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Exosome-Based Drug Delivery Systems: Biological Properties, Engineering Strategies, and Therapeutic Applications in Cancer and Inflammatory Diseases

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Abstract:

Background: Exosomes are membrane-enclosed nanosized vesicles of endosomal origin, ranging from 30 to 150 nanometers in diameter, that are constitutively secreted by virtually all cell types and serve as intercellular communication vehicles through the transfer of proteins, lipids, messenger RNA, microRNA, and non-coding RNA between donor and recipient cells. Their natural origin, intrinsic biocompatibility, capacity to traverse biological barriers including the blood-brain barrier, low immunogenicity, and ability to carry diverse molecular cargo make exosomes highly attractive as drug delivery vehicles. Unlike synthetic nanoparticles, exosomes carry a biological membrane studded with surface proteins that mediate cell-type-specific recognition, uptake, and biological activity in recipient cells, providing a degree of inherent tissue targeting that must be engineered into synthetic systems from scratch.

Objective: This review critically examines the biogenesis, composition, isolation methods, drug loading strategies, surface engineering approaches, characterization techniques, mechanisms of cellular uptake and cargo delivery, and therapeutic applications of exosomes in cancer therapy, inflammatory disease, and neurological conditions, with emphasis on research published up to 2022.

Results and Discussion: Exosomes derived from mesenchymal stem cells, dendritic cells, macrophages, tumor cells, and plant sources have been investigated as natural drug delivery vehicles. Drug loading by electroporation, sonication, co-incubation, and membrane fusion enables encapsulation of small molecules, nucleic acids, and proteins. Surface engineering with targeting ligands, cell-penetrating peptides, and antibody fragments enhances organ and cell-type specificity. Exosome-based delivery of doxorubicin, siRNA, miRNA, and therapeutic proteins has demonstrated superior tumor accumulation, blood-brain barrier penetration, and therapeutic efficacy compared to equivalent free drug or synthetic nanoparticle formulations in preclinical cancer and CNS disease models.

Conclusion: Exosomes represent a biologically sophisticated drug delivery platform whose natural origin confers inherent advantages in biocompatibility, immune evasion, and barrier penetration that are difficult to replicate with synthetic systems. Significant manufacturing, standardization, and regulatory challenges must be addressed before exosome-based drug delivery products can realize their considerable clinical potential.

Keywords: *Exosomes; extracellular vesicles; drug delivery; cancer therapy; siRNA delivery; blood-brain barrier; biomimetic nanocarriers; mesenchymal stem cell exosomes; exosome engineering; tumor targeting*

1. Introduction

Extracellular vesicles are a heterogeneous population of membrane-bound particles released by cells into the extracellular space, classifiable by their size, biogenesis, and surface marker composition into three principal subtypes: exosomes (30 to 150 nm, endosomal origin), microvesicles (100 to 1000 nm, plasma membrane budding), and apoptotic bodies (500 to 5000 nm, apoptotic cell fragmentation). Among these, exosomes have attracted the greatest scientific and pharmaceutical interest as drug delivery vehicles owing to their nanoscale dimensions, homogeneous biogenesis pathway, defined surface proteome, and remarkable capacity to transfer functional molecular cargo between cells across biological distances [1,2].

The discovery that exosomes carry functional RNA — demonstrated by Valadi and colleagues in 2007, who showed that mRNA and miRNA transferred between mast cells via exosomes could be translated or could regulate gene expression in recipient cells — transformed the understanding of exosomes from metabolic waste disposal vehicles to active mediators of intercellular communication. Subsequent work revealed that tumor-derived exosomes contribute to pre-metastatic niche formation, immune suppression, and drug resistance in the tumor microenvironment, while exosomes from mesenchymal stem cells carry regenerative, anti-inflammatory, and neuroprotective molecules that mediate many of the paracrine therapeutic effects attributed to stem cell therapy [3,4].

The pharmaceutical exploitation of exosomes as drug delivery vehicles is motivated by a set of inherent biological properties that synthetic nanocarriers have struggled to replicate despite decades of engineering effort. Exosomes circulate in blood with half-lives of hours to days — substantially longer than most synthetic nanoparticles of equivalent size, which are cleared within minutes to hours by complement opsonization and phagocytic uptake. This extended circulation is attributed to the surface display of CD47, a transmembrane protein that engages SIRPα receptors on phagocytes and delivers a molecular don't-eat-me signal that inhibits phagocytosis. The exosomal lipid bilayer, composed of phosphatidylcholine, sphingomyelin, phosphatidylserine, and cholesterol in proportions similar to the plasma membrane, is fundamentally different from the synthetic lipid bilayers of liposomes, providing greater membrane stability and resistance to osmotic disruption [5,6].

The most compelling pharmacological advantage of exosomes for drug delivery is their demonstrated capacity to cross biological barriers that synthetic nanoparticles traverse only with difficulty or not at all. Exosomes derived from neurons and dendritic cells have been shown to cross the blood-brain barrier through transcytosis mediated by surface proteins including LAMP2b fused with rabies virus glycoprotein-derived peptide, enabling delivery of siRNA and other cargo to brain parenchymal cells in rodent models. Tumor-derived exosomes preferentially home to the organ of origin through surface integrin expression patterns that match the organ's vascular bed surface markers, suggesting mechanisms of organ-selective delivery that go beyond simple size-based EPR accumulation [7,8].

This review systematically examines the biogenesis and molecular composition of exosomes, their isolation and characterization methodologies, strategies for drug loading and surface engineering, mechanisms of cellular uptake and intracellular cargo delivery, and therapeutic applications across cancer, neurological disorders, inflammatory diseases, and cardiovascular conditions. A critical analysis addresses the manufacturing scalability problem, the significant methodological inconsistencies in the exosome literature, and the regulatory landscape for exosome-based therapeutics.

2. Scientific Background

2.1 Exosome Biogenesis

Exosome biogenesis proceeds through the endosomal pathway in a highly regulated multistep process. Following endocytosis of extracellular material or internalization of plasma membrane domains, early endosomes mature into late endosomes, also termed multivesicular bodies (MVBs), characterized by the inward budding of the limiting endosomal membrane to generate intraluminal vesicles (ILVs) within the MVB lumen. This inward budding is driven primarily by the endosomal sorting complex required for transport (ESCRT) machinery, comprising four complexes (ESCRT-0, -I, -II, -III) and associated proteins including VPS4, TSG101, ALIX, and HRS, which sequentially recognize ubiquitylated membrane proteins, deform the endosomal membrane, and pinch off ILVs into the MVB lumen. Lipid raft microdomains enriched in ceramide and lysobisphosphatidic acid provide an ESCRT-independent pathway for ILV budding [9,10].

When MVBs fuse with the plasma membrane — a process regulated by Rab GTPases including Rab27a, Rab27b, Rab35, and Rab11, as well as SNARE proteins — the ILVs are released into the extracellular space as exosomes. MVBs can alternatively be directed to lysosomes for degradation, and the balance between these two fates determines the quantity of exosomes secreted. Cellular stress, calcium

ionophore treatment, hypoxia, and p53 activation all promote exosome secretion by shifting the balance toward MVB-plasma membrane fusion [11].

2.2 Molecular Composition of Exosomes

Exosomes carry a characteristic molecular cargo that reflects both their endosomal biogenesis and the functional state of the parent cell. Tetraspanins CD9, CD63, and CD81 are the canonical exosomal surface markers and are incorporated during ILV budding; their surface expression on exosome membranes is used diagnostically to confirm exosomal identity. Heat shock proteins HSP70 and HSP90, flotillin-1, ALIX, and TSG101 are consistently present in exosomes from diverse cell types and serve as pan-exosomal protein markers. The lipid composition of exosome membranes is enriched in sphingomyelin, cholesterol, and phosphatidylserine relative to the parent cell plasma membrane, and this composition contributes to membrane rigidity and vesicle stability [12,13].

The RNA cargo of exosomes comprises messenger RNA, microRNA, long non-coding RNA, circular RNA, and small nuclear RNA in proportions that reflect the RNA landscape of the parent cell. miRNAs are selectively sorted into exosomes — certain miRNA sequences are enriched or depleted in exosomes relative to their cellular concentration, suggesting active sorting mechanisms involving RNA-binding proteins including hnRNPA2B1, Y-box protein 1, and SYNCRIP. The functional significance of exosomal RNA transfer has been demonstrated in multiple studies showing that exosomal miRNA delivered to recipient cells modulates target gene expression with consequences for proliferation, differentiation, immune activation, and metastatic behavior [14].

2.3 Exosomes as Natural Drug Delivery Vehicles

The properties that make exosomes effective natural intercellular communication vehicles — nanoscale dimensions, lipid bilayer enclosure, surface protein-mediated cellular recognition, capacity to traverse biological barriers, and resistance to immune clearance — collectively position them as biologically sophisticated drug delivery vehicles. The surface CD47 display provides inherent stealth properties that extend circulation half-life without the immunogenicity risks associated with PEGylation. The exosomal membrane's natural origin eliminates the concerns regarding synthetic polymer biocompatibility and degradation product toxicity that apply to PLGA, PEI, and other synthetic nanocarrier materials [15].

3. Classification of Exosome-Based Drug Delivery Systems

3.1 By Parent Cell Source

The parent cell from which exosomes are derived critically determines their surface proteome, endogenous cargo, baseline biodistribution, and therapeutic properties. Mesenchymal stem cell (MSC)-derived exosomes carry anti-inflammatory, regenerative, and immunomodulatory molecules including TGF-beta, hepatocyte growth factor, IDO, and miR-21, making them inherently therapeutic vehicles that have been investigated for tissue repair, stroke recovery, renal injury protection, and graft-versus-host disease. Dendritic cell-derived exosomes express MHC class I and II molecules and co-stimulatory proteins T-cell ligands and represent natural cancer vaccine delivery vehicles. Macrophage-derived exosomes have been exploited for anti-inflammatory drug delivery and for crossing the blood-brain barrier through the natural trafficking of macrophages across the blood-brain barrier. Tumor cell-derived exosomes homologously return to tumor cells through integrin-mediated homing, providing inherent tumor targeting for repurposed tumor exosomes loaded with cytotoxic payloads [16,17].

3.2 By Engineering Strategy

Exosome-based drug delivery systems are classified by the degree and nature of engineering applied. Natural exosomes are collected from conditioned cell culture medium without modification and used directly as therapeutic agents, exploiting their endogenous cargo. Cargo-loaded exosomes are natural exosomes into which exogenous drugs, nucleic acids, or proteins have been incorporated by post-isolation loading methods. Surface-engineered exosomes have targeting ligands, antibodies, or cell-penetrating peptides displayed on their membrane surface to redirect biodistribution or enhance specific cellular uptake. Hybrid exosome-liposomes are created by membrane fusion between exosomes and synthetic liposomes, combining the biological targeting properties of the exosomal membrane with the drug loading capacity and compositional control of synthetic lipid systems. Exosome-mimetic nanovesicles are produced by serial extrusion of cells through membranes of decreasing pore size, generating vesicles with exosome-like size, membrane composition, and surface proteins but at yields orders of magnitude higher than biological exosome production [18,19].

3.3 By Therapeutic Cargo

Exosomes have been loaded with small molecule chemotherapeutics including doxorubicin, paclitaxel, and curcumin; nucleic acid payloads including siRNA, miRNA, antisense oligonucleotides, and CRISPR-Cas9 ribonucleoprotein complexes; therapeutic proteins including catalase, TRAIL, and cytokines; and combinations of the above. The physicochemical properties of the cargo — molecular weight, hydrophilicity, charge, stability — determine the most appropriate loading method and influence encapsulation efficiency and retention during storage and systemic circulation [20].

Table 1: Classification of exosome-based drug delivery systems by parent cell source, surface markers, cargo, and therapeutic applications

Source Cell	Key Surface Markers	Endogenous Cargo	Therapeutic Properties	Drug Delivery Applications
Mesenchymal stem cells	CD44, CD73, CD90, CD105	miR-21, miR-126, TGF-β, HGF	Anti-inflammatory, regenerative	Stroke, cardiac repair, GvHD, kidney injury
Dendritic cells	MHC I/II, CD80, CD86, CD9	Peptide-MHC complexes, cytokines	Immune activation, antigen presentation	Cancer vaccines, immunotherapy
Macrophages	CD14, CD68, integrin α4β1	Anti-inflammatory cytokines, miRNA	Immune modulation, BBB penetration	CNS drug delivery, neuroinflammation
Tumor cells	Integrins (tissue-specific), PD-L1	Oncogenic proteins, miRNA	Homotypic tumor targeting	Re-engineered for cancer drug delivery
Milk (bovine / human)	CD9, CD63, β-casein	Bioactive proteins, miRNA	Oral bioavailability, gut protection	Oral drug and siRNA delivery
Plant-derived (ginger, grape)	Lipids, plant proteins	Plant miRNA, lipids	Anti-inflammatory, colon targeting	Oral IBD therapy, siRNA delivery

MSC: mesenchymal stem cell; HGF: hepatocyte growth factor; TGF-β: transforming growth factor-beta; GvHD: graft-versus-host disease; BBB: blood-brain barrier; IBD: inflammatory bowel disease.

4. Formulation Design and Development

4.1 Parent Cell Selection and Culture Conditions

The selection of parent cell type for exosome production is the foundational formulation decision and determines the baseline biodistribution, immunological properties, and endogenous cargo of the produced exosomes. For cancer drug delivery applications, the parent cell must ideally produce exosomes with tumor-homing surface proteins — tumor-derived exosomes expressing tissue-specific integrins, macrophage-derived exosomes capable of crossing the blood-brain barrier, or HER2-overexpressing cells producing exosomes with natural affinity for HER2-positive tumor cells. The culture conditions of the parent cell — serum concentration, hypoxia, cytokine stimulation, passage number, and cell density — substantially alter the proteome, lipidome, and RNA cargo of secreted exosomes, requiring standardization of production conditions to achieve consistent exosome characteristics [21,22].

Serum-free or exosome-depleted serum media are essential for production of exosomes intended for drug delivery applications, as bovine serum used in standard cell culture contains abundant exosomes of bovine origin that contaminate the preparation and can confound drug loading, biodistribution, and therapeutic effect measurements. Exosome-depleted fetal bovine serum, prepared by ultracentrifugation of serum at 100,000 × g for 18 hours before use, is the standard approach, though serum-free or protein-free culture media are preferred for clinical-grade exosome production to eliminate animal-derived material [23].

4.2 Drug Loading Strategies

Passive co-incubation, the simplest loading approach, involves mixing exosomes with drug in solution and relying on concentration gradient-driven diffusion of hydrophobic drug molecules into the exosomal lipid

bilayer or hydrophilic drug into the exosomal lumen. This method achieves loading efficiencies of 5 to 20% for hydrophobic drugs including curcumin, paclitaxel, and doxorubicin whose logP values favor partitioning into the lipid membrane, but is poorly efficient for hydrophilic molecules and macromolecules [24].

Electroporation uses brief electrical pulses to create transient nanopores in the exosomal membrane through which nucleic acids and other hydrophilic molecules can enter the exosome lumen. It is the most widely applied method for siRNA and miRNA loading, with encapsulation efficiencies of 20 to 40% reported in published studies. However, electroporation causes exosome aggregation and siRNA aggregation that reduce functional delivery, and multiple studies have found that the apparent siRNA encapsulation measured after electroporation is largely attributable to siRNA adsorbed to the exosome surface or aggregated with exosomes rather than truly encapsulated within the lumen [25,26].

Sonication involves exposing exosome-drug mixtures to ultrasonic energy that transiently disrupts and reassembles the exosomal membrane, enabling entrapment of hydrophilic and hydrophobic cargo with loading efficiencies of 20 to 50%. Compared to electroporation, sonication causes less membrane aggregation and achieves more uniform drug distribution, though it can alter exosomal surface protein conformation and biological activity. Saponin permeabilization, using the detergent-like saponin to transiently increase exosomal membrane permeability for passive drug loading, achieves high loading efficiencies of 40 to 60% but causes cholesterol depletion from the exosomal membrane that may alter biodistribution and cellular uptake properties [27].

4.3 Surface Engineering Strategies

Active targeting of exosomes to specific cell types or organs requires display of targeting ligands on the exosomal surface through covalent conjugation, membrane fusion, or genetic engineering of the parent cell to express fusion proteins on the exosomal surface. Genetic engineering — expressing targeting peptides or antibody fragments as genetic fusions with exosomal surface proteins including LAMP2b, Tspan, or PTGFRN in the parent cell — produces exosomes that constitutively display the targeting moiety with appropriate membrane topology, without requiring post-isolation chemical modification that can damage the exosomal membrane or obscure endogenous surface proteins [28,29].

The rabies virus glycoprotein peptide RVG29, which binds the nicotinic acetylcholine receptor expressed on neurons, has been the most extensively investigated targeting peptide for brain-targeted exosome delivery. Expression of RVG-LAMP2b fusion protein in dendritic cells produces exosomes displaying RVG29 on their surface that, when administered intravenously, achieve substantially greater brain accumulation compared to unmodified exosomes and deliver functional siRNA cargo to neurons and microglia [30].

5. Isolation and Preparation Methods

5.1 Differential Ultracentrifugation

Differential ultracentrifugation is the most widely used and historically established method for exosome isolation from conditioned cell culture medium or biological fluids. The protocol involves sequential centrifugation steps at increasing gravitational forces: 300 × g (10 minutes) to remove cells, 2,000 × g (20 minutes) to remove cell debris and apoptotic bodies, 10,000 × g (30 minutes) to pellet microvesicles and large vesicles, and finally 100,000 × g (70 to 120 minutes) to pellet exosomes. The 100,000 × g pellet is typically washed by resuspension and re-centrifugation to remove co-pelleted protein aggregates. This method achieves high exosome yield but poor selectivity, as protein aggregates and non-vesicular particles of similar sedimentation coefficient co-pellet with exosomes [31].

5.2 Density Gradient Ultracentrifugation

Density gradient ultracentrifugation using iodixanol (OptiPrep) or sucrose gradients separates exosomes from co-pelleted protein aggregates and other contaminating particles on the basis of buoyant density. Exosomes have a characteristic buoyant density of 1.13 to 1.19 g/mL in sucrose gradients, distinct from protein aggregates (density > 1.22 g/mL) and lipoproteins (density < 1.12 g/mL). This method provides the highest purity of isolated exosomes but requires long centrifugation times (16 to 18 hours) and specialized equipment, limiting its throughput for large-scale production [32].

5.3 Size Exclusion Chromatography

Size exclusion chromatography using porous agarose or cross-linked dextran columns separates exosomes from smaller proteins and nucleic acids based on hydrodynamic size. Exosomes elute in the void volume of the column in the first fractions, well-separated from smaller protein contaminants that enter and are retarded

within the porous beads. SEC produces high-purity exosome preparations with preserved vesicle structure and surface protein functionality, and is particularly well-suited for clinical-grade exosome preparation owing to its compatibility with aseptic processing and scalability. SEC-isolated exosomes demonstrate superior functional activity in cellular uptake and drug delivery studies compared to differential ultracentrifugation-isolated preparations, attributed to the removal of protein aggregates that compete for cellular uptake [33].

5.4 Precipitation-Based Methods

Polymer precipitation using polyethylene glycol (PEG) at concentrations of 8 to 12% w/v causes exosomes to precipitate from conditioned medium at low centrifugation speeds (1,500 to 10,000 × g), enabling isolation without ultracentrifuge equipment. Commercial precipitation reagents including ExoQuick and Total Exosome Isolation achieve rapid isolation suitable for exploratory research but co-precipitate large amounts of non-vesicular protein aggregates, lipoprotein particles, and nucleic acid complexes, producing low-purity preparations with high contamination that is problematic for functional and drug delivery studies. For in vivo applications, the presence of PEG and contaminating proteins in precipitation-isolated exosome preparations represents an important confound that should preclude their use without further purification steps [34].

5.5 Exosome-Mimetic Nanovesicle Production

Exosome-mimetic nanovesicles are produced by serial extrusion of donor cells through polycarbonate membranes of decreasing pore size (10, 5, and 1 micrometer) under controlled pressure, mechanically fragmenting cells into membrane vesicles that resemble exosomes in size, lipid composition, and surface protein display. This approach produces vesicle yields 100- to 1000-fold higher than biological exosome secretion from the same cell number, addressing the major yield limitation of conventional exosome production. Drug loading can be achieved by incubating drugs with cells before extrusion, resulting in high drug-to-vesicle ratios. The extruded vesicles retain the surface protein complement of the parent cell, including CD47, targeting integrins, and receptor-binding proteins, providing similar immune evasion and targeting properties to authentic exosomes [35].

6. Characterization and Evaluation

6.1 Physical Characterization

Nanoparticle tracking analysis is the preferred method for simultaneous measurement of exosome concentration and size distribution, tracking the Brownian motion of individual vesicles under laser illumination to calculate hydrodynamic diameter and generate particle concentration values in particles per milliliter. Authentic exosome preparations from cell culture supernatants typically contain 10^8 to 10^{11} particles per milliliter with a size distribution mode between 80 and 150 nm. Dynamic light scattering provides a complementary intensity-weighted mean diameter and PDI but is susceptible to interference from large aggregates and does not provide particle concentration. Cryo-electron microscopy provides definitive morphological characterization, confirming the characteristic cup-shaped or spherical morphology of exosomes with an intact lipid bilayer visible at high magnification [36].

6.2 Molecular Characterization

Western blotting for canonical exosomal markers CD9, CD63, CD81, TSG101, and Alix is required to confirm the exosomal identity of isolated preparations. The MISEV2018 guidelines (Minimal Information for Studies of Extracellular Vesicles) require reporting of at least one transmembrane protein, one cytosolic protein, and absence of endoplasmic reticulum and Golgi markers (calnexin, GM130) to confirm exosome purity and distinguish exosomes from contaminating cellular debris. Nano flow cytometry enabling individual vesicle characterization for multiple surface markers simultaneously is an emerging characterization tool that provides population-level surface marker distribution data unobtainable by bulk analysis methods [37].

6.3 Drug Loading Characterization

Drug loading efficiency and encapsulation within exosomes rather than surface adsorption are critical distinctions that require careful characterization. Size exclusion chromatography or membrane ultrafiltration is used to separate free unencapsulated drug from exosomes before quantification of total drug content by HPLC or fluorescence spectrophotometry. The ratio of drug detected in exosome fractions after SEC separation to total drug added defines the true encapsulation efficiency. Surface-adsorbed drug, which would

be released immediately upon dilution in biological fluids and would not benefit from exosome-mediated delivery, must be distinguished from lumenally encapsulated drug, which is protected from degradation and released only intracellularly [38].

6.4 Functional and Biological Evaluation

The biological activity of drug-loaded exosomes is evaluated in cellular uptake studies, endosomal escape imaging, and cytotoxicity assays. Cellular uptake of fluorescently labeled exosomes (PKH26, DiI, DiO-labeled membranes or CFSE-labeled luminal contents) is quantified by flow cytometry and visualized by confocal microscopy. Co-localization of exosome signal with endosomal markers (EEA1, Rab7) and lysosomal markers (LAMP1) versus cytoplasmic localization is assessed to determine the intracellular trafficking fate. Functional delivery of siRNA cargo is confirmed by measuring target gene mRNA and protein levels by RT-PCR and western blotting in recipient cells [39].

6.5 In Vivo Biodistribution and Targeting

In vivo biodistribution of exosomes is assessed by intravenous administration of fluorescently or bioluminescently labeled exosomes in mouse models, followed by ex vivo organ imaging or intravital fluorescence microscopy. Radiolabeled exosomes tracked by SPECT or PET imaging provide quantitative whole-body biodistribution data. Parent cell-specific exosomes are assessed for organ selectivity — tumor-derived exosomes for tumor homing, macrophage-derived exosomes for brain penetration, MSC-derived exosomes for inflamed tissue accumulation. The drug targeting efficiency in tumor or target organ tissue versus plasma is calculated and compared to equivalent liposomal or free drug controls to quantify the targeting advantage of exosomal delivery [40,41].

7. Mechanism of Drug Delivery

7.1 Cellular Uptake Mechanisms

Exosomes are internalized by recipient cells through multiple endocytic pathways whose relative contributions depend on exosome surface protein composition, recipient cell type, and the physicochemical properties of the exosome membrane. Clathrin-mediated endocytosis is the predominant pathway for exosomes bearing ligands for endocytic receptors; macropinocytosis is prominent in macrophages and dendritic cells; phagocytosis is the primary mechanism in professional phagocytes; and membrane fusion with the plasma membrane, while a minor pathway, enables direct cytoplasmic delivery of exosomal contents without endosomal trafficking. Phosphatidylserine exposure on the exosomal outer membrane engages Tim4, BAI1, and other phosphatidylserine receptors on phagocytes to initiate recognition and uptake [42].

The cell-type specificity of exosome uptake is governed by the pattern of surface ligand-receptor interactions between the exosome membrane proteins and the receptor complement of the recipient cell. Tumor-derived exosomes preferentially fuse with tumor cells of the same histological origin through homotypic tetraspanin-tetraspanin interactions. Dendritic cell-derived exosomes displaying MHC-peptide complexes engage T cell receptors on cognate T cells with high specificity. These receptor-ligand interactions provide a biological basis for cell-type-specific exosome uptake that is being deliberately exploited in surface engineering strategies to redirect exosome biodistribution [43].

7.2 Intracellular Trafficking and Cargo Release

Following endocytosis, exosomes are initially localized in early endosomes and subsequently trafficked to late endosomes and lysosomes, where the acidic pH and hydrolytic enzyme activity can degrade both the exosomal membrane and the encapsulated cargo. The proportion of exosomes that escape endosomal degradation and deliver their contents to the cytoplasm is the critical determinant of nucleic acid delivery efficiency. Unlike synthetic lipid nanoparticles that rely on ionizable lipid-mediated endosomal disruption for escape, exosomes are believed to achieve endosomal escape through membrane fusion facilitated by pH-induced conformational changes in surface fusion proteins. However, the molecular mechanisms of exosomal endosomal escape are incompletely characterized and represent an important area of ongoing investigation [44].

7.3 Blood-Brain Barrier Transcytosis

The ability of certain exosome subtypes — particularly those derived from neurons, brain endothelial cells, and macrophages — to cross the blood-brain barrier is attributed to receptor-mediated transcytosis through brain capillary endothelial cells. RVG-displaying exosomes bind nicotinic acetylcholine receptors on brain endothelial cells and are transcytosed across the barrier in a clathrin-dependent manner, releasing their

contents on the abluminal side into the brain parenchyma. Natural MSC-derived exosomes have been reported to cross the blood-brain barrier following intravenous administration in stroke models, with brain accumulation significantly greater than equivalent sized liposomes, attributed to surface proteins mediating endothelial cell recognition and transcytosis [45].

8. Therapeutic Applications

8.1 Cancer Chemotherapy

Exosome-mediated delivery of doxorubicin has been extensively investigated as a means of improving the therapeutic index of this widely used anthracycline by exploiting exosomal tumor tropism and reduced immune recognition relative to synthetic nanoparticles. Doxorubicin-loaded exosomes prepared by electroporation demonstrated superior tumor accumulation and reduced cardiac toxicity compared to free doxorubicin and equivalent liposomal doxorubicin in murine breast cancer models, attributed to the exosomal surface CD47-mediated phagocyte evasion and tumor-homing properties. A landmark study by Tian and colleagues reported that exosomes derived from immature dendritic cells loaded with doxorubicin and surface-modified with an αv integrin-binding peptide achieved tumor-targeted doxorubicin delivery in vivo with significantly improved tumor growth inhibition compared to free doxorubicin [46].

8.2 Nucleic Acid Delivery for Gene Therapy and Gene Silencing

The delivery of siRNA, miRNA, and antisense oligonucleotides to cancer cells and neurons represents one of the most compelling applications of exosome-based drug delivery, exploiting the natural RNA-carrying capacity of exosomes and their ability to cross biological barriers that exclude synthetic RNA delivery systems. Alvarez-Erviti and colleagues demonstrated in a seminal 2011 study that dendritic cell-derived exosomes engineered to display RVG on their surface and loaded with siRNA against BACE1 (a beta-secretase implicated in Alzheimer's disease pathology) via electroporation achieved specific knockdown of BACE1 mRNA and protein in the brains of mice following intravenous administration, without detectable off-target silencing in peripheral tissues [47].

Tumor-suppressive miRNAs that are downregulated in cancer, including miR-34a, miR-21 inhibitor, miR-126, and let-7, have been loaded into exosomes for cancer therapy. MSC-derived exosomes loaded with miR-146b demonstrated significant glioma growth inhibition in orthotopic mouse glioma models following intranasal administration, providing both a CNS-delivery approach and an anti-tumor miRNA payload within a single biologically derived carrier. The tumor-suppressive activity was attributable to miR-146b targeting of epidermal growth factor receptor signaling in glioma cells [48].

8.3 Cancer Immunotherapy and Vaccine Delivery

Dendritic cell-derived exosomes expressing MHC class I and II molecules in complex with tumor-specific peptide antigens, together with co-stimulatory molecules CD80 and CD86, function as cell-free cancer vaccines that activate cytotoxic T lymphocytes and helper T cells against tumor-associated antigens. This application was validated in early clinical trials in the mid-2000s in patients with metastatic melanoma and non-small cell lung cancer, where autologous dendritic cell-derived exosomes loaded with tumor antigen peptides were well tolerated and demonstrated evidence of T cell activation in a proportion of patients. Tumor-derived exosomes expressing PD-L1 on their surface contribute to systemic immune suppression in cancer patients by engaging PD-1 on circulating T cells; repurposing these exosomes after removal of PD-L1 and loading with immunostimulatory cargo converts a mechanism of immune evasion into a vehicle for antitumor immune activation [49,50].

8.4 CNS Disease and Neuroprotection

MSC-derived exosomes have demonstrated neuroprotective and functional recovery-promoting effects in rodent models of ischemic stroke, traumatic brain injury, Parkinson's disease, and spinal cord injury, administered both intravenously and intranasally. The therapeutic effects are mediated by exosomal miRNAs including miR-21, miR-146a, and miR-133b that target neuroinflammatory and apoptotic pathways in neurons and glial cells, as well as by exosomal proteins including TSG-6 that reduce neuroinflammation and promote oligodendrogenesis and axonal remodeling. Catalase, an antioxidant enzyme that reduces oxidative stress in dopaminergic neurons, encapsulated in macrophage-derived exosomes was reported to cross the blood-brain barrier following intravenous or intranasal administration and reduce neurodegeneration in a Parkinson's disease mouse model [51,52].

8.5 Inflammatory Disease and Tissue Repair

MSC-derived exosomes have been most extensively investigated for the treatment of acute respiratory distress syndrome, graft-versus-host disease, kidney acute injury, and inflammatory bowel disease, exploiting their potent anti-inflammatory and immunomodulatory cargo. In a murine lipopolysaccharide-induced acute lung injury model, intratracheal administration of MSC-derived exosomes reduced pulmonary inflammation, improved alveolar fluid clearance, and decreased mortality with efficacy comparable to that of MSC cell therapy, establishing exosomes as a scalable and more practical alternative to cell-based therapy. Plant-derived exosome-like nanoparticles from ginger rhizomes administered orally demonstrated significant attenuation of dextran sulfate-induced colitis in mice, attributed to their cargo of plant miRNAs and lipids that modulate intestinal macrophage polarization toward anti-inflammatory phenotypes [53,54].

9. Recent Advances

9.1 Hybrid Exosome-Liposome Systems

Hybrid systems created by membrane fusion between exosomes and synthetic liposomes have been developed to combine the biological targeting properties and immune evasion capacity of exosomal membranes with the precisely controlled lipid composition, drug loading capacity, and scalable manufacturing of liposomes. Membrane fusion is achieved by co-extrusion of exosome-liposome mixtures through nanopore membranes or by freeze-thaw cycling that disrupts and co-reassembles the two membrane types. Hybrid exosome-liposomes loaded with doxorubicin demonstrated superior tumor accumulation and anticancer efficacy compared to pure liposomes or pure exosomes in breast cancer xenograft models, attributed to the contribution of exosomal surface proteins to tumor cell recognition [55].

9.2 CRISPR-Cas9 Delivery via Exosomes

The delivery of CRISPR-Cas9 ribonucleoprotein complexes or mRNA encoding Cas9 nuclease together with guide RNA represents a frontier application of exosome-based nucleic acid delivery with implications for cancer gene therapy and rare genetic disease correction. Exosomes loaded with Cas9-sgRNA ribonucleoprotein complexes targeting the PCSK9 gene in hepatocytes demonstrated hepatic gene editing efficiency comparable to lipid nanoparticle-delivered Cas9 mRNA in a mouse model, while producing substantially lower off-target editing events in peripheral tissues, attributed to the more restricted biodistribution of exosomes compared to liver-targeting LNPs [56].

9.3 Plant-Derived Exosome-Like Nanovesicles

Nanoparticle-sized vesicles isolated from plant juices, including ginger, grape, grapefruit, and aloe vera, share structural and functional similarities with mammalian exosomes while offering compelling manufacturing advantages: plants produce vastly greater quantities of vesicles than cultured cells, the vesicles can be isolated from plant juice by differential ultracentrifugation without cell culture, and plant-derived vesicles contain bioactive plant lipids, proteins, and miRNAs with defined pharmacological activities. Ginger-derived nanoparticles loaded with siRNA targeting the CXCR4 chemokine receptor demonstrated colon-targeted gene silencing following oral administration in mice, attributed to CD44-mediated uptake in colonic epithelial cells and intestinal macrophages [57].

9.4 Engineered Exosomes for Immune Checkpoint Blockade

Exosomes engineered to display anti-PD-L1 antibody fragments or PD-1 extracellular domain on their surface have been investigated for immune checkpoint blockade delivery that achieves broader tumor infiltration than equivalent antibody therapeutics owing to the nanoparticle dimensions of exosomes enabling penetration into poorly vascularized tumor regions. Tumor-derived exosomes stripped of immunosuppressive PD-L1 and loaded with toll-like receptor agonists convert a natural mechanism of tumor immune evasion into an immunostimulatory vehicle that promotes dendritic cell maturation and antitumor T cell responses. These reprogrammed exosomes demonstrated antitumor efficacy superior to PD-L1 antibody monotherapy in poorly immunogenic murine melanoma and colon cancer models [58].

10. Comparative Analysis

A rigorous comparison of exosomes with other extracellular vesicle populations and with established synthetic nanocarrier platforms reveals the specific contexts in which exosomes offer genuine advantages and those in which the biological complexity of exosome production introduces challenges that synthetic systems overcome more readily. Compared to microvesicles, which bud directly from the plasma membrane

and are larger (100 to 1000 nm), more heterogeneous, and carry different surface protein complements, exosomes offer more homogeneous size distribution, defined biogenesis, and the specific surface protein repertoire of the endosomal compartment including high CD63, CD9, and CD81 expression [59].

The comparison of exosomes with liposomes — the most clinically successful nanocarrier platform with multiple approved products — is particularly instructive. Liposomes offer precise compositional control, scalable GMP manufacturing with established regulatory precedent, high drug loading capacity for lipophilic drugs, and well-characterized pharmacokinetics. Exosomes offer superior immune evasion through CD47-mediated phagocyte inhibition, biological targeting through surface protein-receptor interactions, demonstrated blood-brain barrier transcytosis, and inherent therapeutic cargo — but suffer from batch-to-batch variability, low yield from cell culture, complex and poorly standardized isolation protocols, and the presence of endogenous nucleic acid and protein cargo that may contribute unpredictable biological effects [60].

Table 2: Comparative analysis of exosomes versus synthetic nanocarrier platforms for drug delivery

Parameter	Exosomes	Liposomes	PLGA NPs	Polymeric Micelles	ADCs
Origin	Biological (cell-derived)	Synthetic (lipid)	Synthetic (polymer)	Synthetic (polymer)	Semi-synthetic
Immune evasion	Excellent (CD47)	Moderate (PEG)	Moderate (PEG)	Moderate (PEG)	Low
BBB penetration	Yes (natural)	Limited	Limited	Limited	No
Drug loading capacity	Low–moderate	High	High	Moderate	Low (ADC)
Manufacturing scalability	Poor (low yield)	Excellent	Good	Good	Moderate
Batch consistency	Poor to moderate	Excellent	Good	Good	Excellent
Clinical approval status	Phase 1/2 trials	Multiple approved	None approved	None approved	Multiple approved

BBB: blood-brain barrier; PLGA: poly(lactic-co-glycolic acid); NPs: nanoparticles; ADCs: antibody-drug conjugates.

11. Advantages and Limitations

11.1 Advantages

- Inherent biocompatibility from natural cellular origin eliminates the synthetic polymer toxicity concerns associated with PLGA, PEI, and other synthetic nanocarrier materials
- Surface CD47 display provides phagocyte don't-eat-me signaling that extends systemic circulation half-life without the immunogenicity risks associated with PEGylation in repeat-dosed patients
- Demonstrated capacity to cross the blood-brain barrier through receptor-mediated transcytosis, enabling CNS drug and nucleic acid delivery inaccessible to most synthetic nanocarriers
- Intrinsic therapeutic cargo — anti-inflammatory miRNAs, regenerative growth factors, and immunomodulatory proteins — from therapeutic parent cells (MSCs, dendritic cells) adds therapeutic value beyond the exogenously loaded drug
- Natural RNA-carrying capacity and endosomal origin facilitate intracellular nucleic acid delivery to the cytoplasm, exploiting evolutionary mechanisms for RNA transfer between cells
- Tumor-derived exosomes exhibit organ-selective and tumor-selective homing through surface integrin expression patterns, providing inherent active targeting without antibody conjugation

- Plant-derived exosome-like nanoparticles offer scalable, low-cost production from plant material with demonstrated oral delivery capability and colon-targeting properties

11.2 Limitations

- Extremely low production yield from cell culture — a single dose of exosome-based therapeutic typically requires conditioned medium from millions to billions of cells, creating formidable manufacturing scalability challenges
- Significant batch-to-batch variability in size distribution, surface protein composition, and RNA cargo owing to sensitivity of exosome biogenesis to parent cell culture conditions, passage number, and metabolic state
- Heterogeneous and incompletely characterized endogenous cargo — including oncogenic miRNAs from tumor cell-derived exosomes, immune modulators, and unknown proteins — creates uncertainty about the safety of repeated exosome administration
- Absence of standardized isolation protocols and characterization requirements across the field produces inconsistent data that impedes quantitative comparison of results between research groups and institutions
- Drug loading efficiencies by current methods (electroporation, sonication, co-incubation) are generally low and variable, with significant proportion of drug associated with exosome surface rather than lumenally encapsulated
- The regulatory classification of exosome-based therapeutics — whether as biological drugs, cell therapy products, or combination products — is unresolved in most jurisdictions, creating substantial uncertainty about the clinical development pathway
- Large-scale, GMP-compliant exosome manufacturing processes have not been established for most cell types, and the cost of pharmaceutical-grade exosome production substantially exceeds that of equivalent synthetic nanoparticle systems

12. Clinical Translation and Marketed Products

The clinical development of exosome-based drug delivery has been more modest than the pace of preclinical publication might suggest, reflecting the manufacturing, characterization, and regulatory challenges outlined above. The earliest clinical work with exosomes as drug delivery vehicles was conducted by Gustafsson and colleagues, who pioneered autologous dendritic cell-derived exosome vaccines for metastatic melanoma and non-small cell lung cancer in phase 1 and 2 clinical trials reported in 2005 and 2016, demonstrating safety and immunological activity but limited clinical efficacy in heavily pre-treated patients. These trials established the feasibility of manufacturing patient-derived exosomes to GMP standards and administering them safely to cancer patients [61].

More recent clinical development has focused on MSC-derived exosome formulations for inflammatory conditions, graft-versus-host disease, and COVID-19-associated acute respiratory distress syndrome. A phase 1 study of MSC-derived exosomes for steroid-refractory acute graft-versus-host disease reported complete or partial responses in several patients with minimal adverse effects. Multiple early-phase clinical studies of MSC-derived exosomes for COVID-19 pneumonia and ARDS were initiated and reported preliminary safety data in 2021 and 2022, reflecting the urgency of finding therapeutic options for severe COVID-19 disease [62].

Table 3: Selected clinical trials of exosome-based drug delivery and therapeutic exosome formulations (as of 2022)

Study / Sponsor	Exosome Source	Indication	Payload	Clinical Status / Outcome
Morse et al. (Gustafsson group)	Autologous DCs	NSCLC vaccination	Tumor antigen peptide-MHC	Phase 2 — safe; disease stabilization in 32%

Escudier et al.	Autologous DCs	Metastatic melanoma	MAGE peptide-MHC complexes	Phase 1 — safe; limited efficacy
MSC exosome — GvHD (Yunfeng Li)	Allogeneic MSCs	Steroid-refractory GvHD	Endogenous MSC cargo	Phase 1 — responses in 5/7 patients
Codiak BioSciences (exoSTING)	Engineered HEK293	Solid tumors (intratumoral)	STING agonist encapsulated	Phase 1 ongoing (2022)
MSC exosomes — COVID-19 ARDS	Allogeneic MSCs	COVID-19 pneumonia	Endogenous MSC cargo	Phase 1/2 — safety established (2022)
iExosomes (MD Anderson)	Mesothelial cells	Pancreatic cancer	KRAS siRNA	Phase 1 initiated (2022)

DC: dendritic cell; MSC: mesenchymal stem cell; NSCLC: non-small cell lung cancer; GvHD: graft-versus-host disease; STING: stimulator of interferon genes; KRAS: Kirsten rat sarcoma viral proto-oncogene.

13. Critical Analysis

The exosome drug delivery field is characterized by a fundamental tension between genuinely remarkable biological properties and profound practical limitations in manufacturing, standardization, and functional characterization that have prevented clinical translation at a pace commensurate with the volume and enthusiasm of preclinical publications. A critical examination of the methodology underlying key published findings reveals several areas of concern that deserve explicit discussion.

The claim that exosomes are preferentially taken up by specific target cells — a central premise of many exosome drug delivery studies — is supported primarily by in vitro experiments comparing uptake in one or two cell lines, frequently at concentrations that far exceed what would be achieved in vivo following intravenous administration. When in vivo biodistribution is examined by fluorescence imaging or tissue extraction after intravenous injection, exosomes from most cell sources accumulate predominantly in the liver and spleen through macrophage phagocytosis, with relatively minor accumulation in tumor tissue or brain, analogous to synthetic nanoparticles of similar size. The claimed cell-type-specific targeting observed in vitro does not reliably translate to preferential organ accumulation in vivo, where the vast excess of non-target macrophages in reticuloendothelial organs overwhelms any receptor-mediated selective uptake in the intended target tissue [63].

The electroporation-based siRNA loading method used in the majority of published exosome-siRNA delivery studies has been shown by Kooijmans and colleagues and by subsequent independent groups to produce significant siRNA aggregation and exosome aggregation during electroporation, such that much of the apparent siRNA encapsulation measured by size exclusion chromatography represents siRNA aggregated with the exosome surface rather than lumenally encapsulated siRNA. This surface-associated siRNA would be expected to dissociate rapidly in biological fluids and be rapidly degraded by serum nucleases, making it functionally irrelevant for cytoplasmic delivery. The gene silencing observed in some published studies may therefore reflect exosome-independent delivery of aggregated free siRNA rather than exosome-mediated delivery, which would invalidate the attribution of activity to the exosome carrier [64].

The standardization problem in the exosome field is quantitatively illustrated by the finding of a 2017 study by Webber and Clayton, who prepared exosomes from the same cell line using six different isolation protocols described in published literature and found 8-fold variation in particle yield, 3-fold variation in mean particle size, and qualitatively different protein marker profiles between preparations from the same biological source. These differences would be expected to produce dramatically different in vivo pharmacokinetics and biodistribution, yet the field lacks consensus on which isolation method produces the most pharmacologically relevant exosome preparation. The MISEV2018 guidelines provide a framework for minimal reporting standards but do not prescribe specific isolation methods, leaving substantial methodological heterogeneity in the literature [65].

Finally, the assumption that the endogenous RNA cargo of therapeutic exosomes is universally beneficial requires scrutiny. MSC-derived exosomes used for therapeutic applications contain miRNAs with confirmed roles in cell proliferation, immune modulation, and gene regulation that are poorly characterized in the context of long-term systemic administration to cancer patients or immunocompromised individuals. The complete transcriptomic characterization of therapeutic exosome preparations and evaluation of the biological activity of their endogenous RNA cargo should be considered mandatory safety requirements for clinical development programs [66].

14. Conclusion

Exosomes represent a biologically sophisticated and conceptually compelling drug delivery platform whose natural origin confers inherent advantages in immune evasion, blood-brain barrier penetration, and intercellular communication that synthetic nanocarrier systems have not been able to fully replicate despite substantial engineering effort. The demonstrated capacity of exosomes to carry functional nucleic acids across the blood-brain barrier, to carry endogenous anti-inflammatory and regenerative cargo from MSCs that mediates genuine therapeutic effects in preclinical disease models, and to display cell-type-specific surface proteins that mediate selective cellular uptake provides a strong biological rationale for continued pharmaceutical development of exosome-based delivery systems.

The clinical development programs that have advanced to human trials — dendritic cell exosome vaccines, MSC exosome formulations for graft-versus-host disease and respiratory distress syndrome, and the pioneering iExosomes siRNA delivery trial for pancreatic cancer — have established the safety and preliminary clinical feasibility of exosome-based therapeutics in humans, providing the necessary foundation for more ambitious efficacy-focused trials. The Codiak BioSciences engEx engineered exosome platform, generating exosomes with precisely engineered surface and luminal cargo, represents an important step toward the industrialization and compositional standardization of exosome manufacturing.

The critical examination presented in this review makes clear that the field must honestly address the manufacturing yield problem, the inadequacy of current siRNA loading methods, the in vitro to in vivo translation gap in cellular targeting, and the unknown long-term biological effects of endogenous exosome cargo before exosome-based drug delivery can fulfill its clinical potential. The development of exosome-mimetic nanovesicle production by cell extrusion, hybrid exosome-liposome fusion systems, and cell-free exosome production from membrane fragments represents promising directions for overcoming the yield and scalability limitations that most fundamentally constrain clinical translation of this otherwise remarkable drug delivery platform.

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