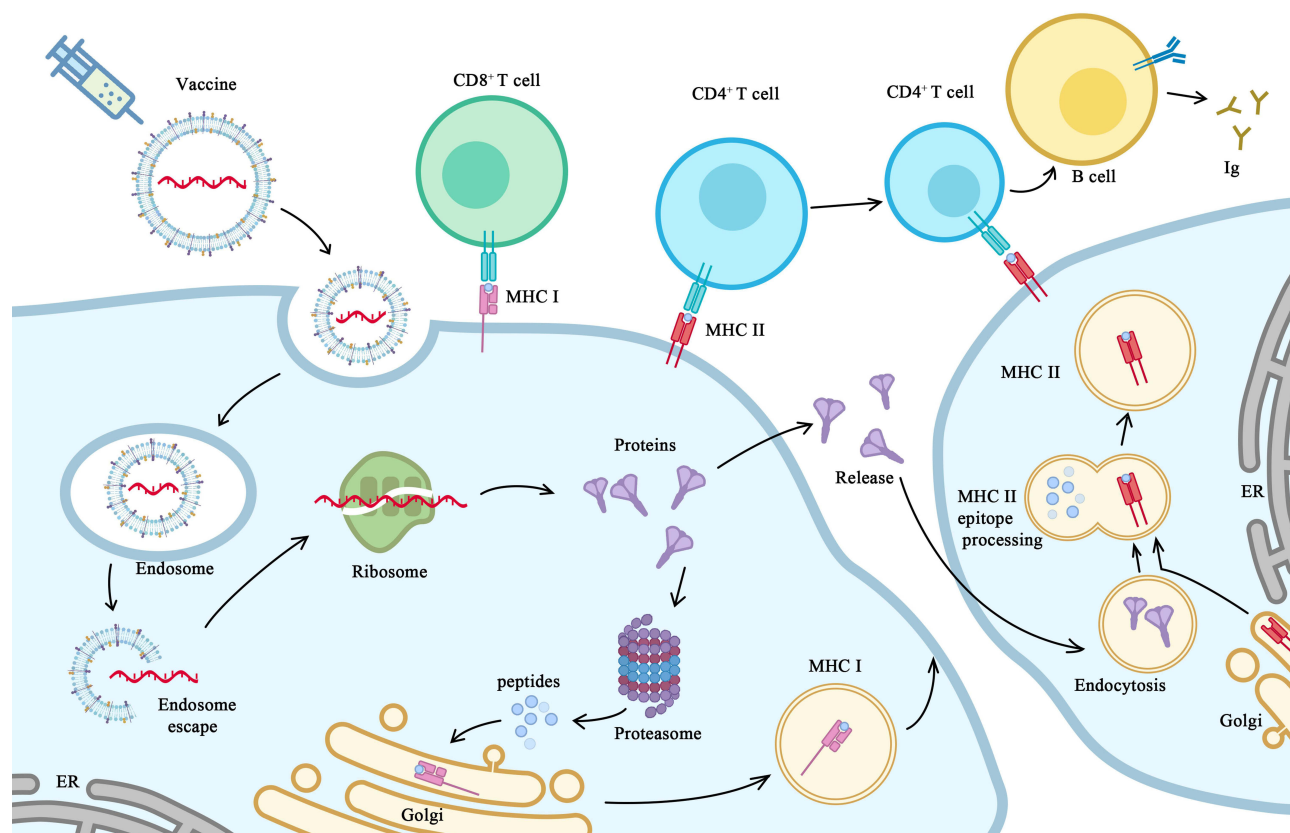


Figure 4 Exosome-Mediated Modulation of Cancer Immune Responses. Exosomes modulate the tumor immune microenvironment through multiple mechanisms, thereby influencing cancer progression and therapeutic responses. Exosomes can carry major MHC I and MHC II molecules as well as tumor-associated antigens (TAAs), which are either taken up by antigen-presenting cells (APCs), such as dendritic cells, or directly interact with T cells. This leads to the activation of CD8⁺ cytotoxic T lymphocytes via MHC I and CD4⁺ helper T cells via MHC II, thus inducing anti-tumor immune responses. The cargoes of exosomes are internalized by recipient cells or APCs through protein-mediated endocytosis or direct fusion with the plasma membrane, facilitating the release of their contents into the cytoplasm.

majority of exosomes internalized by targeted cancer cells are trapped in endosomes and eventually degraded in lysosomes; the proportion of their carried active molecules (especially large RNA or proteins) successfully released into the cytoplasm to exert biological functions is extremely low, greatly reducing the actual therapeutic efficiency of the drugs.¹³⁴ Secondly, off-target effects are the biggest challenge in systemic administration. After systemic administration (such as the most commonly used intravenous injection), the vast majority of natural exosomes are rapidly captured and cleared by the mononuclear phagocyte system (MPS), resulting in a high proportion of the administered dose being highly concentrated in non-target organs such as the liver (Kupffer cells), spleen, and lungs.^{104,135} This significant off-target effect not only greatly reduces the effective therapeutic concentration reaching the tumor site but also easily triggers systemic toxic side effects, such as exosomes loaded with high concentrations of potent chemotherapeutic drugs (such as DOX, PTX) potentially causing severe non-targeted toxic damage to these clearance organs. In addition, the dense extracellular matrix and high interstitial pressure in the tumor microenvironment (TME) further constitute physical barriers, severely limiting the penetration of exosomes into deep tumor tissues and resulting in overall low delivery efficiency.¹³⁶ Therefore, relying solely on the passive targeting of natural exosomes is insufficient to meet the demands of clinical precision treatment. To overcome these translational barriers, advanced surface engineering techniques (such as modifying specific targeting ligands to increase tumor affinity, or introducing “do not eat me” signals like CD47 or surface-attached targeting peptides to evade macrophage phagocytosis and enhance tumor homing) can be utilized to reshape the *in vivo* biodistribution of exosomes, minimizing off-target risks while substantially improving the specific delivery efficiency to tumor sites.

In response to the urgent need to overcome the aforementioned delivery and off-target problems, researchers have developed a variety of exosome systems from specific sources or through modification. For instance, paclitaxel (PTX), a widely used anti-cancer drug, has shown remarkable efficacy, particularly against ovarian cancer, breast cancer, and non-small cell lung cancer. Nevertheless, its use in clinical settings is constrained by factors such as low water solubility, significant toxicity, and the development of drug resistance. Research findings indicate that by delivering PTX through exosomes secreted by M1-type macrophages, it is possible to effectively avoid non-specific clearance, enhance the accumulation of the drug in tumor tissues, activate the NF- κ B pathway, thereby enhancing the tumor immune response, reducing the toxicity of PTX and improving its bioavailability. This dual - action mechanism not only bolsters the immune response against tumors but also mitigates the toxicity of PTX while enhancing its availability in the body.¹³¹ Kim et al showed that paclitaxel-loaded exosomes preferentially accumulated in tumor tissues, enabling targeted drug release while minimizing cytotoxicity and immunogenic responses.¹³⁷ Moreover, when PTX is encapsulated within exosomes derived from cerebral endothelial cells, it gains the ability to effectively cross the cerebrovascular barrier and reach glioblastoma cells. This highlights the remarkable targeting abilities of such exosome - based PTX delivery systems.¹³⁸ Wei et al found that exosomes loaded with doxorubicin (DOX) exhibited a significantly higher IC50 in cardiomyocyte H9C2 cells compared to free DOX, suggesting that exosomes as carriers can substantially mitigate the cardiac toxicity of DOX.¹³⁹ Cisplatin is a commonly used chemotherapy drug. Research indicates that exosomes can deliver cisplatin in a way that prevents endosomal entrapment, reducing drug resistance. In addition, it has been demonstrated that exosomes obtained from M1 macrophages can work in a synergistic manner to boost the therapeutic outcomes of cisplatin in a mouse - based lung cancer model.¹⁴⁰ Exosomes can also deliver antisense oligonucleotides, lncRNAs, shRNAs, and circRNAs to modulate gene expression and inhibit tumor progression. For example, exosome-delivered lncRNA can inhibit the progression of bladder cancer, while exosomes loaded with circRNA BTG, an anti-proliferative factor, can inhibit the growth and spread of glioma cells.^{141,142} Targeted delivery by exosomes significantly reduces the chances of tumor recurrence and metastasis, paving the way for long-term cancer treatment.

In addition to cancer therapy, engineered exosomes show broad potential in other areas, such as neurodegenerative diseases and autoimmune disorders.^{143–145} Exosomes have the ability to traverse physiological barrier, transporting therapeutic substances straight to impaired neurons. This process subsequently facilitates neuronal repair and regeneration. Exosomes can cross the blood-brain barrier, delivering therapeutic molecules directly to damaged neurons, thus promoting neuronal repair and regeneration.¹⁴⁶ For example, in Alzheimer’s disease (AD) research, one study demonstrated that engineered exosomes could enhance the delivery efficiency of antibodies, significantly reducing amyloid

plaque deposition in animal models and improving cognitive function. This research not only provides new directions for Alzheimer's disease therapy but also offers novel approaches for treating other neurological disorders.¹⁴⁷

Exosomes from Diverse Cells in Immune Regulation

In the complex and challenging field of cancer therapy, the regulatory role of the immune system is increasingly gaining attention, with exosomes emerging as a critical intercellular communication mediator. Exosomes secreted by various cellular sources are enriched with bioactive molecules and significantly contribute to modulating cancer-related immune responses.

Exosomes originating from immune cells dynamically modulate the tumor-associated immune landscape. Intercellular signaling among immune cells, including T lymphocytes, macrophages, dendritic cells (DCs), and B lymphocytes. Immune cell-derived exosomes can avoid elimination by the immune system, extending their retention time in peripheral circulation.¹³⁰ CD4⁺T cell-derived exosomes markedly potentiate B cell activation in a dosage-sensitive fashion, subsequently elevating antibody synthesis directed against the hepatitis B surface antigen (HBsAg).¹⁴⁸ Shaobo Ruan et al utilized near-UV LED light (365 nm) to stimulate dendritic cells to secrete exosomes, significantly improving the exosome production rate while maintaining good biocompatibility and immunogenicity. Immunological markers of exosomes, such as CD9 and CD63, were detected using ExoView, revealing that most exosomes expressed CD9, and the number of CD63⁺ exosomes increased. This suggests that light induction has minimal impact on immune marker expression, efficiently inducing the production of immunologically active exosomes, providing a foundation for exosome-based immunotherapies.¹⁴⁹ Exosome-based delivery of MSC-derived exosomes has also been investigated for enhancing the treatment of acute myocardial infarction (AMI). Huang et al found that myocardial delivery of exosomes improved cardiac function, reduced infarct area, and promoted angiogenesis compared to treatment with either exosomes or MSCs alone.¹⁵⁰

Exosomes originating from non-T cell sources also demonstrate multifaceted roles in tumor-associated immune modulation. Specifically, tumor-secreted exosomes can encapsulate particular antigens and RNA, thereby fostering anti-tumor immune responses. Conversely, these exosomes may induce T cell apoptosis, impede maturation of monocytes and establish an inflammatory cancer microenvironment.¹⁵¹ Whitehead et al identified tumor-derived exosomes capable of activating cytotoxic T lymphocytes (CTLs) and suppressing the progression of mastocytomas and breast carcinomas.¹⁵² Additionally, tumor-derived exosomes displaying HSP70/bag-4 on their surface can trigger natural killer cell-mediated granzyme B release, resulting in tumor cell apoptosis.¹⁵³ In a separate study, Huang et al engineered exosomes derived from Luc cells by encapsulating immunogenic cell death (ICD) agents, which activated dendritic cells (DCs) and promoted CD8⁺ T cell expansion.¹⁵⁴ Furthermore, exosomes derived from LPS-activated macrophages promote IFN- γ release in reaction to hepatitis B virus surface antigen in mice.¹⁴⁸ Wang's group employed exosomes secreted by M1 macrophages as a delivery platform, leading to 2.3- to 4.1-fold increase in inflammatory signaling molecule expression.¹⁵⁵ Concurrently, these exosomes triggered the activation of CD8⁺ T lymphocytes and natural killer cells. This dual therapeutic approach of "drug killing + immune activation" not only achieved high concentrations of PTX in tumor tissues but also enhanced therapeutic effects by modulating the tumor microenvironment (TME). Exosomes derived from M2 macrophages can strongly induce endothelial cell proliferation and angiogenesis by activating the HIF1AN/HIF-1 α /VEGFA signaling pathway.¹⁵⁶ Exosomes secreted by mesenchymal stem cells (MSCs) are capable of immune modulation and tissue regeneration. They achieve this by stimulating angiogenesis-related mediators within the tumor microenvironment, which in turn facilitates tumor progression. Exosomes derived from breast cancer cells (eg., MDA-MB-231) have been demonstrated to aberrantly promote CD4⁺ T cell activation and upregulate pro-inflammatory cytokines, highlighting their potential as novel immunotherapeutic agents.¹⁵⁷

Exosome-Based Vaccines

Exosomes have significant potential as vaccines in immunology. In contrast to conventional vaccines, exosomes exhibit multiple benefits: minimal toxicity and antigenicity, release antigens for immune activation, and high versatility.¹⁵⁸ Exosomes are particularly advantageous for immunotherapy as they can transport antigens which stimulate cytotoxic T lymphocytes, enabling their use as autologous vaccine candidates. For example, dendritic cell-derived exosomes

Table 4 Clinical Trials for Exosome-Based Cancer Therapies

NCT Number	Exosome Source	Target Disease	Phase	Status	Cargo/Modification
NCT01159288	Dendritic Cells	Non-Small Cell Lung Cancer (NSCLC)	Phase II	Completed	IFN- γ matured (Dex2) loaded with tumor antigens
NCT03608631	Mesenchymal Stem Cells (MSCs)	Pancreatic Cancer	Phase I	Recruiting	KrasG12D siRNA (iExosomes)
NCT01294072	Plant-derived (Grape)	Colon Cancer	Phase I	Completed	Curcumin
NCT04879810	Plant-derived (Ginger)	Inflammatory Bowel Disease (IBD)	Not Applicable	Completed	Curcumin
NCT04747574	TREx™-293 cells	Coronavirus disease 2019 (COVID-19)	Phase I	Recruiting	CD24
NCT05043181	Mesenchymal Stem Cells (MSCs)	Familial Hypercholesterolemia	Phase I	Not yet recruiting	Ldlr mRNA
NCT03384433	Mesenchymal Stem Cells (MSCs)	Cerebrovascular Disorders	Phase I	Recruiting	miRNA-124
NCT03985696	Tumor B cells	Diffuse large B-cell lymphomas (DLBCL)	Phase I	Active	CD20, PD-LI

express MHC- I and II, which are capable of eliciting anti-tumor immune responses. DC-EXO vaccines show tremendous potential in metastatic melanoma and hepatocellular carcinoma.¹⁵⁹ Compared to DC vaccines, which are rapidly cleared by T lymphocytes, DC-derived exosome vaccines have a relatively longer lifespan and have been tested in multiple Phase I clinical trials.^{160–162} To provide a more comprehensive and clear perspective on the clinical translation of exosome-based therapies, Table 4 summarizes representative ongoing and completed clinical trials globally.

In contrast, tumor-secreted exosomes harbor numerous oncogenic microRNAs (miRNAs) that facilitate tumor growth and dissemination. Consequently, with continued refinement, exosome-based vaccines represent a promising innovation in cancer immunotherapy.¹⁶³ Collectively, the application of exosomes as vaccines or delivery vehicles underscores their significant potential in immunology and therapeutic interventions, providing a secure and efficacious alternative to conventional vaccines.

Translational Challenges in Manufacturing and Safety

As study advances and technologies progress, engineered exosomes are expected to have a broader and deeper application in clinical fields. In the future, exosome-based delivery systems are likely to become an efficient and personalized therapeutic option, offering patients more treatment choices and better therapeutic outcomes. Despite exosomes offer superior drug-encapsulation potential, biocompatibility for delivery, the translation process from laboratory to clinic still faces significant challenges and stringent requirements in chemistry, manufacturing, and control (CMC).

From a regulatory and production perspective, achieving scalable, Good Manufacturing Practice (GMP)-compliant production remains a formidable bottleneck. Unlike synthetic nanoparticles, exosomes are intrinsically highly heterogeneous; their molecular cargo heavily depends on the dynamic metabolic state of the producer cells. This biological complexity results in significant batch-to-batch variability. Furthermore, isolating high-purity exosomes from large-scale bioreactors using current techniques (such as ultracentrifugation or size-exclusion chromatography) is costly and inefficient. More importantly, there is a critical regulatory void regarding standardized potency assays. Accurately defining the “active pharmaceutical ingredient” (API) equivalent of an exosome dose—and ensuring its functional consistency across different batches—remains a major unresolved challenge for quality control (QC).^{164–168}

Equally critical are the safety concerns, particularly regarding the long-term biodistribution and potential pro-tumorigenic risks of specific exosome types. Although exosomes are generally considered highly biocompatible, their systemic administration often leads to rapid sequestration by the mononuclear phagocyte system (MPS), resulting in

massive, unintended accumulation in the liver and spleen. Over time, this off-target biodistribution could trigger cumulative organ toxicity. Furthermore, when utilizing tumor-derived exosomes (eg., as vaccines), there are severe inherent risks. These vesicles may carry oncogenic mutations, pro-tumorigenic miRNAs, or immunosuppressive factors (such as PD-L1). Unintended horizontal gene transfer to healthy recipient cells could paradoxically promote pre-metastatic niche formation, accelerate tumor growth, or trigger unwanted neoangiogenesis.^{169–172} Therefore, meticulous molecular profiling and long-term in vivo safety monitoring are imperative before widespread clinical implementation. While advancing engineered exosome technology, ethical issues and regulatory challenges cannot be overlooked. How to promote the rapid translation of therapeutic exosomes while protecting patient rights is an urgent problem to be addressed in the field of precision medicine. Researchers and relevant institutions need to jointly establish corresponding standards and regulations to facilitate their healthy and orderly development.

Conclusions and Future Perspectives

We highlight the translational potential of exosome-based therapies, particularly their emerging applications as autologous vaccines and targeted nanocarriers in recent clinical trials for cancer treatment. We also explicitly address the challenges of off-target effects in vivo, technical barriers in macromolecular drug loading, regulatory and production issues, as well as long-term safety concerns of tumor-derived exosomes. In summary, exosomes, as a novel class of biological therapeutic carriers, have demonstrated significant potential in overcoming biological barriers and achieving targeted delivery, making them a research hotspot in the field of precision oncology. They serve a dual role as both precise diagnostic markers and multifunctional therapeutic vectors. Exosomes inherently possess the ability to cross biological barriers such as the blood-brain barrier, evade immune clearance, and precisely target receptor cells, offering unique advantages over traditional synthetic nanocarriers and viral vectors. Currently, this field is undergoing a critical transition phase, evolving from early phenomenological observations to standardized and regulated clinical applications.^{118,173}

However, as previously mentioned, merely describing biological phenomena is insufficient to drive substantial breakthroughs in the field. To overcome current developmental bottlenecks, it is imperative to establish a standardized system for large-scale exosome production and systematically evaluate their long-term safety in various disease models. Concurrently, integrating cutting-edge technologies is essential for clinical translation: single-cell multi-omics and high-resolution spatial transcriptomics can deeply elucidate the intrinsic heterogeneity of exosome biosynthesis; artificial intelligence and machine learning algorithms can provide predictive modeling for exosome-target interactions, optimize the rational design of engineered vesicles, and lay the foundation for personalized medicine. To fully realize the translational value of exosome therapies, it is necessary to break through existing technological and biological barriers, shifting from single-discipline research to a paradigm of interdisciplinary collaboration. This requires deep integration of chemistry (surface functionalization), nanotechnology (efficient drug loading), oncology (analysis of tumor microenvironment interactions), and bioinformatics (AI-driven target screening). Only through unified multidisciplinary collaboration can exosomes be transformed from a promising biological phenomenon into standardized, safe, and efficient first-line clinical therapeutic carriers. Finally, it is essential to objectively acknowledge the inherent limitations of this review. Given the current explosive growth in exosome-related research, this review primarily focuses on oncology and engineered delivery systems, which may not comprehensively cover cutting-edge advancements in other therapeutic areas such as cardiovascular diseases and neurodegenerative disorders. Future systematic reviews and meta-analyses based on the MISEV standardized guidelines will provide critical support for further consolidating research conclusions and promoting standardized development in this field.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The author(s) report no conflicts of interest in this work.

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