Expanding the Role of Exosomes in Drug, Biomolecule, and Nanoparticle Delivery

Ongun Mehmet Saka[1], Devrim Demir Dora[2], Gunes Kibar[3,4], Atakan Tevlek[3,5*]

[1]Department of Pharmaceutical Technology, Faculty of Pharmacy, Ankara University, Ankara, 06800, Turkey
[2]Department of Pharmacology, Faculty of Medicine, Akdeniz University, Antalya, 07070, Turkey
[3]Micro Nano Particles (MNP) Research Group, Materials and Engineering Department, Adana Alparslan Turkes Science and Technology University, Adana, 01250, Turkey
[4]UNAM-National Nanotech. Research Center and Institute of Materials Science&Nanotech. I.D. Bilkent University, Ankara, 06800, Turkey
[5]Department of Medical Biology, Faculty of Medicine, Atılım University, Ankara, 06830, Turkey

Exosomes are nanoscale extracellular vesicles released by diverse cell types, serving essential functions in intercellular communication and physiological processes. These vesicles have garnered considerable interest in recent years for their potential as drug delivery systems, attributed to their natural origin, minimal immunogenicity, high biocompatibility, and capacity to traverse biological barriers, including the blood-brain barrier. Exosomes can be obtained from diverse biological fluids, rendering them accessible and versatile vehicles for therapeutic medicines. This study emphasizes the burgeoning significance of exosomes in drug administration, concentrating on their benefits, including improved stability, target selectivity, and the capacity to encapsulate various biomolecules, such as proteins, nucleic acids, and small molecules. Notwithstanding their potential applications, other problems remain, including as effective drug loading, industrial scalability, and the standardization of isolation methodologies. Overcoming these hurdles via new research is essential for fully harnessing the promise of exosomes in therapeutic applications, especially in the treatment of intricate diseases like cancer and neurological disorders.

Keywords: Exosomes, drug delivery, biomolecules, nanoparticles.

***Corresponding Author:**

Dr. Atakan Tevlek (PhD)

atakan.tevlek@atilim.edu.tr

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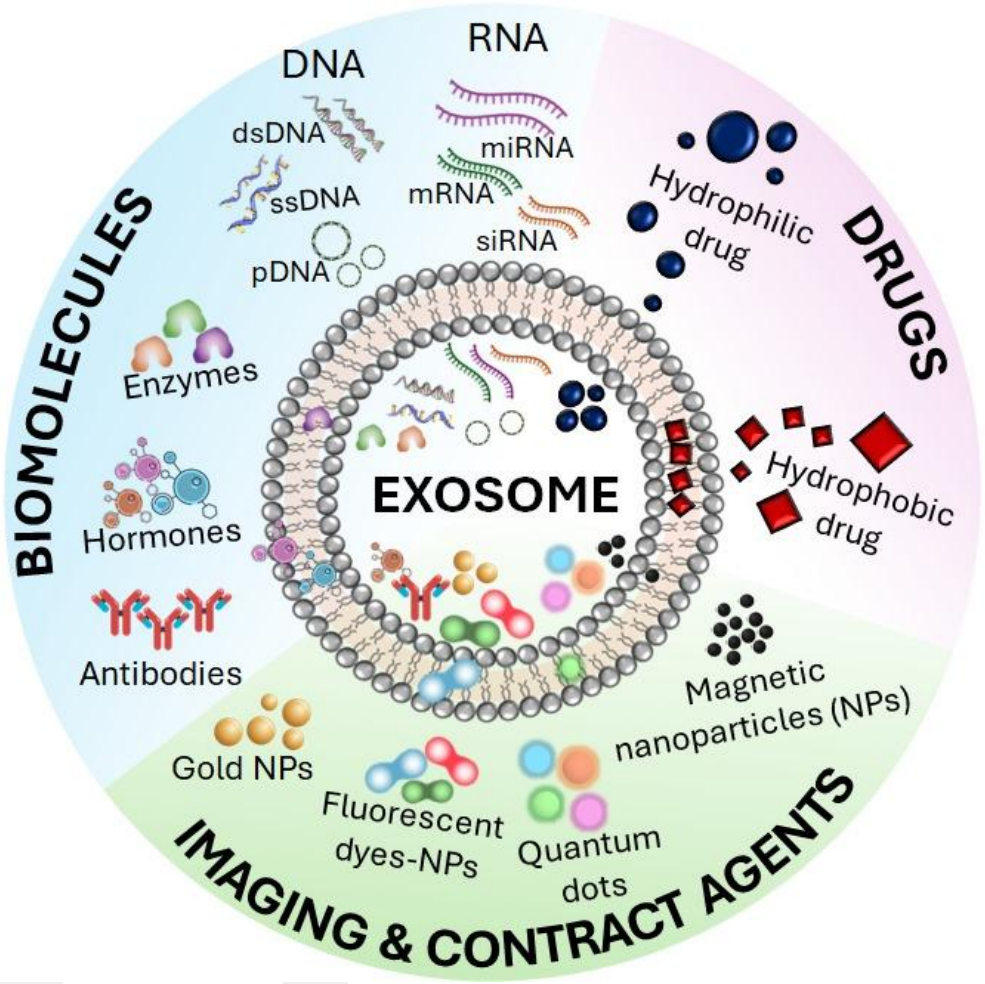
Graphical Abstract



1. Introduction

Exosomes were initially discovered in sheep reticulocytes in 1987, highlighting their significance as small vesicles released from multi-vesicular structures during reticulocyte development [1,2]. Initially, exosomes were considered as cellular mechanisms responsible for eliminating unnecessary cellular components, portraying them as structures of limited significance that solely focus on removing garbage [3]. As studies progressed, it became evident that exosomes were not merely biological byproducts but played significant roles in intercellular communication. Subsequently, exosomes have been found to encompass a diverse array of biomolecules, including proteins, lipids, and nucleic acids, suggesting their potential as effective carriers for genetic information and signaling molecules. In fact, recent research has revealed the comprehensive treatment possibilities of exosomes in many diseases such as autoimmune, cardiovascular, ocular and neurological diseases [4,5]. They are present not just in sheep reticulocytes but also in several cell types and biological fluids, including blood, urine, saliva, breast milk, tumor effusions, bronchoalveolar fluid, seminal fluid, amniotic fluid, and cerebrospinal fluid [6]. Their content, derived from bioactive chemicals, serves various essential functions in cellular communication and disease progression [7]. Target cells can absorb these chemicals, which subsequently affect biological processes such as cell differentiation, proliferation, and survival [8]. Furthermore, exosomes have the essential capability to traverse biological barriers, such as the blood-brain barrier, rendering them highly advantageous as natural drug carriers for delivering therapeutic agents to the central nervous system [9,10]. Moreover, exosomes have shown promise as biomarkers for disease diagnosis and prognosis, highlighting their importance in clinical oncology [11]. In conclusion, the ability of exosomes to convey molecular cargo between cells underscores their biological importance in both physiological and pathological states.

Exosomes have been recognized as optimal vehicles for the delivery of therapeutic drugs because of their low immunogenicity, good biocompatibility, stability, and advantageous mechanical properties, as evidenced by numerous research studies [12–14]. In the realm of brain cancer treatment, exosomes are regarded as promising "Trojan horse" nanocarriers owing to their biocompatibility, stability, and substantial loading capacity, effectively tackling difficulties such as medication resistance and the permeability of the blood-brain barrier [15]. Furthermore, exosomes have enhanced biological activity and therapeutic efficacy relative to non-exosomal cargo, highlighting its potential in cancer treatment [16]. Notwithstanding problems such as drug loading constraints, exosomes continue to be a significant asset in the progression of cancer therapy, particularly for gastrointestinal malignancies, where they function as efficient drug delivery mechanisms [17]. Thus far, it has been examined as a possible vehicle for therapeutic agents, including several RNA molecules and

immunomodulators, particularly anticancer pharmaceuticals, and its efficacy in diagnostic methodologies has been substantiated [18]. Studies indicate that exosomes, particularly those secreted by tumors, can be identified and analyzed in the plasma of cancer patients, providing information regarding disease status and progression [19]. The clinical significance of exosomes in delivering established tumor markers and their increased expression in tumor patients relative to normal controls underscores their role in improving clinical treatment and patient outcomes [20]. Moreover, despite the respectable characteristics of exosomes, their application as drug delivery vehicles faces with several obstacles [21]. These include difficulties associated with extraction and separation, encapsulation and loading, immunogenicity and clearance, heterogeneity and characterization, storage and stability, along with regulatory and technological issues [22–24].

This review offers a thorough examination of exosomes, elucidating their biological importance in intercellular communication and therapeutic potential. The manuscript commences with an examination of the physiological functions and origins of exosomes, which is subsequently followed by an examination of their isolation from a variety of biological fluids. Key sections address recent developments in the field of biomolecule and drug delivery applications, with a particular emphasis on the strategic integration of active compounds into exosomes, such as nucleic acids, proteins, and small molecules. Furthermore, this review discusses targeted delivery strategies, including exosome engineering and surface modifications, to improve selectivity and improve therapeutic efficacy. In order to illustrate the current status and potential for transference into clinical practice, clinical trials that demonstrate exosome-based therapies are also examined. In conclusion, the review emphasizes the potential of exosomes as versatile instruments for both diagnostics and therapy by highlighting their role in the delivery of imaging agents. By addressing these multifaceted aspects, this article aims to provide a comprehensive comprehension of the most recent developments and obstacles in the field of exosome research, as well as to contribute valuable knowledge for future therapeutic innovations.

2. Applications of Exosomes as Delivery Systems

Research on exosomes has rapidly expanded over recent years, revealing their significant potential in diagnostics, therapeutics, and drug delivery systems. Exosomes have emerged as a promising platform for drug delivery systems due to their unique ability to facilitate targeted and efficient transport of therapeutic agents. Biocompatibility, targeting ability, stability and cargo loading are the main properties of exosomes as drug delivery systems [25]. Compared to synthetic nanoparticles, exosomes are naturally biocompatible and have low

immunogenicity, as they are secreted from cells. Because exosomes have surface proteins on their surface, they can be further modified to improve delivery to particular tissues or cells [26]. They are stable in the bloodstream and can protect their cargo from degradation. Their biocompatibility and the presence of surface proteins that enable specific cell targeting make exosomes ideal for delivering therapeutics directly to target cells while minimizing off-target effects and toxicity. Furthermore, exosomes can cross biological barriers, such as the blood-brain barrier [27].

2.1. Biomolecule Delivery

These nanovesicles can be engineered to encapsulate a variety of biomolecules, including small molecules, proteins, and nucleic acids. They encapsulate different types of nucleic acids, such as DNA and RNA, including messenger RNA (mRNA), microRNA (miRNA), long non-coding RNA (lncRNA), and small interfering RNA (siRNA), all of which play pivotal roles in gene regulation and cellular communication [28]. In addition, exosomes are rich in proteins, encompassing membrane proteins like tetraspanins and integrins, cytosolic proteins involved in signaling and structural functions, heat shock proteins (HSPs) that aid in stress responses, and growth factors and cytokines that modulate immune responses and cell growth. Structurally, exosomes contain a lipid bilayer made up of phospholipids, sphingolipids, and cholesterol, which not only provide membrane integrity but also influence their stability and interaction with target cells [29]. Moreover, exosomes carry metabolites and small molecules, including metabolic enzymes and signaling molecules like ATP and prostaglandins, reflecting the metabolic state of their parent cells. They can also transport other bioactive compounds such as peptides, vitamins, and co-factors that support cellular processes and biochemical functions [30]. This broad spectrum of cargo allows exosomes to facilitate intercellular communication and holds significant potential for diagnostic and therapeutic applications in various medical fields. Table 1 provides recent literature examples for biomolecule delivery of exosomes.

2.2. Drug Delivery

The basic principles underlying the use of exosomes in drug delivery systems revolve around their biocompatibility, targeting capabilities, natural cargo-loading mechanisms, stability, ability to cross biological barriers, and engineering potential [31]. More importantly, they exhibit a remarkable dual capability as carriers for both hydrophobic and hydrophilic drugs, owing to their natural lipid bilayer structure [32]. Hydrophobic drugs such as paclitaxel and curcumin can be encapsulated within exosomes, facilitating their dispersion in aqueous environments, although this does not guarantee an increase in their intrinsic solubility in water. Conversely, hydrophilic drugs like doxorubicin and methotrexate can be loaded into the aqueous interior of

exosomes, protected from enzymatic degradation and clearance mechanisms [33]. This versatile property of exosomes makes them promising candidates for targeted drug delivery of various therapeutic agents such as chemotherapeutic agents, anti-inflammatory drugs, antibiotics, antimicrobials and various drug loaded nanoparticles due to improved bioavailability, reduced systemic toxicity, and enhanced therapeutic efficacy [34]. Table 2 provides recent literature examples of exosomes for drug delivery while Table 3 provides clinical trials of them. As research and technology advance, the application of exosomes in drug delivery continues to show great promise, offering innovative solutions for treating complex diseases and improving patient outcomes.

Table 1. Applications of exosomes for biomolecule delivery.

Types of Biomolecules	Source of Exosome	Delivered Biomolecules	Loading Mechanism	Target	Outcome	Reference
Enzyme	umbilical mesenchymal stem cell-derived extracellular vesicles	N-acetylgalactosamine-6-sulfate sulfatase (GALNS) enzyme	incubation	Morquio A fibroblasts	a potential treatment option for Morquio A syndrome	[35]
Hormone	bone marrow mesenchymal stem cells (BMMSCs)-derived exosomes	17β-estradiol	incubation, sonication	BMMSCs	a potential treatment for osteoporosis	[36]
Antibody	tumor antigen-stimulated dendritic cell-derived exosomes (tDC-Exo)	anti-CD3 and anti-EGFR	incubation	human dendritic cells	an efficient endogenous T cells activation for solid tumor theraphy	[37]
Antibody fragment	FreeStyle 293F cells	crystallizable (Fc) portion of human IgG	endogeneous loading	Huh-7 cells	a targeted cancer therapy using tumour-specific therapeutic antibodies to guide the EVs to tumour cells and to deliver antitumoural drugs	[38]

Growth factor	mesenchymal stem cells derived exosomes	human fibroblast growth factor 1 (FGF1)	endogeneous loading	mouse and human fibroblasts	a foundation for developing novel wound-healing therapies	[39]
mRNA	bovine milk-derived exosomes	SARS-CoV-2 receptor-binding domain (RBD) mRNA	incubation	293T cells	a development of new mRNA-based oral vaccine delivery system SARS-COV-2	[40]
siRNA	THP-1 monocytes derived M1 macrophage derived exosomes	angiopep-2 (An2)-functionalized signal transducers and activators of transcription 3 (STAT3) siRNA	incubation	U87MG cells	a promising strategy for treatment of one of the aggressive brain disease, glioblastoma	[41]
miRNA	A549 cells derived exosomes	miR-449a	incubation	A549 cells, Hela cells and HepG2 cells	a targeted cancer therapy by using miRNA based genetic drugs	[42]
DNA plasmid	placental tissue derived exosomes	piggyBac plasmid DNA	electroporation, incubation	not applicable	a foundation of potential of placental exosomes as DNA delivery vehicles	[43]

Protein	HEK293FT cell-derived exosomes	CRISPR/Cas9	chimeric exosomes	human MSCs	a development of promising CRISPR–Cas9 delivery system for in vivo gene manipulation	[44]
biomolecule combination	A549 non-small lung cancer cells derived exosomes	PD-L1 antibody + siRNA	incubation	A549 and H460 cells	a development of targeted exosomes carrying siRNA for gene therapy	[45]
biomolecule combination	Human embryonic kidney (HEK) 293T cells and human lung cells (HLCS	GFP-encoding mRNA and RFP protein labeled nanoparticles	electroporation	heart, liver, kidney,spleen, cecum and brain tissue of CD1 mice	a foundation of an inhalation-based system delivering mRNA and protein drugs with enhanced pulmonary bioavailability and therapeutic efficacy	[46]

biomolecule combination	HEK293FT cells derived exosomes	plasmid DNA and cell-penetrating peptides (CPPs)	incubation and transfection	HEK293FT and A549 cells	a development of a technique providing high transfection efficacy and bioavailability of the encapsulated nucleic acid delivery	[47]

Table 2. Applications of exosomes for drug delivery.

Properties of Drug	Loaded Drug	Area of Use	Mechanism of Action	Carrier Exosome	Intendent Use	Reference
Hydrophilic	Doxorubicin	Various cancers (e.g., breast, bladder, lymphomas)	Inhibits DNA replication by intercalating into DNA	mature dendritic cell (mDCs) derived exosomes	targeted to breast cancer cells and breast cancer stem cells	[48]
	Methotrexate	Cancer, autoimmune diseases, ectopic pregnancy	Inhibits dihydrofolate reductase, affecting DNA synthesis	human primary glioma cell line U87 derived exosomes	selective target binding as well as therapeutic effects for brain tumor treatment	[49]
	Cisplatin	Various cancers (e.g., testicular, ovarian, bladder, lung)	Forms DNA cross-links, inducing apoptosis	Umbilical Cord Blood-Derived M1 Macrophage Exosomes	targeted treatment for ovarian cancers	[50]
	Carboplatin	Various cancers (e.g., ovarian, lung, head and neck, testicular)	Forms DNA cross-links, inducing apoptosis	Y79 primary eye tumor cells derived exosomes	Targeted drug delivery system for the treatment of retinoblastoma	[51]
	Dexamethasone	Anti-inflammatory, autoimmune diseases, allergies	Glucocorticoid receptor agonist, suppresses inflammation	retinal pigment epithelial cells (RPEs) derived exosomes	treatment of proliferative vitreoretinopathy	[52]

	Vancomycin	Bacterial infections	Inhibits bacterial cell wall synthesis	RAW264.7 macrophage cells derived mannosylated exosomes	treatment of intracellular methicillin-resistant *Staphylococcus aureus*	[53]
	Ciproflaxacin	Bacterial infections (e.g., UTIs, respiratory infections)	Inhibits bacterial DNA gyrase and topoisomerase IV	human B-lymphoid cells derived exosomes and non-pathogenic *myxobacteria* SBSr073 derived exosomes	delivery of antibiotic for the treatment of antibiotic resistant enteropathogenic *Shigella*	[54]
	Amphotericin B	Fungal infections	Binds to ergosterol in fungal cell membranes, causing leakage	*Leishmania major* derived exosomes	delivery of antifungal agents for the treatment cutaneous leishmaniasis	[55]
	Donepezil	Alzheimer's disease (AD)	Acetylcholinesterase inhibitor, increases acetylcholine levels, improving cognitive function	human plasma derived exosomes	brain-targeted delivery for the treatment of AD	[56]

Hydrophobic	Paclitaxel	Ovarian, breast, lung, pancreatic cancers	Stabilizes microtubules, preventing cell division	murine 4T1 breast cancer cells derived exosomes	drug delivery for future targeted cancer therapy	[57]
	Coenzyme Q10	Neurodegenerative diseases, cardiovascular diseases	Involved in mitochondrial electron transport chain, antioxidant	adipose-derived stem cells derived exosomes	increasing the delivery of CoQ10 in the treatment of Alzheimer's	[58]
	Curcumin	Inflammatory conditions, cancer prevention	Inhibits multiple signaling pathways related to inflammation and cancer	human adipose derived mesenchymal stem cells derived exosomes	effective therapeutic strategy for rheumatoid arthritis	[59]
	Docetaxel	Breast, lung, prostate, gastric cancers	Promotes microtubule polymerization, inhibiting depolymerization	human umbilical cord blood-mesenchymal stem cell derived exosomes	enhanced inhibition of proliferative and migratory aggressiveness of the murine triple-negative breast cancer TNBC 4T1 cells	[60]

	Lapatinib	Breast cancer, other solid tumors	Inhibits EGFR and HER2/neu tyrosine kinases, reducing cell proliferation	non-cancerous epithelial breast cells MCF10 A	reducement of cellular proliferation of HER2-positive breast cancer cell line SKBR 3 cells	[61]
	Rifampicin	Tuberculosis, bacterial infections	Inhibits bacterial RNA polymerase	bone marrow derived mesenchymal stem cells derived exosomes	high targeting capacity of the drug in the treatment of central nervous system tuberculosis	[62]
	Tofacitinib	Autoimmune diseases (e.g., rheumatoid arthritis)	A Janus kinase (JAK) inhibitor that modulates the immune response, reducing inflammation and immune system activity	A-431 human epidermoid carcinoma keratinocyte type cell line derived exosomes	targeted drug delivery for treatment of psoriasis	[63]

Table 3. Clinical trials of exosomes used as drug delivery systems [64].

Clinical Trial	Date	Disease	Drug	Exosome Source	Phase	Status	Sponsor
NCT01294072	S: 2011.01	Colon Cancer	Curcumin	Plant (Grape)	I	Recruiting	University of Louisville
NCT04879810	S: 2018.03.01 C: 2022.08.02	Irritable Bowel Disease	Curcumin	Plant (Ginger)	NA	Completed	University of Louisville
NCT03384433	S: 2019.04.17 C: 2021.12.17	Acute Ischemic Stroke	miRNA-124	MSCs	I/II	Recruiting	Isfahan University of Medical Sciences
NCT04747574	S: 2020.09.25 C: 2021.03.25	SARS-CoV-2 Pneumonia	EXO-CD24	T-REx™-293 cells	I	Completed	Tel-Aviv Sourasky Medical Center
NCT04356300	S: 2020.09.01 C: 2030.09.01	Multiple Organ Failure	Exosome of MSC	MSCs	I/II	Not yet recruiting	Fujian Medical University
NCT03608631	S: 2021.01.27 EC:2025.04.30	Pancreatic Adenocarcinoma	KRAS G12D siRNA	MSCs	I	Recruiting	M.D. Anderson Cancer Center
NCT05043181	S: 2021.12 EC: 2026.12	Familial Hypercholesterolemia	Ldlr mRNA Exosomes	MSCs	I	Not yet recruiting	Tang-Du Hospital Collaborator: Air Force Military Medical University, China

S: Date of start

C: Date of completion

EC: Estimated completion

MSCs: Mesenchymal Stem Cells

Ldlr mRNA: Low Density Lipoprotein Receptor mRNA

2.2.1. Approaches of Incorporating Active Compounds into Exosomes

Exosomes have arisen as attractive nanocarriers for therapeutic applications owing to their natural origin, little immunogenicity, and inherent capacity to target specific cells and tissues [65]. In contrast to synthetic nanoparticles, exosomes have biological characteristics that confer biocompatibility and stability in circulation, hence diminishing the possibility of rapid clearance or adverse immunological reactions [66]. To fully utilize their potential as delivery vehicles, safe and reliable strategies for loading active substances into exosomes are crucial. These techniques facilitate the accurate encapsulation and regulated release of therapeutic substances, rendering exosomes appropriate for various applications, including as targeted delivery of drugs, biomolecule transport, and imaging [67].

Incorporating active compounds into exosomes requires an individually tailored strategy, as the loading efficiency markedly differs based on the drug type and the selected method. Current methods—such as incubation, electroporation, sonication, extrusion, and chimeric exosome formation—are persistently refined to enhance loading efficiency while preserving the structural integrity and functionality of exosomes (Figure 1) [68].

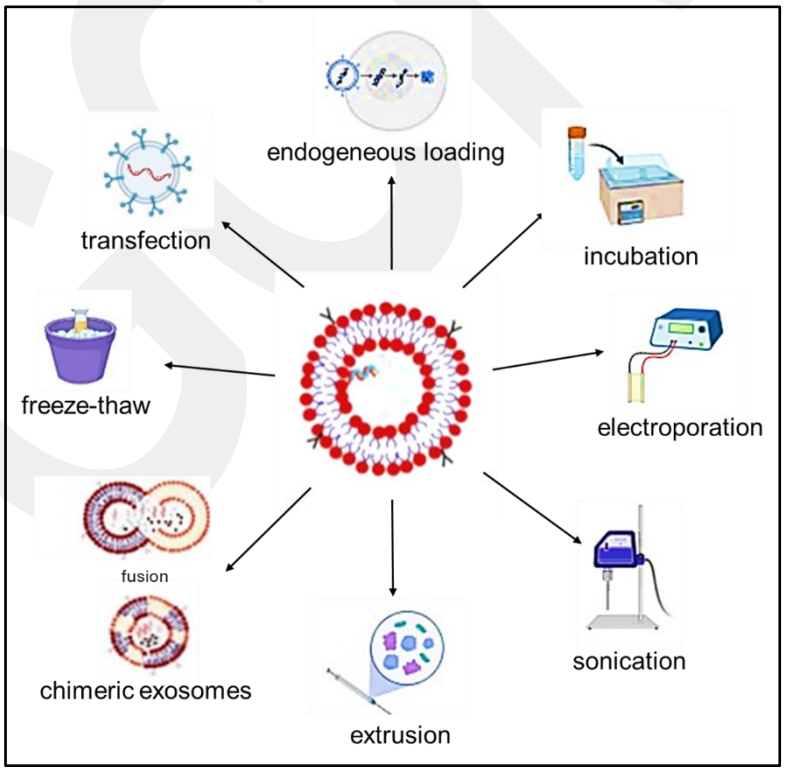


Figure 1. Approaches for active compound incorporation into the exosomes.

The incubation method, as its name suggests, relies on the co-incubation of exosomes with small molecules over a certain period. Although this method offers advantages such as ease of application and no harm to exosome integrity, its applicability is primarily limited to certain types of active substances. This method is particularly advantageous for hydrophobic active substances that interact with the lipid surfaces of exosomes. The loading efficiency with this method depends on the polarity of the small molecule. Low to medium molecular weight lipophilic small molecules are more readily loaded into exosomes by incubation. Consequently, the loading capacity is contingent on both the hydrophobic properties of the active substance and the incubation time [69,70].

The process of electroporation involves the loading of small-molecule drugs into exosomes through the application of an external electric field just beyond the exosome membrane. This is achieved by using short high-voltage pulses to overcome the barrier of the membrane. Under appropriate conditions, the lipid layer instantaneously ruptures, creating a transient state of membrane permeability, which allows small-molecule drugs to diffuse through the exosome membrane [71,72].

The application of the sonication method, which utilises sound energy, results in the redistribution of the membranes of exosomes. This restructuring leads to the formation of a membrane microviscosity that retains them within the membranes. Consequently, the membrane integrity is compatible, allowing the diffusion of hydrophilic or hydrophobic molecules or bioactive chemicals across lipid bilayers. Consequently, in many cases, the sonication method provides a high efficiency, which is superior to that of incubation and electroporation [73,74].

In the extrusion-based technique, in brief, the active substance and isolated exosomes are passed together through the extruder at a specific temperature and pore size (100-400 nm). Extrusion is a crucial technique in the field of exosome research that offers high loading efficiency, size control and scalability, and this process disrupts the exosome membrane, allowing efficient loading of the active ingredient [75,76].

The chimeric exosome approach is based on the principle of creating a hybrid system with carrier systems such as liposomes, with the aim of overcoming the low encapsulation ability of exosomes. Hybridisation is performed with liposome formulations due to their loading capacity and allowing surface modification. Furthermore, the relatively high cytotoxic properties of liposome formulations can also be tolerated with this preparation method. Exosome-liposome hybrids are being created to increase the loading capacity of exosomes and significantly increase their half-life in plasma [69,77].

Freeze and thaw cycle is a simple and effective strategy to load active substances directly into exosomes. In essence, it involves repeatedly freezing exosomes at low temperatures and then thawing them at room temperature. This process results in multiple ruptures and repairs of the exosomal plasma membrane. During this continuous rupture and repair process, the active substance is loaded into the exosome. The freeze-thaw cycle is a gentle procedure that does not damage the membrane structure of the exosomes and makes it a suitable option for mass production since exosomes remain stable at low temperatures [78]. Although an agitation process is applied, the preservation of the biological activity of exosomes during the process increases the interest in this method [79,80].

Transfection is the process by which different molecules, such as active substances, proteins or oligonucleotides, can be loaded into exosomes using chemical or biological reagents. Exofect, a commercialised exosome transfection kit, is highly effective in transferring small molecules and nucleic acids directly into exosomes [81]. Therapeutic molecules can be loaded into the exosomal lumen under controlled transfection processes, and the therapy stability of this method is higher than other installation methods. However, the efficiency is not constant. On occasion, instances of underloading have been documented. Transfection agents can indicate gene expression in exosomes produced from donors. This may result in the extent of biological functions of charged molecular acid agents. Consequently, some of the reported potencies of transfection agents have promising prospects for safe transfection methods [82].

Endogenous loading is an engineered method for drug loading that utilises exosome donor cells. This method is simple and convenient but only suitable for drugs with low cytotoxicity. In this approach, small-molecule drugs are co-incubated with donor cells or treated with other loading strategies such that they can be absorbed by the cells through the lipid bilayer and encapsulated in exosomes [83,84].

The selection of the aforementioned approaches may include certain limitations and risks, including the exertion of physical force, variations in pH levels, and the potential for surfactants to harm the membrane. Choosing the optimal strategy for chemical integration is essential for enhancing therapeutic efficacy. The incubation approach is beneficial for hydrophobic tiny compounds, whereas electroporation is typically favored for nucleic acids and bigger macromolecules. Each approach poses distinct issues, especially with scalability, load capacity, and the maintenance of exosome activity. Through the advancement and enhancement of these methodologies, researchers seek to harness the complete therapeutic potential of exosomes in intricate ailments, like cancer and neurodegenerative disorders, where conventional drug delivery strategies encounter considerable constraints.

2.2.2. Targeted Delivery Strategies

Exosomes have an inherent ability to target specific cells or tissues, a property derived from their parent cells. They carry surface molecules that facilitate recognition and binding to specific cell types, allowing for more precise delivery of their cargo. This natural targeting ability should be further enhanced through engineering, where exosomes can be modified to express surface ligands or antibodies that improve their affinity for target cells, thus increasing the efficacy and precision of drug delivery [85]. Exosomes must first bind to the recipient cell, in other words, the generated exosomes must bind to particular target cell types depending on the intended indication to exert a therapeutic effect [86]. Therefore, modification techniques have to be developed to decorate the exosomal surface with targeting moieties to direct their transport to particular cell types and organs, thereby overcoming this condition [87]. These natural and engineered modifications significantly enhance the ability of exosomes to interact with target cells (Figure 2). In subsequent sections of the article, this section will be addressed under the headings "Exosome Surface Modification" and "Exosome Ligand Modification."

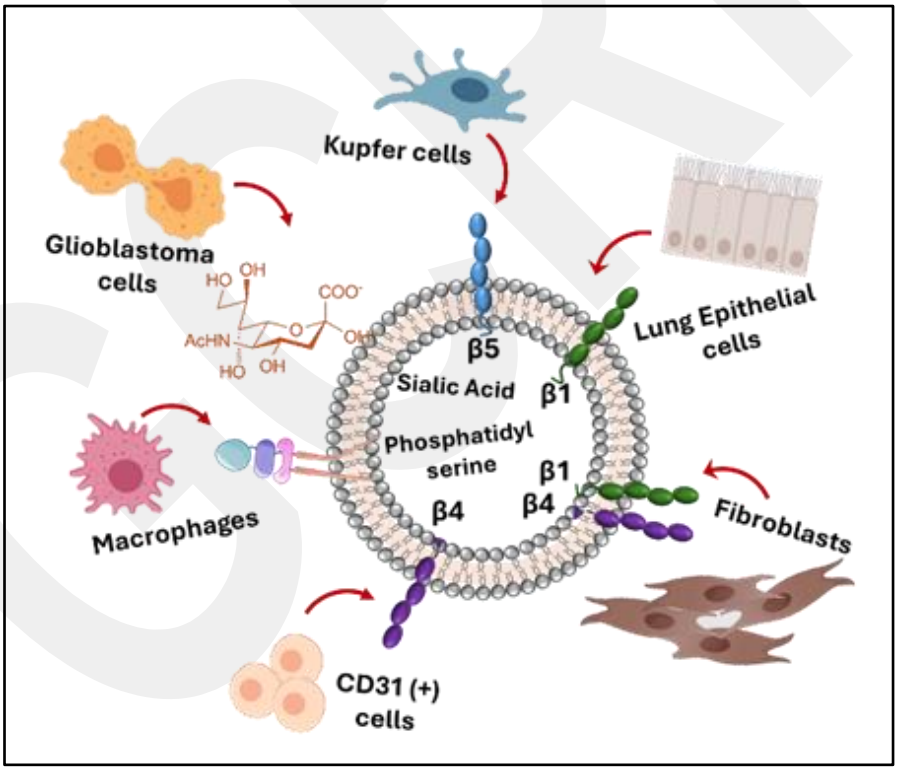


Figure 2. Exosome surface modifications and cellular targeting: Engineered ligands, antibodies, or peptides added to the exosome surface enhance targeted binding to specific cell types, leading to more effective drug delivery.

Exosomes' targeting behavior is largely determined by their protein composition [88]. Exosome formulations that exhibit integrin α6 in association with β1 and β4 subunits, for instance, have been demonstrated to specifically target surfactant protein C-positive epithelial cells in the lungs and S100-A4-positive fibroblasts. Kupffer cells in the liver and CD31-positive endothelial cells in the brain are the preferred targets of exosome formulations expressing the β5 and β4 subunits, respectively [89]. Additionally, numerous investigations have indicated that the targeting behavior of exosomes is influenced by their lipid makeup [90]. It has been demonstrated that exosomes may target macrophages with great effectiveness when they have phosphatidylserine on their surface [91]. Moreover, it has been reported that exosome uptake is inhibited when macrophage uptake is competitively mediated by various carrier systems including phosphatidylserine. Different negatively charged lipid compounds have produced similar outcomes [92]. On the other hand, exosome targeting is also actively influenced by glycan profiles [93]. It has been demonstrated that targeting of glioblastoma cells is dependent on the different glycan derivatives carried by exosomes. The targeting of regions rich in lectin receptors can occur because of sialic acid derivatization [94].

2.2.1. Surface Modification of Exosomes

Exosomes hold significant potential for therapeutic development due to their intrinsic regulatory properties and natural capacity for delivering bioactive molecules. Enhancing the surface of exosomes through modification can further improve their targeting specificity, thus increasing their efficiency *in vivo* and minimizing adverse effects [95]. Exosome surface alterations are generally accomplished using diverse membrane modification techniques, such as genetic, chemical, metabolic, and enzymatic ligation [96]. These strategies are depicted in Figure 3. These approaches aim to optimize therapeutic performance and reduce off-target impacts.

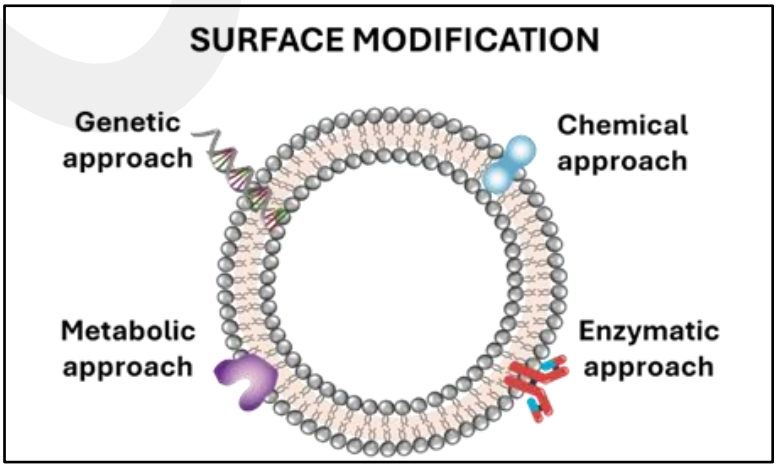


Figure 3. Approaches for exosome surface modification: Various techniques such as genetic engineering, chemical conjugation, metabolic labeling, and enzymatic modification are employed to enhance exosome targeting and functionality.

In the genetic approach, exosomes typically exhibit membrane proteins like as CD63, CD81, CD9, and Lamp2b, to which peptides or proteins can be conjugated at their extracellular domains, facilitating the presentation of these molecules on the exosome surface [97]. A variety of membrane proteins, including Lamp2b, tetraspanins, the transmembrane domain of PDGFR, syntenin-1, PTGFRN, and CXCR4, have been used to display targeting ligands on exosomes [98]. Surface modification of exosomes can also be achieved by targeting membrane lipids. In this strategy, targeting moieties are genetically fused to proteins or peptides that specifically bind to lipid components of the exosome membrane [99]. The transfected cells then release modified exosomes through these protein-lipid interactions. In a recent study, GAG-based fusion proteins were labeled and their effects on cell uptake mechanisms were evaluated in detail [100]. The results of this study indicated that surface proteins are effective in the pharmacokinetics of exosomes. Additionally, different studies have shown that surface proteins of exosomes prevent their uptake by macrophages [101,102]. In a study, metabolic engineering was used to covalently replace exosome surface azide groups with diphenylcyclooctin-dithiophosphate to improve macrophage identification in ischemic mice [103]. In chemical approach, click chemistry or physical interaction with lipids, which allows for the organization of exosomes at the surface. These modifications facilitate the purification process and promote receptor-mediated endocytosis, which in turn facilitates specific cellular uptake and enhances the in vivo targeting ability of exosomes [104]. Surface protein components of exosomes play a role in determining their circulation kinetics and biodistribution profiles. One of the most employed methods for increasing the circulation time of nanocarriers is the modification of these particles with polyethylene glycol (PEG) [105]. This approach involved coating exosome formulations with PEG that had been functionalized with anti-EGFR nanobodies. The PEGylation method resulted in longer plasma half-lives and less nonspecific interactions for exosomes. Furthermore, the inclusion of nanobodies was found to improve binding selectively to cells expressing EGFR, hence increasing targeted delivery efficiency [106]. The enzymatic approach leverages protein ligases, enzymes capable of ligating proteins or peptides with specific amino acid sequences. Surface modification of exosomes is achieved through enzymatic ligation between exosome membrane proteins and targeting peptides or proteins, creating permanent covalent bonds without the need for genetic or chemical modification [107]. Pham et al. modified exosomes via attaching Sortase A and OaAEP1 protein ligases, with the number of modified molecules per vesicle varying based on

the type of exosome and the presence of enzyme-recognition motifs on the membrane surface [108].

2.2.2. Ligand Modification of Exosomes

These interactions are based on the principle of increasing the interaction with natural receptors on the surface of the target area by binding ligands to exosomes. In order to ensure targeting, studies have been conducted which bind transferrin to exosomes produced from blood reticulocytes, and avidin-biotin to exosomes produced from the umbilical cord [109]. Exosomes have also been functionalized with targeting peptides postproduction. A peptide (ApoA1) containing an ApoA-I mimetic sequence that associates with phospholipids was employed to target glioma cells. A peptide specific to the neuropilin-1 receptor (RGERPPR), expressed on glioma and tumour vascular endothelial cells, was also employed to enhance targeting. The results of the targeting studies indicated that the CP05 peptide was capable of binding to the exosome surface. The CP05 peptide has been employed to attach N1ND, a functional domain of the high mobility group nucleosome-binding protein 1 (HMGN1) which enhances dendritic cells' capacity to stimulate T cells and is beneficial for immunotherapy-based cancer treatment. M12 (muscle targeting peptide) and RVG peptides were also fused with CP05-modified exosomes for their enhanced delivery to peripheral muscles and the brain, respectively [4,16].

2.3. Delivery of Imaging Agents

Accurate and effective imaging is fundamental to the early detection of diseases, precise diagnosis, and the management of therapeutic interventions. Although conventional contrast agents are indispensable for enhancing visual clarity in medical imaging, they come with inherent limitations, such as toxicity and the potential for adverse allergic reactions [110]. These limitations underscore the need for alternative imaging agents that can achieve high-quality imaging with minimized side effects. Exosomes, with their unique biological properties—namely, natural biocompatibility, low toxicity, and the intrinsic ability to cross biological barriers—represent a promising new class of imaging agents that address these limitations [111]. Furthermore, exosomes can be engineered to carry multiple types of imaging agents simultaneously, enabling comprehensive visualization of complex biological processes, which is valuable for both diagnostic and therapeutic purposes [112].

In recent years, the incorporation of specific imaging agents into exosomes has enabled the advancement of high-resolution imaging across various medical fields. Exosome-based contrast agents have shown great potential in disciplines such as oncology, neurology, cardiovascular imaging, infectious disease detection, and the monitoring of inflammatory conditions [113]. These exosome-based agents have been successfully integrated into various imaging modalities, including magnetic resonance imaging (MRI), computed tomography (CT), fluorescence imaging, ultrasound, as well as nuclear imaging techniques like single photon emission computed tomography (SPECT) and positron emission tomography (PET) [114].

For instance, exosomes modified to carry magnetic nanoparticles, such as superparamagnetic iron oxide (SPIO), can significantly enhance contrast in MRI scans while improving specificity through targeted accumulation in diseased tissues and organs. This targeted approach not only increases the sensitivity of MRI but also minimizes the likelihood of false positives, thereby enhancing diagnostic accuracy [115]. Similarly, in CT imaging, exosomes containing iodine or gold nanoparticles can significantly enhance contrast, especially in tumors and vascular structures, hence improving the assessment of vascular disorders and tumor features [116]. In fluorescence imaging, fluorescent dye- or quantum dot-labeled exosomes provide real-time monitoring of exosome biodistribution, which is crucial for investigating cellular absorption mechanisms and assessing treatment responses at the cellular level. This fluorescence-based imaging facilitates dynamic, in vivo observations of biological processes, offering significant benefits for research and therapeutic applications [117,118]. Nuclear imaging, a noninvasive approach, enhances the utility of exosomes as imaging agents [119]. Exosomes can be accurately tracked by radiolabeling them with widely utilized radionuclides, such iodine-131, indium-111, and technetium-99m, enabling the acquisition of extremely precise images at cellular and tissue levels with gamma cameras or PET/CT equipment [120,121].

Despite the potential benefits of exosome-based contrast agents, numerous hurdles must be resolved prior to their widespread implementation in clinical practice. Maintaining the uniform quality and efficacy of exosome preparations via standardized production methods is essential, as is pursuing additional study on their long-term metabolism, biodistribution, and possible impacts on human health [122]. Moreover, regulatory frameworks must be established to ensure the safe and effective application of exosome-based imaging agents in clinical environments [123]. In a nutshell exosomes constitute an exciting area in medical imaging, providing a versatile, biocompatible, and powerful platform capable of considerably boosting diagnostic and therapeutic imaging applications.

3. Discussion: Challenges and Future Directions in Exosome-Based Delivery Systems

Exosomes have emerged as a transformative platform in drug delivery due to their unique biological properties and versatility. However, despite the significant potential, translating exosome-based therapies from bench to bedside presents several formidable challenges. These obstacles must be systematically addressed to fully harness the therapeutic capabilities of exosomes and integrate them into clinical practice effectively.

One of the primary challenges in exosome-based drug delivery is the scalable production of therapeutic-grade exosomes [124]. Current production methods often struggle to generate enough for clinical applications, primarily due to the complexity of isolating exosomes from cell cultures and biological fluids. Traditional methods, such as ultracentrifugation, are labor-intensive and have limited scalability. To overcome this, there is a pressing need to develop robust bioreactor systems that can support large-scale exosome production while maintaining high purity and functionality. Furthermore, the use of defined cell sources is critical to ensure consistency and reproducibility across batches. Quality control measures, including stringent characterization protocols, are essential to verify the biological activity and safety of produced exosomes, thereby meeting regulatory standards and ensuring patient safety [125].

Effective drug loading is pivotal for the success of exosome-based delivery systems [126]. Current techniques for encapsulating therapeutic agents within exosomes, such as electroporation, sonication, and co-incubation, often face limitations in loading efficiency and may impact the integrity of the exosomal membrane. Achieving high loading efficiency while preserving the exosome's structure and function is crucial. Innovative approaches, such as genetic modification of parent cells or advanced nanotechnology methods, could enhance the incorporation of drugs into exosomes. Additionally, ensuring the stability of these loaded exosomes throughout storage and delivery is imperative to maintain their therapeutic efficacy [127]. Addressing these technical challenges will significantly enhance the performance of exosomes as drug carriers and broaden their application in various therapeutic domains.

Understanding how exosomes interact with and traverse biological barriers is critical to their success as drug delivery vehicles. Exosomes possess an inherent ability to cross barriers such as the blood-brain barrier, but the precise mechanisms by which they navigate these and other complex environments are not fully understood [128]. Further research is needed to elucidate how exosomes recognize and bind to target cells, and how they are internalized and trafficked within these cells. By leveraging their natural targeting capabilities, exosomes can be engineered to enhance their delivery to specific tissues or disease sites, thereby improving the precision and efficacy of therapies. Exploring the roles of surface molecules and engineering

exosomes with specific ligands or antibodies can significantly advance targeted drug delivery, making treatments more effective and reducing off-target effects.

Consistency and reliability in the isolation and characterization of exosomes are paramount to their clinical application [129]. Variability in isolation methods, including ultracentrifugation, size-exclusion chromatography, precipitation-based approaches, and microfluidic techniques, can lead to differences in the purity, yield, and composition of exosomal preparations, which can affect their therapeutic potential [130]. Ultracentrifugation, although extensively utilized, frequently co-isolates protein clumps and non-exosomal vesicles, which may impact subsequent uses. Size-exclusion chromatography provides enhanced purity, while potentially yielding smaller quantities. Precipitation-based kits, while expeditious and user-friendly, may introduce polymer impurities that disrupt functional studies. Microfluidic technologies, as nascent platforms, have potential for high-purity exosome isolation with little sample processing [131]. Standardized protocols for exosome isolation, that balance efficiency, purity, and scalability must be established to ensure reproducibility. Characterization methods, including particle sizing, protein profiling, and RNA analysis, should be standardized to accurately assess the quality and functionality of exosomes. The development of these standardized practices will facilitate regulatory approval processes and enable the widespread clinical adoption of exosome-based therapies.

Before exosomes can be widely used in clinical settings, comprehensive biosafety evaluations are necessary to address potential risks [132]. These include assessing the potential for immune reactions, the risk of transferring oncogenic or pathogenic materials, and the long-term safety of exosome-based treatments. Given that exosomes can carry biomolecules reflective of their parent cells, there is a concern about the inadvertent transfer of harmful components. Rigorous preclinical studies and risk assessments are required to evaluate the safety profile of exosomes and mitigate any adverse effects. Establishing regulatory frameworks and guidelines for the safe use of exosomes in therapy is essential to ensure patient safety and build confidence in these innovative treatment modalities.

In conclusion, addressing the challenges of scalable production, efficient drug loading, biological targeting, standardization, and biosafety is crucial to unlocking the full potential of exosomes in drug delivery. By advancing research and developing innovative solutions to these issues, exosome-based therapies can become a cornerstone in treating a wide range of diseases, offering new hope and improved outcomes for patients.

4. Conclusion

Exosomes are gaining recognition as effective delivery vehicles in biomedicine owing to their natural origin, biocompatibility, and multifunctional capabilities. These tiny vesicles effectively transport various therapeutic agents, such as proteins, nucleic acids, and small molecules, rendering them suitable for targeted medication administration. Their capacity to adeptly traverse biological barriers and selectively target specific cells improves the accuracy and efficacy of treatments. The biocompatibility of exosomes reduces immune rejection and adverse responses, benefiting long-term therapies. Notwithstanding these advantages, numerous problems must be resolved to completely utilize exosomes as delivery vehicles. Scalable manufacturing and reproducible isolation techniques are essential to fulfill clinical requirements. Enhancing methods for the efficient loading of therapeutic compounds into exosomes and maintaining their stability during delivery is crucial. Moreover, comprehending the interaction of exosomes with biological barriers would enhance their targeting efficacy. Standardization of isolation and characterization techniques is essential for ensuring uniform quality and functionality, while thorough biosafety assessments are crucial for mitigating potential dangers. Confronting these issues via focused research will realize the complete potential of exosomes in clinical applications. Exosome-based delivery systems are poised to transform treatments by offering precise, efficient, and biocompatible solutions for many diseases, addressing intricate medical requirements through new methodologies.

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Competing Interest

The corresponding author declares that this manuscript is original, has not been published before, and is not currently being considered for publication elsewhere. There is no conflict of interest.

Availability of data and materials

No new data were generated or analyzed in this study.

5. References

[1] Y. Li, L. Meng, B. Li, Y. Li, T. Shen, B. Zhao, The Exosome Journey: From Biogenesis to Regulation and Function in Cancers., J Oncol 2022 (2022) 9356807. https://doi.org/10.1155/2022/9356807.

[2] X.-R. Guo, Y. Ma, Z.-M. Ma, T.-S. Dai, S.-H. Wei, Y.-K. Chu, X.-G. Dan, Exosomes: The role in mammalian reproductive regulation and pregnancy-related diseases., Front Physiol 14 (2023) 1056905. https://doi.org/10.3389/fphys.2023.1056905.

[3] Y. Yao, Y. Jiang, J. Song, R. Wang, Z. Li, L. Yang, W. Wu, L. Zhang, Q. Peng, Exosomes as Potential Functional Nanomaterials for Tissue Engineering., Adv Healthc Mater 12 (2023) e2201989. https://doi.org/10.1002/adhm.202201989.

[4] R. Tenchov, J.M. Sasso, X. Wang, W.-S. Liaw, C.-A. Chen, Q.A. Zhou, Exosomes–Nature's Lipid Nanoparticles, a Rising Star in Drug Delivery and Diagnostics., ACS Nano 16 (2022) 17802–17846. https://doi.org/10.1021/acsnano.2c08774.

[5] S. Singh, D. Paul, V. Nath, R. A, Exosomes: current knowledge and future perspectives., Tissue Barriers 12 (2024) 2232248. https://doi.org/10.1080/21688370.2023.2232248.

[6] J. Cao, M. Zhang, F. Xie, J. Lou, X. Zhou, L. Zhang, M. Fang, F. Zhou, Exosomes in head and neck cancer: Roles, mechanisms and applications., Cancer Lett 494 (2020) 7–16. https://doi.org/10.1016/j.canlet.2020.07.005.

[7] X. Zhang, D. Liu, Y. Gao, C. Lin, Q. An, Y. Feng, Y. Liu, D. Liu, H. Luo, D. Wang, The Biology and Function of Extracellular Vesicles in Cancer Development., Front Cell Dev Biol 9 (2021) 777441. https://doi.org/10.3389/fcell.2021.777441.

[8] Y. Xia, X.-T. He, X.-Y. Xu, B.-M. Tian, Y. An, F.-M. Chen, Exosomes derived from M0, M1 and M2 macrophages exert distinct influences on the proliferation and differentiation of mesenchymal stem cells., PeerJ 8 (2020) e8970. https://doi.org/10.7717/peerj.8970.

[9] M. Heidarzadeh, Y. Gürsoy-Özdemir, M. Kaya, A. Eslami Abriz, A. Zarebkohan, R. Rahbarghazi, E. Sokullu, Exosomal delivery of therapeutic modulators through the blood-brain barrier; promise and pitfalls., Cell Biosci 11 (2021) 142. https://doi.org/10.1186/s13578-021-00650-0.

[10] H. Choi, K. Choi, D.-H. Kim, B.-K. Oh, H. Yim, S. Jo, C. Choi, Strategies for Targeted Delivery of Exosomes to the Brain: Advantages and Challenges., Pharmaceutics 14 (2022). https://doi.org/10.3390/pharmaceutics14030672.

[11] S. Zhou, T. Hu, G. Han, Y. Wu, X. Hua, J. Su, W. Jin, Y. Mou, X. Mou, Q. Li, S. Liu, Accurate Cancer Diagnosis and Stage Monitoring Enabled by Comprehensive Profiling of Different Types of Exosomal Biomarkers: Surface Proteins and miRNAs., Small 16 (2020) e2004492. https://doi.org/10.1002/smll.202004492.

[12] F. Yang, M. Wang, X. Guan, Exosomes and mimics as novel delivery platform for cancer therapy., Front Pharmacol 13 (2022) 1001417. https://doi.org/10.3389/fphar.2022.1001417.

[13] H. Kim, H. Jang, H. Cho, J. Choi, K.Y. Hwang, Y. Choi, S.H. Kim, Y. Yang, Recent Advances in Exosome-Based Drug Delivery for Cancer Therapy., Cancers (Basel) 13 (2021). https://doi.org/10.3390/cancers13174435.

[14] Y. Liang, L. Duan, J. Lu, J. Xia, Engineering exosomes for targeted drug delivery., Theranostics 11 (2021) 3183–3195. https://doi.org/10.7150/thno.52570.

[15] D.I. Avgoulas, K.S. Tasioulis, R.M. Papi, A.A. Pantazaki, Therapeutic and Diagnostic Potential of Exosomes as Drug Delivery Systems in Brain Cancer., Pharmaceutics 15 (2023). https://doi.org/10.3390/pharmaceutics15051439.

[16] S. Sen, J. Xavier, N. Kumar, M.Z. Ahmad, O.P. Ranjan, Exosomes as natural nanocarrier-based drug delivery system: recent insights and future perspectives., 3 Biotech 13 (2023) 101. https://doi.org/10.1007/s13205-023-03521-2.

[17] F. Xie, Y. Huang, Y. Zhan, L. Bao, Exosomes as drug delivery system in gastrointestinal cancer., Front Oncol 12 (2022) 1101823. https://doi.org/10.3389/fonc.2022.1101823.

[18] G. Patel, T.G. Agnihotri, M. Gitte, T. Shinde, S.S. Gomte, R. Goswami, A. Jain, Exosomes: a potential diagnostic and treatment modality in the quest for counteracting cancer., Cell Oncol (Dordr) 46 (2023) 1159–1179. https://doi.org/10.1007/s13402-023-00810-z.

[19] T. Wu, Y. Liu, N.M. Ali, B. Zhang, X. Cui, Effects of Exosomes on Tumor Bioregulation and Diagnosis., ACS Omega 8 (2023) 5157–5168. https://doi.org/10.1021/acsomega.2c06567.

[20] Y.-L. Tai, K.-C. Chen, J.-T. Hsieh, T.-L. Shen, Exosomes in cancer development and clinical applications., Cancer Sci 109 (2018) 2364–2374. https://doi.org/10.1111/cas.13697.

[21] J. Wang, D. Chen, E.A. Ho, Challenges in the development and establishment of exosome-based drug delivery systems., J Control Release 329 (2021) 894–906. https://doi.org/10.1016/j.jconrel.2020.10.020.

[22] A. Butreddy, N. Kommineni, N. Dudhipala, Exosomes as Naturally Occurring Vehicles for Delivery of Biopharmaceuticals: Insights from Drug Delivery to Clinical Perspectives., Nanomaterials (Basel) 11 (2021). https://doi.org/10.3390/nano11061481.

[23] A. Rajput, A. Varshney, R. Bajaj, V. Pokharkar, Exosomes as New Generation Vehicles for Drug Delivery: Biomedical Applications and Future Perspectives., Molecules 27 (2022). https://doi.org/10.3390/molecules27217289.

[24] H.I. Kim, J. Park, Y. Zhu, X. Wang, Y. Han, D. Zhang, Recent advances in extracellular vesicles for therapeutic cargo delivery, Exp Mol Med 56 (2024) 836–849. https://doi.org/10.1038/s12276-024-01201-6.

[25] T. Li, X. Li, G. Han, M. Liang, Z. Yang, C. Zhang, S. Huang, S. Tai, S. Yu, The Therapeutic Potential and Clinical Significance of Exosomes as Carriers of Drug Delivery System., Pharmaceutics 15 (2022). https://doi.org/10.3390/pharmaceutics15010021.

[26] D. Goutas, A. Pergaris, N. Goutas, S. Theocharis, Utilizing Exosomal-EPHs/Ephrins as Biomarkers and as a Potential Platform for Targeted Delivery of Therapeutic Exosomes, Int J Mol Sci 23 (2022). https://doi.org/10.3390/ijms23073551.

[27] T. Li, X. Li, G. Han, M. Liang, Z. Yang, C. Zhang, S. Huang, S. Tai, S. Yu, The Therapeutic Potential and Clinical Significance of Exosomes as Carriers of Drug Delivery System, Pharmaceutics 15 (2023). https://doi.org/10.3390/pharmaceutics15010021.

[28] M.N. Huda, M. Nafiujjaman, I.G. Deaguero, J. Okonkwo, M.L. Hill, T. Kim, M. Nurunnabi, Potential Use of Exosomes as Diagnostic Biomarkers and in Targeted Drug Delivery: Progress in

Clinical and Preclinical Applications., ACS Biomater Sci Eng 7 (2021) 2106–2149. https://doi.org/10.1021/acsbiomaterials.1c00217.

[29] D.K. Jeppesen, A.M. Fenix, J. Franklin, J. Higginbotham, Q. Zhang, L. Zimmerman, D. Liebler, J. Ping, Q. Liu, R. Evans, W. Fissell, J.G. Patton, L. Rome, D. Burnette, R. Coffey, Reassessment of Exosome Composition, Cell 177 (2019) 428-445.e18. https://doi.org/10.1016/j.cell.2019.02.029.

[30] Y. Zhang, J.-Y. Bi, J. Huang, Y. Tang, S. Du, P. Li, Exosome: A Review of Its Classification, Isolation Techniques, Storage, Diagnostic and Targeted Therapy Applications, Int J Nanomedicine 15 (2020) 6917–6934. https://doi.org/10.2147/IJN.S264498.

[31] H.B. Koh, H.J. Kim, S.-W. Kang, T.-H. Yoo, Exosome-Based Drug Delivery: Translation from Bench to Clinic., Pharmaceutics 15 (2023). https://doi.org/10.3390/pharmaceutics15082042.

[32] S. Li, Y. Tang, Y. Dou, The Potential of Milk-derived Exosomes for Drug Delivery., Curr Drug Deliv (2020). https://doi.org/10.2174/1567201817666200817112503.

[33] N. Samed, V. Sharma, A. Sundaramurthy, Hydrogen bonded niosomes for encapsulation and release of hydrophilic and hydrophobic anti-diabetic drugs: An efficient system for oral anti-diabetic formulation, Appl Surf Sci (2017). https://doi.org/10.1016/J.APSUSC.2017.11.055.

[34] Y. Huang, X. Guo, Y. Wu, X. Chen, L. Feng, N. Xie, G. Shen, Nanotechnology's frontier in combatting infectious and inflammatory diseases: prevention and treatment, Signal Transduct Target Ther 9 (2024) 34. https://doi.org/10.1038/s41392-024-01745-z.

[35] M. Flanagan, I. Pathak, Q. Gan, L. Winter, R. Emnet, S. Akel, A.M. Montaño, Umbilical mesenchymal stem cell-derived extracellular vesicles as enzyme delivery vehicle to treat Morquio A fibroblasts, Stem Cell Res Ther 12 (2021) 1–15. https://doi.org/10.1186/s13287-021-02355-0.

[36] M.S. Gholami Farashah, M. Javadi, J. Soleimani Rad, S.K. Shakouri, S. Asnaashari, S. Dastmalchi, S. Nikzad, L. Roshangar, 17β-Estradiol-Loaded Exosomes for Targeted Drug Delivery in Osteoporosis: A Comparative Study of Two Loading Methods., Adv Pharm Bull 13 (2023) 736–746. https://doi.org/10.34172/apb.2023.072.

[37] M. Fan, H. Liu, H. Yan, R. Che, Y. Jin, X. Yang, X. Zhou, H. Yang, K. Ge, X.-J. Liang, J. Zhang, Z. Li, A CAR T-inspiring platform based on antibody-engineered exosomes from antigen-feeding dendritic cells for precise solid tumor therapy., Biomaterials 282 (2022) 121424. https://doi.org/10.1016/j.biomaterials.2022.121424.

[38] O.P.B. Wiklander, D.R. Mamand, D.K. Mohammad, W. Zheng, R. Jawad Wiklander, T. Sych, A.M. Zickler, X. Liang, H. Sharma, A. Lavado, J. Bost, S. Roudi, G. Corso, A.J. Lennaárd, M. Abedi-Valugerdi, I. Mäger, E. Alici, E. Sezgin, J.Z. Nordin, D. Gupta, A. Görgens, S. EL Andaloussi, Antibody-displaying extracellular vesicles for targeted cancer therapy, Nat Biomed Eng (2024). https://doi.org/10.1038/s41551-024-01214-6.

[39] M.D. Hade, C.N. Suire, Z. Suo, Significant Enhancement of Fibroblast Migration, Invasion, and Proliferation by Exosomes Loaded with Human Fibroblast Growth Factor 1, ACS Appl Mater Interfaces 16 (2024) 1969–1984. https://doi.org/10.1021/acsami.3c10350.

[40] Q. Zhang, M. Wang, C. Han, Z. Wen, X. Meng, D. Qi, N. Wang, H. Du, J. Wang, L. Lu, X. Ge, Intraduodenal Delivery of Exosome-Loaded SARS-CoV-2 RBD mRNA Induces a Neutralizing

Antibody Response in Mice, Vaccines (Basel) 11 (2023). https://doi.org/10.3390/vaccines11030673.

[41] S.-F. Liang, F.-F. Zuo, B.-C. Yin, B.-C. Ye, Delivery of siRNA based on engineered exosomes for glioblastoma therapy by targeting STAT3, Biomater Sci 10 (2022) 1582–1590.

[42] W. Zhou, M. Xu, Z. Wang, M. Yang, Engineered exosomes loaded with miR-449a selectively inhibit the growth of homologous non-small cell lung cancer, Cancer Cell Int 21 (2021) 1–11. https://doi.org/10.1186/s12935-021-02157-7.

[43] M. Kang, C. Hisey, B. Tsai, Y. Nursalim, C. Blenkiron, L.W. Chamley, Placental Extracellular Vesicles Can Be Loaded with Plasmid DNA, Mol Pharm 20 (2023) 1898–1913. https://doi.org/10.1021/acs.molpharmaceut.2c00533.

[44] Y. Lin, J. Wu, W. Gu, Y. Huang, Z. Tong, L. Huang, J. Tan, Exosome–Liposome Hybrid Nanoparticles Deliver CRISPR/Cas9 System in MSCs, Advanced Science 5 (2018) 1700611. https://doi.org/https://doi.org/10.1002/advs.201700611.

[45] X. Lin, L. Lin, J. Wu, W. Jiang, J. Wu, J. Yang, C. Chen, A targeted siRNA-loaded PDL1-exosome and functional evaluation against lung cancer., Thorac Cancer 13 (2022) 1691–1702. https://doi.org/10.1111/1759-7714.14445.

[46] K.D. Popowski, B. López de Juan Abad, A. George, D. Silkstone, E. Belcher, J. Chung, A. Ghodsi, H. Lutz, J. Davenport, M. Flanagan, J. Piedrahita, P.-U.C. Dinh, K. Cheng, Inhalable exosomes outperform liposomes as mRNA and protein drug carriers to the lung, Extracellular Vesicle 1 (2022) 100002. https://doi.org/https://doi.org/10.1016/j.vesic.2022.100002.

[47] J. Nebogatova, H.H. Härk, A. Puskar, L. Porosk, P. Guazzi, M. Dowaidar, Ü. Langel, K. Kurrikoff, A Method for Using Cell-Penetrating Peptides for Loading Plasmid DNA into Secreted Extracellular Vesicles, Biomolecules 13 (2023). https://doi.org/10.3390/biom13121751.

[48] S. Paul, S. Bhagat, L. Dash, H. Das Mohapatra, S. Jena, S.K. Verma, A. Dutta, ExoDS: a versatile exosome-based drug delivery platform to target cancer cells and cancer stem cells, Front Bioeng Biotechnol 12 (2024) 1–14. https://doi.org/10.3389/fbioe.2024.1362681.

[49] Z. Ye, T. Zhang, W. He, H. Jin, C. Liu, Z. Yang, J. Ren, Methotrexate-Loaded Extracellular Vesicles Functionalized with Therapeutic and Targeted Peptides for the Treatment of Glioblastoma Multiforme, ACS Appl Mater Interfaces 10 (2018) 12341–12350. https://doi.org/10.1021/acsami.7b18135.

[50] X. Zhang, J. Wang, N. Liu, W. Wu, H. Li, W. Lu, X. Guo, Umbilical Cord Blood-Derived M1 Macrophage Exosomes Loaded with Cisplatin Target Ovarian Cancer In Vivo and Reverse Cisplatin Resistance, Mol Pharm 20 (2023) 5440–5453. https://doi.org/10.1021/acs.molpharmaceut.3c00132.

[51] J. Attem, R.V.L. Narayana, R. Manukonda, S. Kaliki, G.K. Vemuganti, Small extracellular vesicles loaded with carboplatin effectively enhance the cytotoxicity of drug-resistant cells from Y79 cells-in vitro, Biomedicine & Pharmacotherapy 173 (2024) 116403. https://doi.org/https://doi.org/10.1016/j.biopha.2024.116403.

[52] P. Zhao, J. Wang, H. Huang, Z. Chen, H. Wang, Q. Lin, Exosomes-based dual drug loaded nanocarrier for targeted and multiple proliferative vitreoretinopathy therapy, Regen Biomater (2024) rbae081. https://doi.org/10.1093/rb/rbae081.

[53] X. Yang, B. Xie, H. Peng, G. Shi, B. Sreenivas, J. Guo, C. Wang, Y. He, Eradicating intracellular MRSA via targeted delivery of lysostaphin and vancomycin with mannose-modified exosomes, Journal of Controlled Release 329 (2021) 454–467. https://doi.org/https://doi.org/10.1016/j.jconrel.2020.11.045.

[54] E. Heinrich, O. Hartwig, C. Walt, A. Kardani, M. Koch, L.P. Jahromi, J. Hoppstädter, A.K. Kiemer, B. Loretz, C.-M. Lehr, G. Fuhrmann, Cell-Derived Vesicles for Antibiotic Delivery—Understanding the Challenges of a Biogenic Carrier System, Small 19 (2023) 2207479. https://doi.org/https://doi.org/10.1002/smll.202207479.

[55] A. Davari, H. Hajjaran, A. Khamesipour, M. Mohebali, F. Mehryab, S. Shahsavari, F. Shekari, Amphotericin B-Loaded Extracellular Vesicles Derived from Leishmania major Enhancing Cutaneous Leishmaniasis Treatment through In Vitro and In Vivo Studies., Iran J Parasitol 18 (2023) 514–525. https://doi.org/10.18502/ijpa.v18i4.14260.

[56] J.-M.R.M.H.C.Z.M.R.B.K.S.A.P. Rummenigge Oliveira Silva Hermine Counil, C. Ramassamy, Donepezil-Loaded Nanocarriers for the Treatment of Alzheimer's Disease: Superior Efficacy of Extracellular Vesicles Over Polymeric Nanoparticles, Int J Nanomedicine 19 (2024) 1077–1096. https://doi.org/10.2147/IJN.S449227.

[57] J. Liu, Y. Tang, Y. Li, X. Hu, S. Huang, W. Xu, X. Hao, M. Zhou, J. Wu, D. Xiang, Paclitaxel-loaded hybrid exosome for targeted chemotherapy of triple-negative breast cancer, Europe PMC (2022).

[58] M. Sheykhhasan, R. Amini, S. Soleimani Asl, M. Saidijam, S.M. Hashemi, R. Najafi, Neuroprotective effects of coenzyme Q10-loaded exosomes obtained from adipose-derived stem cells in a rat model of Alzheimer's disease., Biomed Pharmacother 152 (2022) 113224. https://doi.org/10.1016/j.biopha.2022.113224.

[59] X. He, C. Zhang, S. Amirsaadat, A.T. Jalil, M.M. Kadhim, M. Abasi, Y. Pilehvar, Curcumin-Loaded Mesenchymal Stem Cell–Derived Exosomes Efficiently Attenuate Proliferation and Inflammatory Response in Rheumatoid Arthritis Fibroblast-Like Synoviocytes, Appl Biochem Biotechnol 195 (2023) 51–67. https://doi.org/10.1007/s12010-022-04090-5.

[60] M. Basak, B. Sahoo, D.K. Chaudhary, S. Narisepalli, S. Tiwari, D. Chitkara, A. Mittal, Human umbilical cord blood-mesenchymal stem cell derived exosomes as an efficient nanocarrier for Docetaxel and miR-125a: Formulation optimization and anti-metastatic behaviour, Life Sci 322 (2023) 121621. https://doi.org/https://doi.org/10.1016/j.lfs.2023.121621.

[61] N.S. Değirmenci, M. Uslu, O.K. Kırbaş, F. Şahin, E. Önay Uçar, Lapatinib loaded exosomes as a drug delivery system in breast cancer, J Drug Deliv Sci Technol 75 (2022) 103584. https://doi.org/https://doi.org/10.1016/j.jddst.2022.103584.

[62] J.H.Y.Z.W.C.Q.T.Y.A.R.C. Han Li Yinan Ding, C. Hu, Angiopep-2 Modified Exosomes Load Rifampicin with Potential for Treating Central Nervous System Tuberculosis, Int J Nanomedicine 18 (2023) 489–503. https://doi.org/10.2147/IJN.S395246.

[63] P. Dehghani, J. Varshosaz, M. Mirian, M. Minaiyan, M. Kazemi, M. Bodaghi, Keratinocyte Exosomes for Topical Delivery of Tofacitinib in Treatment of Psoriasis: an In Vitro/ In Vivo Study in Animal Model of Psoriasis, Pharm Res 41 (2024) 263–279. https://doi.org/10.1007/s11095-023-03648-0.

[64] www.clinicaltrials.gov, (2024).

[65] S. Han, H. Yang, X. Ni, Y. Deng, Z. Li, X. Xing, M. Du, Programmed release of vascular endothelial growth factor and exosome from injectable chitosan nanofibrous microsphere-based PLGA-PEG-PLGA hydrogel for enhanced bone regeneration, Int J Biol Macromol 253 (2023) 126721. https://doi.org/https://doi.org/10.1016/j.ijbiomac.2023.126721.

[66] C. Kang, P. Han, J.S. Lee, D. Lee, D. Kim, Anchor, Spacer, and Ligand-Modified Engineered Exosomes for Trackable Targeted Therapy, Bioconjug Chem 31 (2020) 2541–2552. https://doi.org/10.1021/acs.bioconjchem.0c00483.

[67] Z. Chen, M. Xiong, J. Tian, D. Song, S. Duan, L. Zhang, Encapsulation and assessment of therapeutic cargo in engineered exosomes: a systematic review, J Nanobiotechnology 22 (2024). https://doi.org/10.1186/s12951-023-02259-6.

[68] R. Gul, H. Bashir, M. Sarfraz, A.J. Shaikh, Y.A. Bin Jardan, Z. Hussain, M.H.H. Bin Asad, F. Gulzar, B. Guan, I. Nazir, M.I. Amirzada, Human plasma derived exosomes: Impact of active and passive drug loading approaches on drug delivery, Saudi Pharmaceutical Journal 32 (2024) 102096. https://doi.org/https://doi.org/10.1016/j.jsps.2024.102096.

[69] H. Zeng, S. Guo, X. Ren, Z. Wu, S. Liu, X. Yao, Current Strategies for Exosome Cargo Loading and Targeting Delivery, Cells 12 (2023). https://doi.org/10.3390/cells12101416.

[70] X. Xi, S. Xia, R. Lu, Drug loading techniques for exosome-based drug delivery systems., Pharmazie 76 2 (2021) 61–67. https://doi.org/10.1691/ph.2021.0128.

[71] P. Ruzgys, M. Jakutavičiūtė, S. Chopra, S. Šatkauskas, Enhancement of drug electrotransfer by extracellular plasmid DNA., Arch Biochem Biophys 666 (2019) 156–160. https://doi.org/10.1016/j.abb.2018.12.010.

[72] T.N. Lamichhane, S. Jay, Production of Extracellular Vesicles Loaded with Therapeutic Cargo., Methods in Molecular Biology 1831 (2018) 37–47. https://doi.org/10.1007/978-1-4939-8661-3_4.

[73] T.N. Lamichhane, A. Jeyaram, D. Patel, B. Parajuli, N.K. Livingston, N. Arumugasaamy, J. Schardt, S. Jay, Oncogene Knockdown via Active Loading of Small RNAs into Extracellular Vesicles by Sonication, Cell Mol Bioeng 9 (2016) 315–324. https://doi.org/10.1007/S12195-016-0457-4.

[74] C. Liu, W. Zhang, Y. Li, J. Chang, F. Tian, F. Zhao, Y. Ma, J. Sun, Microfluidic Sonication to Assemble Exosome Membrane-Coated Nanoparticles for Immune Evasion-Mediated Targeting., Nano Lett (2019). https://doi.org/10.1021/acs.nanolett.9b02841.

[75] P. Guo, S. Busatto, J. Huang, G. Morad, M. Moses, A Facile Magnetic Extrusion Method for Preparing Endosome-Derived Vesicles for Cancer Drug Delivery, Adv Funct Mater 31 (2021). https://doi.org/10.1002/adfm.202008326.

[76] X. Wang, S. Hu, D. Zhu, J. Li, K. Cheng, G. Liu, Comparison of extruded cell nanovesicles and exosomes in their molecular cargos and regenerative potentials., Nano Res 16 (2023) 7248–7259. https://doi.org/10.1007/s12274-023-5374-3.

[77] L. Zheng, B. Hu, D. Zhao, W. Liu, Q. Liu, Y. Huang, S. Ruan, Recent progresses of exosome–liposome fusions in drug delivery, Chinese Chemical Letters 35 (2024) 108647. https://doi.org/https://doi.org/10.1016/j.cclet.2023.108647.

[78] S. Ahmadian, N. Jafari, A. Tamadon, A. Ghaffarzadeh, R. Rahbarghazi, M. Mahdipour, Different storage and freezing protocols for extracellular vesicles: a systematic review, Stem Cell Research and Therapy 15 (2024). https://doi.org/10.1186/s13287-024-04005-7.

[79] K. Ko, Y.-I. Yoo, J.Y. Kim, B. Choi, S. Park, W. Park, W. Rhim, D. Han, Attenuation of Tumor Necrosis Factor-α Induced Inflammation by Umbilical Cord-Mesenchymal Stem Cell Derived Exosome-Mimetic Nanovesicles in Endothelial Cells, Tissue Eng Regen Med 17 (2020) 155–163. https://doi.org/10.1007/s13770-019-00234-7.

[80] Y. Shi, S. Guo, Y. Liang, L. Liu, A. Wang, K. Sun, Y. Li, Construction and evaluation of liraglutide delivery system based on milk exosomes: a new idea for oral peptide delivery., Curr Pharm Biotechnol (2021). https://doi.org/10.2174/1389201022666210820114236.

[81] R.D. de Abreu, C. V Ramos, C. Becher, M. Lino, C. Jesus, P.A. da Costa Martins, P.A.T. Martins, M.J. Moreno, H. Fernandes, L. Ferreira, Exogenous loading of miRNAs into small extracellular vesicles, J Extracell Vesicles 10 (2021). https://doi.org/10.1002/jev2.12111.

[82] M. Wallen, F. Aqil, W. Spencer, R.C. Gupta, Exosomes as an Emerging Plasmid Delivery Vehicle for Gene Therapy., Pharmaceutics 15 (2023). https://doi.org/10.3390/pharmaceutics15071832.

[83] L. Li, L. Zhang, M. Knez, Comparison of two endogenous delivery agents in cancer therapy: Exosomes and ferritin., Pharmacol Res 110 (2016) 1–9. https://doi.org/10.1016/j.phrs.2016.05.006.

[84] L. Huang, D. Wang, N. Gu, X.-E. Zhang, Construction of engineered exosomes with high loading efficiency of cellular endogenous proteins., Sheng Wu Gong Cheng Xue Bao 35 8 (2019) 1537–1545. https://doi.org/10.13345/j.cjb.190124.

[85] H. Chen, L. Wang, X. Zeng, H. Schwarz, H.S. Nanda, X. Peng, Y. Zhou, Exosomes, a New Star for Targeted Delivery, Front Cell Dev Biol 9 (2021). https://doi.org/10.3389/fcell.2021.751079.

[86] H. Kim, E.H. Kim, G. Kwak, S. Chi, S.H. Kim, Y. Yang, Exosomes: Cell-Derived Nanoplatforms for the Delivery of Cancer Therapeutics, Int J Mol Sci 22 (2020). https://doi.org/10.3390/ijms22010014.

[87] H. Choi, Y. Choi, H.Y. Yim, A. Mirzaaghasi, J.-K. Yoo, C. Choi, Biodistribution of Exosomes and Engineering Strategies for Targeted Delivery of Therapeutic Exosomes, Tissue Eng Regen Med 18 (2021) 499–511. https://doi.org/10.1007/s13770-021-00361-0.

[88] U. Sterzenbach, U. Putz, L.-H. Low, J. Silke, S. Tan, J. Howitt, Engineered Exosomes as Vehicles for Biologically Active Proteins., Mol Ther 25 6 (2017) 1269–1278. https://doi.org/10.1016/j.ymthe.2017.03.030.

[89] S. Rana, S. Yue, D. Stadel, M. Zöller, Toward tailored exosomes: The exosomal tetraspanin web contributes to target cell selection, Int J Biochem Cell Biol 44 (2012) 1574–1584. https://doi.org/https://doi.org/10.1016/j.biocel.2012.06.018.

[90] J. Donoso-Quezada, S. Ayala-Mar, J. González-Valdez, The role of lipids in exosome biology and intercellular communication: Function, analytics and applications, Traffic 22 (2021) 204–220. https://doi.org/10.1111/tra.12803.

[91] R.S. Flannagan, J. Canton, W. Furuya, M. Glogauer, S. Grinstein, The phosphatidylserine receptor TIM4 utilizes integrins as coreceptors to effect phagocytosis., Mol Biol Cell 25 (2014) 1511–1522. https://doi.org/10.1091/mbc.E13-04-0212.

[92] A. Matsumoto, Y. Takahashi, M. Nishikawa, K. Sano, M. Morishita, C. Charoenviriyakul, H. Saji, Y. Takakura, Role of Phosphatidylserine-Derived Negative Surface Charges in the Recognition and Uptake of Intravenously Injected B16BL6-Derived Exosomes by Macrophages., J Pharm Sci 106 (2017) 168–175. https://doi.org/10.1016/j.xphs.2016.07.022.

[93] A. Shimoda, Y. Tahara, S. Sawada, Y. Sasaki, K. Akiyoshi, Glycan profiling analysis using evanescent-field fluorescence-assisted lectin array: Importance of sugar recognition for cellular uptake of exosomes from mesenchymal stem cells., Biochem Biophys Res Commun 491 3 (2017) 701–707. https://doi.org/10.1016/j.bbrc.2017.07.126.

[94] J. Berenguer, T. Lagerweij, X.W. Zhao, S. Dusoswa, P. van der Stoop, B. Westerman, M.C. de Gooijer, M. Zoetemelk, A. Zomer, M.H.W. Crommentuijn, L.E. Wedekind, À. López-López, A. Giovanazzi, M. Bruch-Oms, I.H. van der Meulen-Muileman, R.M. Reijmers, T.H. van Kuppevelt, J.-J. García-Vallejo, Y. van Kooyk, B.A. Tannous, P. Wesseling, D. Koppers-Lalic, W.P. Vandertop, D.P. Noske, V.W. van Beusechem, J. van Rheenen, D.M. Pegtel, O. van Tellingen, T. Wurdinger, Glycosylated extracellular vesicles released by glioblastoma cells are decorated by CCL18 allowing for cellular uptake via chemokine receptor CCR8., J Extracell Vesicles 7 (2018) 1446660. https://doi.org/10.1080/20013078.2018.1446660.

[95] H. Zhang, W. Yan, J. Wang, S. Xie, W.A. Tao, C.-W. Lee, X. Zhang, G. Zhang, Y. Liu, D. Wei, J. Hu, H. Liu, F. Liu, Y. Nie, X. Chen, H. Xu, J. Xia, S. Wang, Surface functionalization of exosomes for chondrocyte-targeted siRNA delivery and cartilage regeneration, Journal of Controlled Release 369 (2024) 493–505. https://doi.org/https://doi.org/10.1016/j.jconrel.2024.04.009.

[96] F. Tan, X. Li, Z. Wang, J. Li, K. Shahzad, J. Zheng, Clinical applications of stem cell-derived exosomes, Signal Transduct Target Ther 9 (2024). https://doi.org/10.1038/s41392-023-01704-0.

[97] Y. Li, C. Guo, Q. Huang, N. Zhou, J. Wang, Y. Huang, X. Shen, X. Yu, H. Shen, Exploration for sorting serum exosome as a measure of spinal cord injury severity, J Radiat Res Appl Sci 17 (2024) 101048. https://doi.org/https://doi.org/10.1016/j.jrras.2024.101048.

[98] Q. Liu, D. Li, X. Pan, Y. Liang, Targeted therapy using engineered extracellular vesicles: principles and strategies for membrane modification, J Nanobiotechnology 21 (2023) 334. https://doi.org/10.1186/s12951-023-02081-0.

[99] X. Ren, R. Xu, C. Xu, J. Su, Harnessing exosomes for targeted therapy: strategy and application, Biomaterials Translational 5 (2024) 46–58. https://doi.org/10.12336/biomatertransl.2024.01.005.

[100] Y. Ji, L. Mi, M. Zhao, X. He, Y. Hu, Y. Gao, C. Yin, K. Xu, Innovative Diagnosis and Therapeutic Modalities: Engineered Exosomes in Autoimmune Disease., Int J Nanomedicine 19 (2024) 3943–3956. https://doi.org/10.2147/IJN.S452184.

[101] S.M. Hallal, Á. Tűzesi, L.A. Sida, E. Xian, D. Madani, K. Muralidharan, B. Shivalingam, M.E. Buckland, L. Satgunaseelan, K.L. Alexander, Glioblastoma biomarkers in urinary extracellular vesicles reveal the potential for a 'liquid gold' biopsy, Br J Cancer 130 (2024) 836–851. https://doi.org/10.1038/s41416-023-02548-9.

[102] T. Cheng, M. Mao, Y. Liu, L. Xie, F. Shi, H. Liu, X. Li, The potential therapeutic effect of human umbilical cord mesenchymal stem cell-derived exosomes in bronchopulmonary dysplasia, Life Sci 357 (2024) 123047. https://doi.org/https://doi.org/10.1016/j.lfs.2024.123047.

[103] H. Zhang, J. Wu, J. Wu, Q. Fan, J. Zhou, J. Wu, S. Liu, J. Zang, J. Ye, M. Xiao, T. Tian, J. Gao, Exosome-mediated targeted delivery of miR-210 for angiogenic therapy after cerebral ischemia in mice, J Nanobiotechnology 17 (2019) 1–13. https://doi.org/10.1186/s12951-019-0461-7.

[104] Y. Ji, L. Mi, M. Zhao, X. He, Y. Hu, Y. Gao, C. Yin, K. Xu, Innovative Diagnosis and Therapeutic Modalities: Engineered Exosomes in Autoimmune Disease., Int J Nanomedicine 19 (2024) 3943–3956. https://doi.org/10.2147/IJN.S452184.

[105] O. Mehmet Saka, A. Bozkir, Formulation and in vitro characterization of PEGylated chitosan and polyethylene imine polymers with thrombospondin-I gene bearing pDNA, J Biomed Mater Res B Appl Biomater 100 (2012) 984–992.

[106] J.S. Suk, Q. Xu, N. Kim, J. Hanes, L.M. Ensign, PEGylation as a strategy for improving nanoparticle-based drug and gene delivery, Adv Drug Deliv Rev 99 (2016) 28–51.

[107] F.B.H. Rehm, T.J. Harmand, K. Yap, T. Durek, D.J. Craik, H.L. Ploegh, Site-Specific Sequential Protein Labeling Catalyzed by a Single Recombinant Ligase, J Am Chem Soc 141 (2019) 17388–17393. https://doi.org/10.1021/jacs.9b09166.

[108] T.C. Pham, M.K. Jayasinghe, T.T. Pham, Y. Yang, L. Wei, W.M. Usman, H. Chen, M. Pirisinu, J. Gong, S. Kim, B. Peng, W. Wang, C. Chan, V. Ma, N.T.H. Nguyen, D. Kappei, X.-H. Nguyen, W.C. Cho, J. Shi, M.T.N. Le, Covalent conjugation of extracellular vesicles with peptides and nanobodies for targeted therapeutic delivery, J Extracell Vesicles 10 (2021) e12057. https://doi.org/https://doi.org/10.1002/jev2.12057.

[109] S. Salunkhe, M. Basak, D. Chitkara, A. Mittal, others, Surface functionalization of exosomes for target-specific delivery and in vivo imaging & tracking: Strategies and significance, Journal of Controlled Release 326 (2020) 599–614.

[110] Z. Huo, B. Chen, Z. Wang, Y. Li, L. He, B. Hu, H. Li, P. Wang, J. Yao, F. Xu, Y. Li, X. Yang, Enhanced plasmonic scattering imaging via deep learning–based super-resolution reconstruction for exosome imaging, Anal Bioanal Chem (2024). https://doi.org/10.1007/s00216-024-05550-z.

[111] M.L. Hill, S.J. Chung, H.J. Woo, C.R. Park, K. Hadrick, M. Nafiujjaman, P.P.P. Kumar, L. Mwangi, R. Parikh, T. Kim, Exosome-Coated Prussian Blue Nanoparticles for Specific Targeting and Treatment of Glioblastoma, ACS Appl Mater Interfaces (2024). https://doi.org/10.1021/acsami.4c02364.

[112] Z. Guo, W. Liu, T. Liu, J. Wang, H. Jiang, X. Liu, Y. Weizmann, X. Wang, Engineered exosome hybrid copper nanoscale antibiotics facilitate simultaneous self-assembly imaging and elimination of intracellular multidrug-resistant superbugs, Chinese Chemical Letters 35 (2024) 109060. https://doi.org/https://doi.org/10.1016/j.cclet.2023.109060.

[113] Y. Liu, M. Li, J. Gu, H. Huang, H. Xie, C. Yu, S. Roy, X. Chen, T. Kuang, Y. Zhang, S. Jiang, B. Guo, Engineering of exosome-liposome hybrid-based theranostic nanomedicines for NIR-II fluorescence imaging-guided and targeted NIR-II photothermal therapy of subcutaneous glioblastoma, Colloids Surf B Biointerfaces 245 (2025) 114258. https://doi.org/https://doi.org/10.1016/j.colsurfb.2024.114258.

[114] Y. Wu, X. Shan, Y. Zhang, W. Shi, X. Zhu, M. Yu, S. Xie, L. Guo, Z. Chen, C. Zhang, D. Zhou, Z. Cai, Bioorthogonal surface-edited exosomes for targeted multimodal imaging and synergic chemophototherapy of oral cancer, Chemical Engineering Journal 497 (2024) 154653. https://doi.org/https://doi.org/10.1016/j.cej.2024.154653.

[115] K.O. Jung, H. Jo, J.H. Yu, S.S. Gambhir, G. Pratx, Development and MPI tracking of novel hypoxia-targeted theranostic exosomes, Biomaterials 177 (2018) 139–148. https://doi.org/https://doi.org/10.1016/j.biomaterials.2018.05.048.

[116] L. Gong, Y. Weng, W. Zhou, K. Zhang, W. Li, J. Jiang, J. Zhu, In vivo CT imaging of gold nanoparticle-labeled exosomes in a myocardial infarction mouse model, Ann Transl Med 9 (2021) 504–504. https://doi.org/10.21037/atm-21-981.

[117] V. Vinduska, C.E. Gallops, R. O'connor, Y. Wang, X. Huang, Exosomal surface protein detection with quantum dots and immunomagnetic capture for cancer detection, Nanomaterials 11 (2021). https://doi.org/10.3390/nano11071853.

[118] A. Pramanik, S. Patibandla, Y. Gao, L.R. Corby, M.M. Rhaman, S.S. Sinha, P.C. Ray, Bio-Conjugated Magnetic-Fluorescence Nanoarchitectures for the Capture and Identification of Lung-Tumor-Derived Programmed Cell Death Lighand 1-Positive Exosomes, ACS Omega 7 (2022) 16035–16042. https://doi.org/10.1021/acsomega.2c01210.

[119] C.T. Yang, R.C. Lai, V.J.X. Phua, S.E. Aw, B. Zhang, W.K. Sim, S.K. Lim, D.C.E. Ng, Standard Radio-Iodine Labeling Protocols Impaired the Functional Integrity of Mesenchymal Stem/Stromal Cell Exosomes, Int J Mol Sci 25 (2024). https://doi.org/10.3390/ijms25073742.

[120] Z.T. Rosenkrans, A.S. Thickens, J.A. Kink, E. Aluicio-Sarduy, J.W. Engle, P. Hematti, R. Hernandez, Investigating the In Vivo Biodistribution of Extracellular Vesicles Isolated from Various Human Cell Sources Using Positron Emission Tomography, Mol Pharm 21 (2024) 4324–4335. https://doi.org/10.1021/acs.molpharmaceut.4c00298.

[121] Y.H. Chung, Y.P. Ho, S.S. Farn, W.C. Tsai, Z.X. Li, T.Y. Lin, C.C. Weng, In vivo SPECT imaging of Tc-99m radiolabeled exosomes from human umbilical-cord derived mesenchymal stem cells in small animals, Biomed J 47 (2024). https://doi.org/10.1016/j.bj.2024.100721.

[122] J. Wang, Z.J. Chen, Z.Y. Zhang, M.P. Shen, B. Zhao, W. Zhang, Y. Zhang, J.G. Lei, C.J. Ren, J. Chang, C.L. Xu, M. Li, Y.Y. Pi, T.L. Lu, C.X. Dai, S.K. Li, P. Li, Manufacturing, quality control, and GLP-grade preclinical study of nebulized allogenic adipose mesenchymal stromal cells-derived extracellular vesicles, Stem Cell Res Ther 15 (2024). https://doi.org/10.1186/s13287-024-03708-1.

[123] C.K. Wang, T.H. Tsai, C.H. Lee, Regulation of exosomes as biologic medicines: Regulatory challenges faced in exosome development and manufacturing processes, Clin Transl Sci 17 (2024). https://doi.org/10.1111/cts.13904.

[124] X. Li, A.L. Corbett, E. Taatizadeh, N. Tasnim, J. Little, C. Garnis, M. Daugaard, E. Guns, M. Hoorfar, I.T.S. Li, Challenges and opportunities in exosome research—Perspectives from biology, engineering, and cancer therapy, APL Bioeng 3 (2019). https://doi.org/10.1063/1.5087122.

[125] C.-K. Wang, T.-H. Tsai, C.-H. Lee, Regulation of exosomes as biologic medicines: Regulatory challenges faced in exosome development and manufacturing processes, Clin Transl Sci 17 (2024). https://api.semanticscholar.org/CorpusID:271765630.

[126] S. Fu, Y. Wang, X. Xia, J.C. Zheng, Exosome engineering: Current progress in cargo loading and targeted delivery, NanoImpact 20 (2020) 100261. https://doi.org/10.1016/j.impact.2020.100261.

[127] V. Poinsot, N. Pizzinat, V. Ong-Meang, Engineered and Mimicked Extracellular Nanovesicles for Therapeutic Delivery, Nanomaterials 14 (2024). https://doi.org/10.3390/nano14070639.

[128] R. Elliott, M. He, Unlocking the Power of Exosomes for Crossing Biological Barriers in Drug Delivery, Pharmaceutics 13 (2021). https://doi.org/10.3390/pharmaceutics13010122.

[129] J. Chen, P. Li, T. Zhang, Z. Xu, X. Huang, R. Wang, L. Du, Review on Strategies and Technologies for Exosome Isolation and Purification, Front Bioeng Biotechnol 9 (2022). https://doi.org/10.3389/fbioe.2021.811971.

[130] Y.T. Tang, Y.Y. Huang, L. Zheng, S.H. Qin, X.P. Xu, T.X. An, Y. Xu, Y.S. Wu, X.M. Hu, B.H. Ping, Q. Wang, Comparison of isolation methods of exosomes and exosomal RNA from cell culture medium and serum, Int J Mol Med 40 (2017) 834–844. https://doi.org/10.3892/ijmm.2017.3080.

[131] L.L. Yu, J. Zhu, J.X. Liu, F. Jiang, W.K. Ni, L.S. Qu, R.Z. Ni, C.H. Lu, M.B. Xiao, A Comparison of Traditional and Novel Methods for the Separation of Exosomes from Human Samples, Biomed Res Int 2018 (2018). https://doi.org/10.1155/2018/3634563.

[132] D. Perocheau, L. Touramanidou, S. Gurung, P. Gissen, J. Baruteau, Clinical applications for exosomes: Are we there yet?, Br J Pharmacol 178 (2021) 2375–2392. https://doi.org/10.1111/bph.15432.