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REVIEW

# The Application of Exosomes From Different Sources Loaded with Natural Small-Molecule Compounds in Disease

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**Abstract:** Exosomes, as naturally derived nanoscale vesicles, possess inherent biological functions that make them highly efficient drug delivery vehicles. Their cellular origin dictates their bioactivity, targeting specificity, and regulatory effects, which are critical for therapeutic applications. Natural small-molecule compounds, despite their therapeutic potential, are often limited by poor solubility, instability, toxicity, and rapid clearance. Exosomes can overcome these limitations by enhancing drug stability, improving bioavailability, and enabling targeted delivery to disease sites, while also contributing therapeutic effects through their intrinsic biological properties. This review summarizes advances in the application of exosomes from diverse cellular sources for delivering natural compounds in the treatment of various diseases, and discusses the dual functional mechanisms of exosomes as both carriers and therapeutic agents, highlighting the advantages of integrating exosomes with natural small-molecule compounds. The review aims to explore novel strategies for improving the utilization of natural small-molecule compounds and to inspire new perspectives for the development of targeted nanomedicines in the future.

**Keywords:** exosome, natural small-molecule compounds, drug delivery systems, cellular sources, disease treatment

## Introduction

Exosomes are nanosized vesicles with a diameter ranging from 40 to 160 nm, secreted by virtually all cell types.[1] During cellular growth and development, exosomes function as mediators of intercellular material exchange and information transfer.[2,3] The process begins with the internalization of the plasma membrane via endocytosis, forming early endosomes.[1,4] These early endosomes mature into late endosomes with the assistance of the Golgi apparatus.[1,4] In late endosomes, intraluminal vesicles (ILVs) are formed through inward budding of the endosomal membrane, resulting in the formation of multivesicular bodies (MVBs) (Figure 1).[5] The sorting of cargo into ILVs is tightly regulated by three major mechanisms: the endosomal sorting complexes required for transport (ESCRT) machinery (comprising ESCRT-0, -I, -II, and -III), lipid-based domains such as ceramide and cholesterol, and tetraspanin proteins (CD63, CD81, and CD9).[6,7] Once sorting is complete, MVBs either fuse with lysosomes for ILV degradation or with the plasma membrane to release ILVs as exosomes into the extracellular space.[3] The cargo sorted into exosomes primarily includes nucleic acids, proteins, lipids, cytokines, growth factors, and metabolites.[8–10] By carrying these bioactive molecules, exosomes play a key role in intercellular communication and modulation of intracellular signaling pathways, thereby influencing a wide range of physiological and pathological processes. Exosomes can be internalized by recipient cells through clathrin-dependent endocytosis, and clathrin-independent pathways such as caveolin-mediated uptake, macropinocytosis, phagocytosis, and lipid raft-mediated internalization (Figure 1).[11] Exosomes can also directly fuse with the plasma membrane.[1] Additionally, exosomes membrane proteins can activate signaling molecules on the surface of recipient cells,

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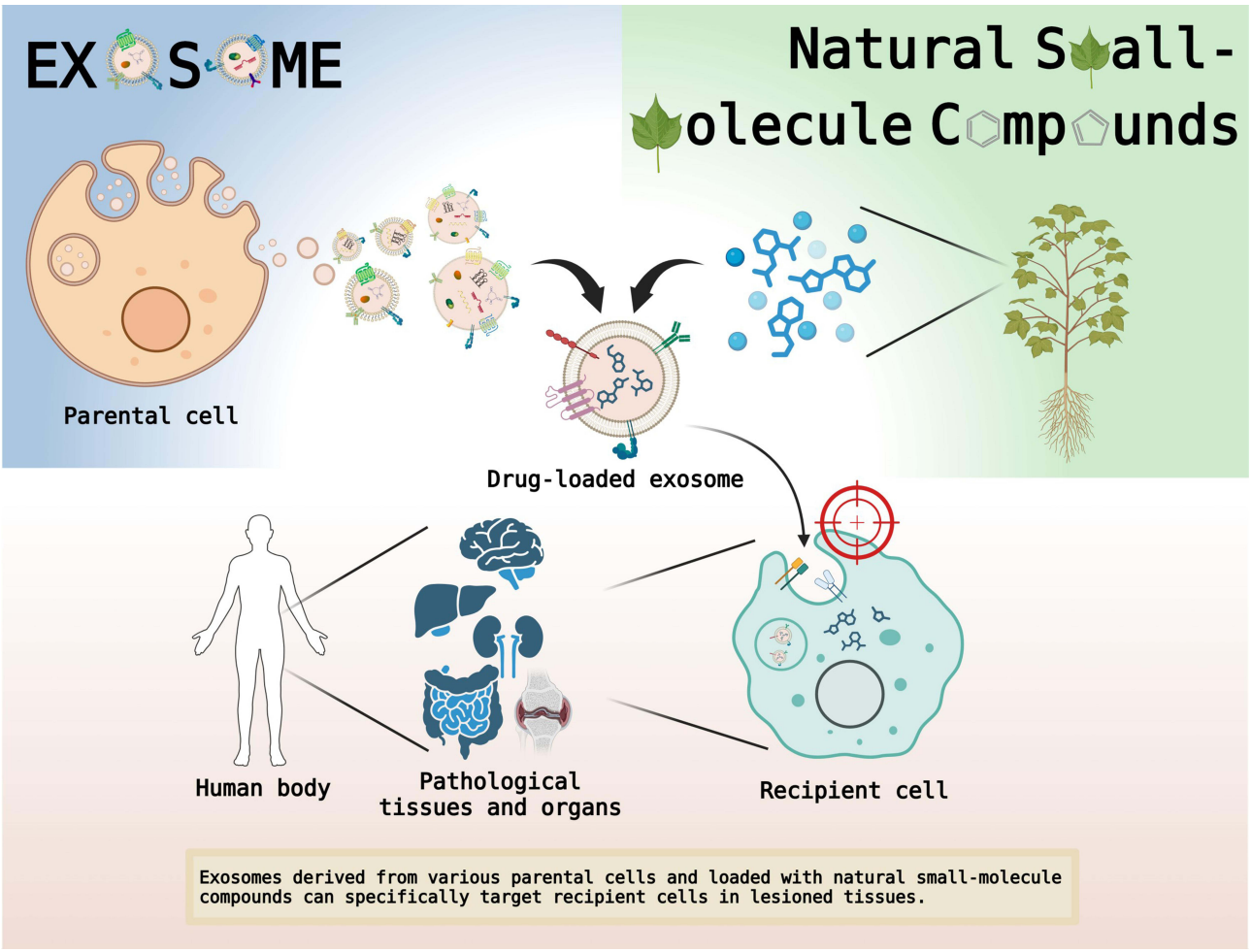
**Graphical Abstract**




facilitating intercellular communication.[12] The bilayer membrane structure and nanoscale size of exosomes also confer protection to their cargo, preventing immune system clearance and degradation, thus prolonging their circulation time and preserving their biological activity.[1] As a result, exosomes are increasingly being investigated as potential novel drug delivery vehicles in therapeutic development.

Traditional Chinese medicine, as an essential component of conventional medicine, has provided a wide range of bioactive compounds, such as alkaloids, polysaccharides, and terpenoids, which have been extensively utilized in disease treatment and the development of new drugs.[13–16] However, the limitations of natural small-molecule compounds, such as poor water solubility, rapid hydrolysis, high toxicity, and lack of target specificity, pose significant challenges for their clinical application.[17] To address these challenges, researchers have employed strategies such as drug structural modification and drug delivery systems to improve the properties and efficacy of natural small-molecule compounds.[18] Among these strategies, nanocarriers are one of the most powerful systems for encapsulating and delivering therapeutic agents.[19] Nanocarriers utilize various materials to alter the physicochemical properties of drugs. Functional groups on the carrier molecules can be modified, enabling conjugation with drug molecules, targeting ligands, or the formation of copolymers.[20] This ultimately enhances the chemical stability and solubility of the drug, reduces the required therapeutic dose, controls the drug release rate, delivers the drug to specific sites of action, and improves the bioavailability of the drug.[21]

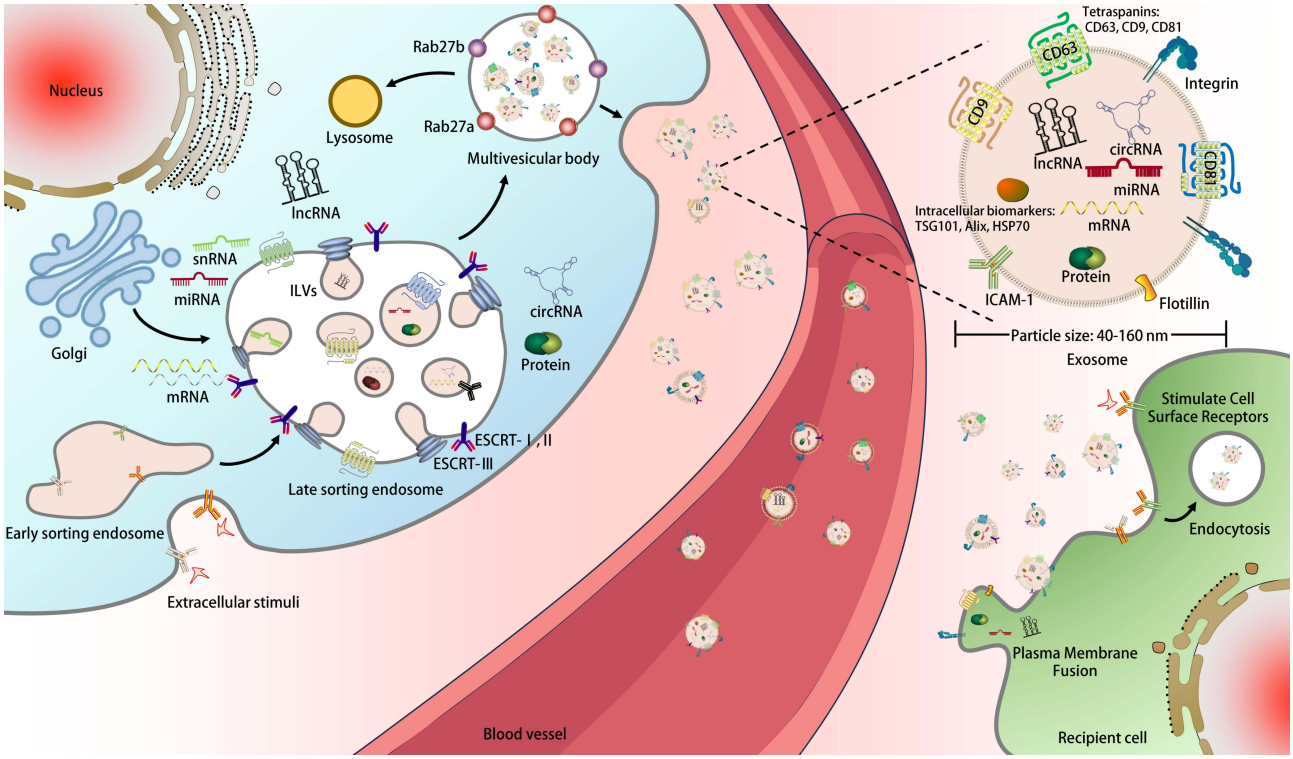



**Figure 1** Biogenesis and Intercellular Trafficking of Exosomes. Upon extracellular stimulation, the plasma membrane invaginates to form early sorting endosomes. These structures mature into late sorting endosomes through interactions with the Golgi apparatus, eventually evolving into MVBs. Exosomes are subsequently released extracellularly under the regulation of membrane trafficking proteins Rab27a/b. Following their secretion, exosomes are transported via systemic circulation to recipient cells. The regulatory effects of exosomes on recipient cells are mediated through three primary mechanisms: endocytosis, membrane fusion, or activation of surface signaling molecules by exosomal membrane proteins.

**Abbreviations**: ILVs, intraluminal vesicles; snRNA, small nuclear RNA; miRNA, micro-RNA; lncRNA, long non-coding RNA; ESCRT, endosomal sorting complexes required for transport; ICAM, intercellular adhesion molecule.

Compared to traditional nanocarriers, exosomes, as naturally occurring endogenous carriers, demonstrate superior biocompatibility, low immunogenicity, and high delivery efficiency.[22,23] Moreover, exosomes possess unique bioactivity and homing capabilities not found in conventional nanocarriers.[24,25] The bioactivity of exosomes varies depending on their cellular origin, inheriting the unique properties of the parent cells. For instance, exosomes derived from mesenchymal stem cells (MSC-Exos) possess regenerative capabilities, including self-renewal and multi-lineage differentiation potential, as well as the ability to modulate both innate and adaptive immunity. Moreover, MSC-Exos exhibit superior secretion capacity compared to exosomes from other cell types.[26] Exosomes derived from macrophages (MφExos) exhibit distinct bioactivities depending on the macrophage phenotype (M1 or M2), playing unique roles in the tumor immune microenvironment and inflammation.[27–29] The homing capability of exosomes is attributed to specific proteins or lipid molecules on their membrane surface, which confer natural targeting properties.[30] Exosomes derived from M2 macrophages (M2Exos) display stronger anti-inflammatory activity and inflammation-targeting capability compared to M0 and M1 exosomes, due to the specific interaction between lymphocyte Function-Associated Antigen (LFA)-1 and very late Antigen (VLA)-4 on their surface and intercellular adhesion molecule (ICAM)-1 and vascular Cell Adhesion Molecule (VCAM)-1 receptors on lipopolysaccharide (LPS)-activated macrophages.[31] This allows for tissue-specific targeting without the need to manipulate exosomes.[32] Due to the numerous advantages of exosomes as drug delivery carriers, they have now been widely applied for the targeted delivery of natural compounds derived from natural medicine in the treatment of diseases.[33,34] This review will provide an overview of the current research on the targeted delivery of natural compounds derived from natural medicine using exosomes, based on the bioactivity of different types of exosomes. It will highlight the advantages and significance of combining exosomes with natural compounds, aiming to provide insights for the development of future targeted nanoparticle-based drug delivery systems.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## Sources of Exosomes

The sources of natural exosomes are primarily categorized into animal-derived and plant-derived exosomes. Animal-derived exosomes are further subdivided into normal exosomes and tumor exosomes, depending on whether they originate from a normal or tumor microenvironment. Nearly all types of normal cells, including endothelial cells, mesenchymal stem cells (MSCs), and immune cells (T cells, B cells, macrophages), are capable of producing exosomes.[35–38] Exosomes display heterogeneity due to variations in their parental cell types. They differ in size, yield, the content of various biomolecules (miRNAs, proteins, metabolites), functionality, and drug loading capacity.[1] This heterogeneity is determined by the microenvironment and intrinsic biological characteristics of the parent cells.[1] The receptors expressed on the surface of exosomes vary, and this variation modulates the effects of exosomes on recipient cells. This functional heterogeneity enables exosomes to convey distinct biological information, promoting cell survival, inducing apoptosis, and modulating immune responses.[1] Functional heterogeneity is also evident in exosomes derived from tumor cells.[39] Tumor-derived exosomes can induce epithelial-mesenchymal transition, promote immune suppression, enhance angiogenesis and vascular permeability, establish pre-metastatic niches, and transfer drug resistance molecules.[40]

Exosomes are also widely distributed in bodily fluids, such as saliva, plasma, urine, breast milk, amniotic fluid, and bile.[41–44] Bodily fluid-derived exosomes have increasingly been recognized for their potential as valuable tools for early diagnosis.[45] Liquid biopsy for cancer predominantly uses exosomes from plasma and urine, due to their stability in these fluids and ability to carry RNA for intercellular communication.[45] The use of exosome-derived RNA combined with ctDNA detection methods results in a tenfold increase in the copy number of mutations when exosomal RNA and ctDNA are analyzed together. Compared to ctDNA detection alone, this approach significantly enhances the likelihood of detecting mutations in samples, which is particularly beneficial in the early stages of disease when ctDNA circulation levels are very low.[46] Urine has garnered widespread attention as a source of non-invasive biomarker discovery material due to its ease of collection, making it an ideal alternative to invasive tissue biopsies. Exosomes in urine are released from various renal epithelial cell types across the urinary space, carrying molecular biomarkers indicative of renal dysfunction and structural damage. Studies have identified potential tumor biomarkers by analyzing the lipid composition differences in urine-derived exosomes between healthy individuals and patients with renal cell carcinoma.[47] Milk-derived exosomes have attracted attention as drug carriers due to their excellent biocompatibility, cost-effectiveness, and scalable source. Researchers have explored their potential for both hydrophilic and lipophilic agents, including chemotherapy drugs.[48] For instance, leveraging the stability of milk-derived exosomes in acidic environments, exosomes isolated from mixed raw milk during the mid-lactation phase of dairy cows have been utilized to develop oral exosome-based formulations encapsulating paclitaxel (PTX). This approach has been shown to enhance therapeutic efficacy while mitigating systemic and tissue toxicity.[49] Additionally, milk-derived exosomes may participate in anti-cancer pathways, such as the inhibition of the nuclear factor kappa-B (NF-κB) signaling pathway, thereby exhibiting greater anti-tumor activity compared to other nanocarrier materials.[50]

## Features and Methods of Exosome-Based Drug Delivery

### Features of Exosome-Based Drug Delivery

Exosomes exhibit a range of biological activities, primarily through their role as transport carriers, loading various cargoes to reach target cells and triggering a series of signaling events. Leveraging this characteristic, exosomes have become widely studied as natural small-molecule compounds carriers. An important advantage of exosome-based drug loading lies in its capacity to traverse multiple membrane barriers.[51] Drug delivery systems typically encounter three levels of membrane barriers within the organism: the blood-brain barrier and mucosal barrier at the tissue level, the plasma membrane barrier at the cellular level, and the endosomal/lysosomal membrane barrier at the subcellular level.[52] These structures represent major obstacles to the accumulation of drugs at their target sites. The blood-brain barrier is one of the most critical biological barriers in the human body. It serves as a dynamic physiological interface between the brain and the circulatory system, regulating the local brain microenvironment essential for normal neuronal function.[53] Composed of tightly interconnected capillary endothelial cells surrounded by pericytes and astrocytic end-feet, this

structure protects the brain from toxins and pathogens.[53] However, it also prevents therapeutics from crossing the barrier to treat neurological disorders. As naturally derived vesicles secreted by bodily cells, exosomes have been demonstrated to cross the blood-brain barrier and are involved in the pathogenesis and progression of various neurological diseases.[53] Current research indicates that exosomes traverse the blood-brain barrier via three mechanisms: macropinocytosis, lipid raft-mediated or nonspecific exosome-endothelial interactions, and receptor-mediated transcytosis.[54] Additionally, exosomes possess the potential to actively migrate to pathological sites. Their membranes contain tetraspanins that facilitate fusion with the target cell membrane, enabling direct content release into the cytoplasm and thereby avoiding entrapment in endosomes/lysosomes.[11,55] These properties have led a growing number of researchers to engineer exosomes into drug carriers that target neurons, including specific neuronal subpopulations, thereby enabling precise delivery of therapeutic agents.

Utilizing exosomes as drug carriers bypasses many design and manufacturing challenges associated with conventional synthetic nanocarriers. However, exogenous exosomes are rapidly cleared by the mononuclear phagocyte system, predominantly in the liver and spleen.[56,57] To address this issue, researchers have drawn inspiration from the mechanisms by which tumor cells evade immune phagocytosis, focusing on myeloid-specific immune checkpoints such as CD47.[58] The CD47–SIRPα axis represents a critical molecular interaction that inhibits the activation of macrophages and other myeloid cells by delivering a "do not eat me" signal, thereby suppressing phagocytosis.[59,60] Employing exosomes derived from tumor cells with high CD47 expression, such as ovarian cancer cell-derived exosomes, or other unmodified exosome types, including those from human foreskin fibroblasts, human MSCs, Jurkat T cells, and platelets, can reduce nonspecific clearance by non-target cells and enhance targeted delivery efficiency.[58] Other similarly functioning anti-phagocytic molecules include CD31/PECAM-1, expressed on exosomes from endothelial cells, endothelial progenitor cells, and platelets; CD24, detected on exosomes isolated from normal human urine and amniotic fluid; and PD-L1, present on exosomes derived from tumor cells, immune cells, and MSCs.[58] Therefore, selecting exosomes with intrinsic anti-phagocytic properties based on cellular origin is crucial to prolong circulation time.

## Methods of Exosome-Based Drug Delivery

### Endogenous Drug Loading

Exosome-mediated delivery of active ingredients derived from natural small-molecule compounds predominantly involves small molecule compounds. The exosome loading techniques for these small molecule drugs can be categorized into endogenous and exogenous strategies (Figure 2).[61] The endogenous approach achieves drug loading through the pre-treatment of parent cells, wherein free drugs are internalized by cells and subsequently incorporated into exosomes via

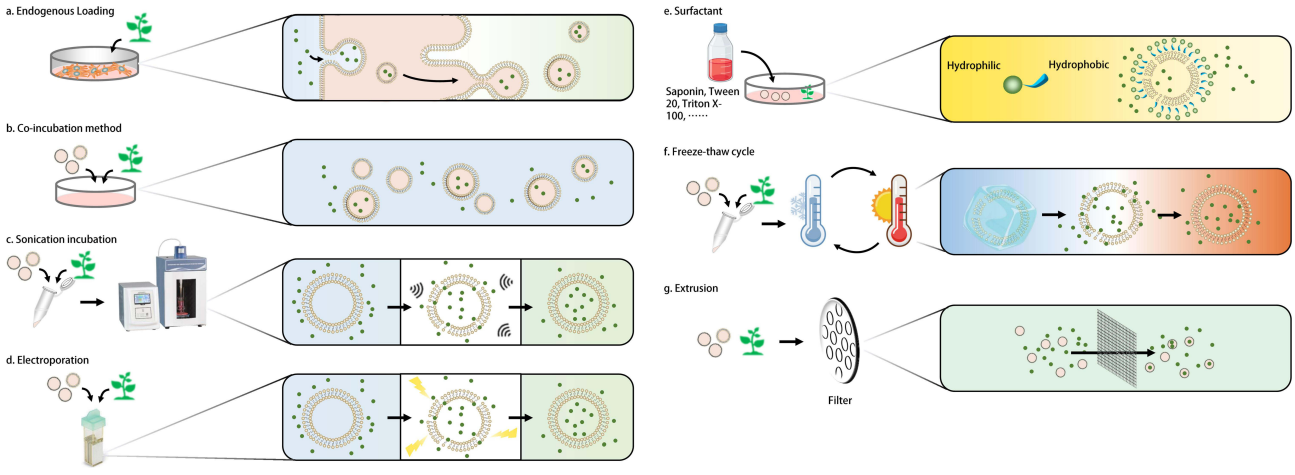


**Figure 2** Technical Strategies for Exosomal Drug Loading. Method (**a**), termed endogenous loading, involves pre-treating cells with the drug prior to exosome isolation. The drug is subsequently incorporated into exosomes through cellular uptake and secretion processes. The remaining six methods (**b**–**g**) are exogenous loading strategies, where isolated exosomes are subjected to physical or chemical interventions to facilitate drug encapsulation. Method (**b**), the co-incubation approach, exploits the lipid solubility of drugs to integrate them into the exosomal membrane. Methods (**c**) to (**g**) disrupt membrane integrity through external forces, enabling drug diffusion into exosomal vesicles. Created in BioRender. Xiaomeng, Z. (2025) https://BioRender.com/ eerrv97.


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the MVBs pathway (Figure 2).[61,62] For instance, PTX-treated human bone marrow MSCs (BMSCs) secrete exosome mimetics containing PTX, with the PTX/exosome protein ratio increasing in a dose-dependent manner (25–100 μg/mL), reaching 38.9, 76.1, and 74.22 ng/μg, respectively.[63]

## Exogenous Drug Loading

Exogenous drug loading, on the other hand, refers to the process of introducing drugs into pre-isolated exosomes using membrane permeabilization techniques. This method is currently more commonly used for loading small molecule drugs and typically includes co-incubation, sonication, electroporation, surfactant-assisted permeation, freeze-thaw cycles, and extrusion methods.

The co-incubation method relies on passive drug loading driven primarily by a concentration gradient, whereby free drug molecules diffuse across the exosomal lipid bilayer into the vesicle lumen or associate with the lipid membrane. This process is strongly influenced by the physicochemical properties of the drug, such as molecular weight, lipophilicity, hydrogen bonding capacity, and surface charge, as well as by the lipid composition and fluidity of the exosomal membrane. For hydrophobic drugs, co-incubation can significantly enhance apparent solubility, stability, and bioavailability by embedding the drug within the lipid bilayer or lumenal compartments. For example, co-incubating curcumin (Cur) with exosomes derived from the EL-4 murine lymphoma cell line for just 5 mins increased its solubility, stability, and plasma concentration compared with free Cur[64] Co-incubation is the most convenient method for loading drugs into exosomes, but due to the absence of additional intervention, the loading efficiency is influenced by the drug's molecular size, net charge, and hydrophobicity, leading to considerable variability in loading efficiency.[24]

The sonication method uses pulsed focused ultrasound technology to create transient pores and cracks on the exosome membrane, facilitating drug penetration (Figure 2). MφExos exhibited significantly higher drug loading efficiency for PTX via sonication compared to co-incubation and electroporation methods.[65,66] This is attributed to the high temperature generated during sonication, which reduces the rigidity of the exosome membrane and increases its permeability to the drug.

Electroporation involves mixing exosomes with drug molecules in a conductive buffer and applying electric pulses to create reversible pores on the membrane surface, allowing drug molecules to pass through (Figure 2).[67] Using electroporation under conditions of 400V, 150mF, and a 1ms discharge duration, triptolide (TP) was loaded into exosomes derived from human synovial fibroblasts (hRAF-Exos), achieving a drug loading efficiency of 81.22%[68] The voltage, pulse, and time parameters of electroporation vary based on the type of exosome and the loading cargo, with applied voltages ranging from 0.1 to 1000kV.[66,69]

The surfactant-assisted permeation method utilizes surfactants such as saponins or Tween 20 to induce the formation of small pores in the lipid membrane without disrupting the bilayer structure, enhancing membrane permeability and aiding drug penetration (Figure 2).[24] Using 0.2% saponin detergent, the Cur loading efficiency in milk-derived exosomes was three times higher compared to co-incubation.[70] However, residual surfactants may induce toxicity.

Freeze-thaw cycles exploit phase change stress to disrupt the membrane structure (Figure 2). Exosomes and the drug solution are rapidly frozen in liquid nitrogen or at −70°C, followed by thawing at room temperature, repeated at least three times[71] When the temperature drops below 0°C, water inside the exosomes freezes and expands, generating stress that creates gaps in the exosome membrane[61] Upon heating, the ice melts, and the drug molecules pass through these gaps into the exosomes. The membrane pores shrink as the expansion stress decreases, completing the encapsulation[61] Using plasma-derived exosomes (Plasma-Exos) to load methotrexate via freeze-thaw resulted in an encapsulation rate of 64% and 72 h release rate of 60%. Although the loading efficiency was lower than that of sonication (99%), freeze-thaw treatment preserved significantly higher cell viability of the exosomes.[72] This suggests that freeze-thaw may cause less damage to the exosome membrane structure compared to sonication. However, some argue that the repetitive cooling and heating process not only requires a longer preparation time but also has a significant negative impact on the biological activity of the exosomes, limiting its applicability.[61]

The extrusion method relies on mechanical force to disrupt membrane integrity (Figure 2). By mixing exosomes with drug molecules and passing the mixture repeatedly through polycarbonate membranes with pore sizes of 100–400 nm, the membrane integrity is compromised, allowing drug molecules to penetrate the exosome[73] This process results in

higher encapsulation efficiency and a more uniform particle size distribution[74] During extrusion, the exosome surface proteins' zeta potential and structure also change.[75]

The drug loading methods listed above have their advantages and disadvantages (Table 1). In current research on exosome drug loading, multiple drug loading strategies are typically tested at the initial stages, from which the most suitable method for the study object is selected. One study evaluated six loading methods, co-incubation, electroporation, extrusion, freeze-thaw, sonication, and surfactant-assisted permeation (Triton X-100 and Tween-20 treatments), using HEK293T cell-derived exosomes to load doxorubicin.[76] The results indicated that co-incubation and electroporation methods achieved higher encapsulation rates and biological activity[76] In research on exosomes derived from RAW264.7 macrophages loaded with PTX for the treatment of multidrug-resistant cancers, sonication achieved higher loading efficiency than electroporation and co-incubation methods[66] The exosome loading efficiency resulting from different strategies may vary depending on factors such as the drug molecule's size, surface net charge distribution, and hydrophobicity.

## Drug Loading Efficiency of Exosomes

The structure of mammalian cell-derived exosomes is generally consistent across different cell types; therefore, variations in drug loading efficiency depend more on the characteristics of the drug molecules and the loading techniques employed. A common feature of many natural small-molecule compounds is their hydrophobicity. One study compared the loading efficiency of the hydrophilic drug doxorubicin with that of the hydrophobic drug PTX into exosomes.[65] The overall loading concentration of hydrophilic drugs was lower than that of hydrophobic agents, which may be attributed to the relatively tight and highly structured lipid bilayer of exosomes. When doxorubicin was loaded under pH conditions close to its isoelectric point, thereby reducing its charge and increasing hydrophobicity, its loading efficiency improved significantly.[65] These findings suggest that higher lipophilicity of a drug correlates with improved exosomal loading efficiency. In a study using exosomes derived from 293T cells loaded with the natural compounds crocin and Cur via

**Table 1** Techniques for Loading Drugs Into Exosomes: Advantages and Disadvantages

| Method | Advantage | Disadvantage |
|---|---|---|
| Endogenous loading | Non-destructive to exosome membrane.<br>Avoids additional purification steps post-loading. | Extremely low loading efficiency.<br>Drug must be non-toxic and permeable to parent cells.[77]<br>High risk of drug modification/degradation by cellular machinery. |
| Co-incubation | Simplest procedure; maintains exosome structure.<br>Ideal for hydrophobic drugs that integrate into the lipid bilayer.[78] | Passive diffusion leads to low and variable efficiency.<br>Highly dependent on drug's lipophilicity and molecular weight.[78]<br>Leakage is common during storage or in vivo. |
| Sonication incubation | High loading efficiency for a wide range of molecules.[77,79]<br>Relatively fast process. | May cause damage to exosome membrane structure. |
| Electroporation | High efficiency for hydrophilic/highly charged molecules.[80,81] | Can induce drug aggregation due to local pH changes, and requires low-conductivity buffers.[82]<br>Risk of damaging membrane proteins, affecting targeting capability. |
| Surfactant | Can achieve moderate to high efficiency by solubilizing the membrane.[83] | Residual surfactants (eg, saponin, Triton X-100) are highly cytotoxic and difficult to remove completely.[79] |
| Freeze-thaw Cycles | Simple and low-cost; no specialized equipment needed. | Multiple cycles are required, which is time-consuming.<br>Formation of ice crystals can compromise membrane integrity and bioactivity.<br>Low to moderate efficiency; can lead to heterogeneous drug distribution.[83] |
| Extrusion | Produces homogeneous exosome populations with uniform size. | High shear forces can strip surface proteins and abolish natural targeting function.<br>The mechanical stress may cause complete loss of exosome identity and function.[83] |


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sonication and freeze-thaw cycles for cancer treatment,[84] spectrophotometric measurements of the optical density (OD) revealed a more pronounced decrease in OD values for Cur (initial OD = 1.85; OD after sonication = 0.9; OD after freeze-thaw = 0.4) than for crocin (initial OD = 2.93; OD after sonication = 1.86; OD after freeze-thaw = 1.09), indicating higher loading efficiency for Cur. According to PubChem data, Cur (XLogP3-AA = 3.2) exhibits significantly greater lipophilicity than crocin (XLogP3-AA = −2.5), supporting the role of hydrophobic interactions in promoting drug incorporation into the phospholipid bilayer of exosomes. In contrast, hydrophilic drugs may rely more on electrostatic interactions for attachment to the exosomal surface or permeate into the interior driven by concentration gradients. Such associations may lead to instability in loading efficiency, as drugs could be released during washing steps or changes in the solvent environment.

The choice of loading method also significantly influences drug loading efficiency. The various loading techniques each present distinct advantages and limitations. In current research on exosome-based drug loading, it is common practice to test multiple methods during the initial phase to identify the most suitable approach for the specific drug under investigation. Based on existing studies involving natural small-molecule compounds, sonication generally yields higher loading efficiency under comparable experimental conditions. For example, sonication was superior to co-incubation and electroporation for loading PTX.[66] Similarly, in studies comparing co-incubation and sonication for loading luteolin (Lut), berberine (Ber), and the hydrophilic drug doxorubicin, sonication demonstrated higher efficiency.[85,86] Furthermore, loading conditions significantly affect efficiency. Under identical sonication parameters, performing the procedure at room temperature resulted in higher PTX loading efficiency than on ice, likely due to enhanced molecular motion.[65] Similarly, when using co-incubation, direct mixing of an exosome suspension with a PTX solution in ethanol yielded better loading than first forming a PTX film by rotary evaporation before mixing.[65]

Based on the above, the following factors can be inferred to influence exosomal drug loading efficiency: (1) Drugs should possess a degree of hydrophobicity to facilitate integration into the lipid bilayer. (2) External energy input is often required to reduce membrane rigidity and enhance permeability. (3) Low temperatures should be avoided as they suppress molecular motion. (4) Sufficient mixing and contact between the drug and exosomes are necessary.

# Exosome-Loaded Natural Drugs in Disease Applications

## Mesenchymal Stem Cells-Derived Exosomes

MSC-Exos have garnered significant attention in the fields of regenerative medicine, cancer therapy, and immunomodulation. This is largely owing to the properties of their parent MSCs, which possess self-renewal and differentiation capabilities.[87–90] Accumulating evidence has demonstrated that the biological and therapeutic effects of MSCs are predominantly mediated through paracrine mechanisms via the secretion of bioactive molecules, including growth factors, chemokines, cytokines, and extracellular vesicles such as exosomes.[91] Current research data reveal that their tissue origins for isolation include bone marrow (51%), umbilical cord/placental tissues (23%), adipose tissue (13%), embryonic stem cells or induced pluripotent stem cells (iPSCs) (8%), and other sources (5%).[92] The tissue-specific origin of MSC-Exos critically determines their biological and functional heterogeneity, which governs their specific therapeutic efficacy in natural small-molecule compounds delivery for disease treatment.

### Bone Marrow Mesenchymal Stem Cells-Derived Exosomes

#### Osteoarthritis

BMSCs are adult stem cells originating from bone marrow, capable of differentiating into various types of tissue cells, including osteoblasts, chondrocytes, adipocytes, and even neural cells.[93] BMSCs have the potential to promote the healing of bone, cartilage, muscles, ligaments, and tendons, and clinical studies have begun to investigate the use of BMSCs-derived exosomes (BMSC-Exos) in the treatment of orthopedic diseases.[94,95] Research teams have started to explore the use of BMSC-Exos loaded with the active ingredient icariin (ICA) from the herb Epimedium to treat osteoarthritis (Figure 3).[96] ICA itself can induce chondrogenic differentiation of stem cells through multiple target pathways such as NF-κB, Wnt/β-catenin, and mitogen-activated protein kinase (MAPK), promoting chondrocyte proliferation, reducing matrix metalloproteinase (MMP) expression, and enhancing extracellular matrix secretion, thereby repairing cartilage.[97–99] This study not only utilizes BMSC-Exos as a cellular delivery carrier for ICA, overcoming the

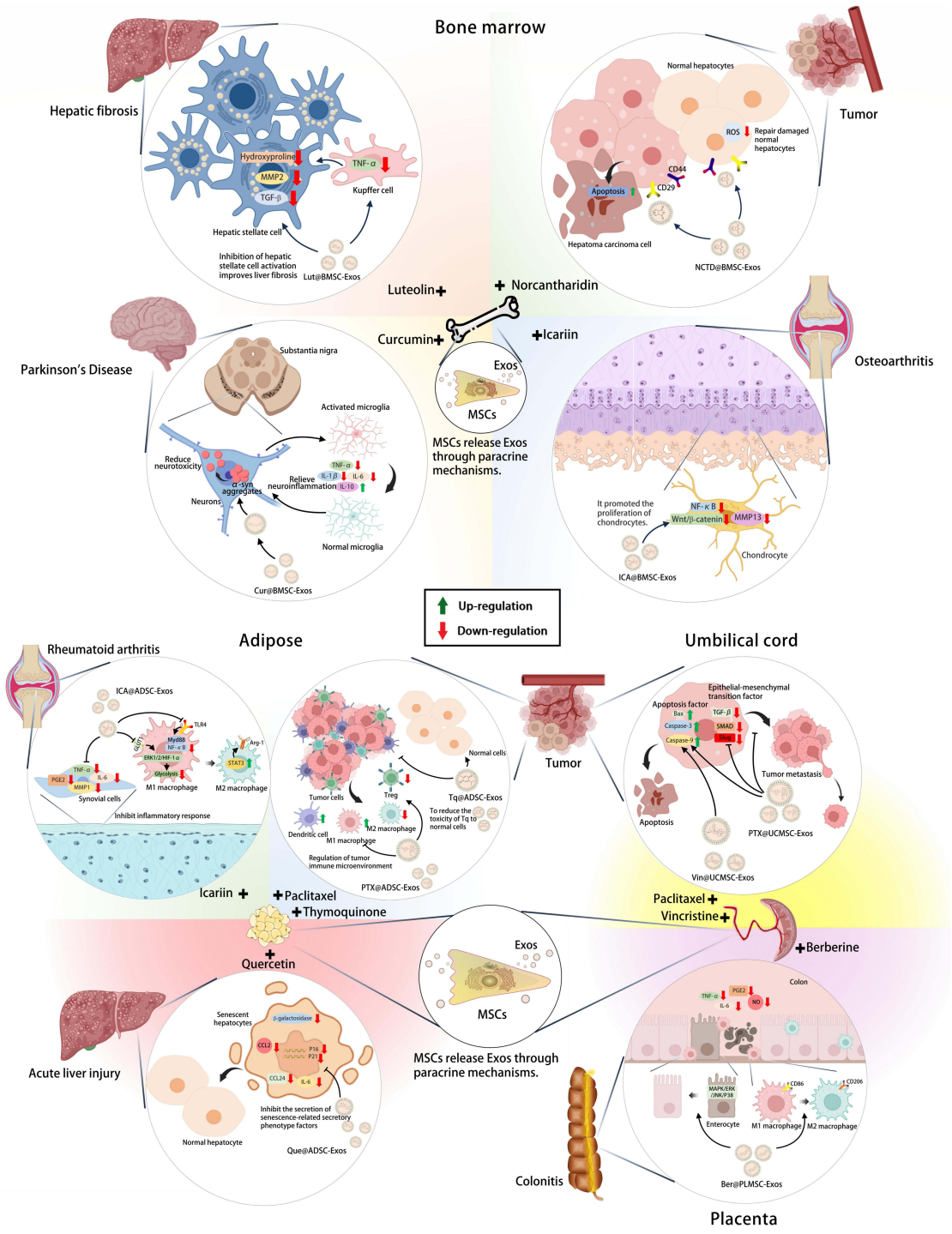


**Figure 3** Molecular Mechanisms Underlying Drug-Loaded MSC-Exos of Different Tissue for Targeted Disease Intervention. MSC-Exos of diverse tissue origins exhibit therapeutic potential through drug-loading strategies for targeted disease modulation. BMSC-Exos loaded with luteolin, norcantharidin, curcumin, or icariin, which mitigate hepatic fibrosis, tumors, Parkinson's disease, and osteoarthritis. These effects are mediated by mechanisms such as suppression of hepatic stellate cell activation, induction of hepatocellular carcinoma apoptosis, inhibition of neuroinflammation via microglial regulation, and promotion of chondrocyte proliferation. ADSC-Exos, loaded with icariin, paclitaxel, thymoquinone, or quercetin, target rheumatoid arthritis, tumors, and acute liver injury by inhibiting fibroblast and macrophage-driven inflammation, modulating the tumor immune microenvironment, and reducing senescence-associated protein secretion in hepatocytes. UCMSC-Exos, carrying paclitaxel or vincristine, induce tumor cell apoptosis and suppress epithelial-mesenchymal transition in cancer therapy. PLMSC-Exos loaded with berberine alleviate colitis by attenuating intestinal epithelial inflammation and promoting M1-to-M2 macrophage polarization. The green colored upward arrow indicates an increase; the red colored downward arrow indicates a decrease and the black colored plus sign represents the drugs carried by the exosomes. Created in BioRender. Xiaomeng, Z. (2025) https://BioRender.com/ bfzdn7g.
**Abbreviations**: Exos, exosomes; MSCs, mesenchymal stem cells; ROS, reactive oxygen species; MMP, matrix metalloproteinase; PGE2, Prostaglandin E2; NO, nitric oxide.


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limitations of ICA's low solubility and poor permeability, but also increases the uptake rate of SW1353 cells with a chondrocyte phenotype for the drug.[96] Additionally, ICA and BMSC-Exos work synergistically to promote chondrocyte proliferation and migration, as well as to inhibit MMP13 secretion in the OA rat models, achieving the goal of repairing damaged cartilage tissue.[96] The synergistic effect is attributed to the inherent biological functions of BMSC-Exos, which can deliver functional nucleic acids (such as lncRNA MEG-3 and various miRNAs) to inhibit chondrocyte apoptosis and inflammatory pathways (Wnt/β-catenin and PI3K/AKT/mTOR), while also ameliorating the inflammatory microenvironment within the joint cavity by modulating macrophage polarization (shifting from M1 to M2 phenotype), thereby creating favorable conditions for cartilage repair.[100,101]

### Liver Fibrosis

The pathogenesis of liver fibrosis is mainly believed to involve the pathological activation of hepatic stellate cell (HSC), which differentiate into myofibroblasts under disease conditions.[102] To address this issue, studies have utilized exosomes derived from BMSC-Exos as carriers to deliver the natural bioactive compound Lut (Lut@BMSC-Exos) for the treatment of liver fibrosis (Figure 3).[85] Although Lut, a plant-derived flavonoid, exhibits significant anti-inflammatory effects, its clinical application is limited by low water solubility and rapid metabolism. Compared to free Lut, the Lut@BMSC-Exos significantly ameliorates carbon tetrachloride ($CCl_4$)-induced liver fibrosis in rats, reducing pro-fibrotic markers including hydroxyproline, transforming growth factor-β (TGF-β), and MMP2 of liver tissue relative to the model group. Lut@BMSC-Exos also suppresses levels of the inflammatory cytokine TNF-α produced by Kupffer cells, which accelerates HSC activation. These findings indicate that BMSC-Exos not only improve the bioavailability of Lut but also enhance its anti-fibrotic and anti-inflammatory activities. This effect is also related to the intrinsic ability of BMSC-Exos to directly suppress hepatic stellate cell activation. BMSC-Exos can ameliorate $CCl_4$-induced liver fibrosis by inhibiting hepatic stellate cell activation through the Wnt/β-catenin pathway.[103] The miR-26a carried by BMSC-Exos promotes ferroptosis in HSC LX2 by directly binding to SLC7A11 mRNA and downregulating SLC7A11 protein expression.[104] miR-192-5p delivered by BMSC-Exos further suppresses HSC activation through downregulation of PPP2R3A.[105] Additionally, circCDK13 contained in BMSC-Exos acts as a sponge for miR-17-5p and inhibits the progression of liver fibrosis by reducing the expression of the miR-17-5p/KAT2B axis.[106]

### Tumor

In cancer research, the application of BMSC-Exos as drug carriers has gained significant attention. Based on the "bone marrow-pancreatic cancer axis" theory in pancreatic cancer research, BMSC-Exos originating from the bone marrow have been observed to migrate and frequently localize within the tumor microenvironment.[107,108] BMSC-Exos exhibit the ability to deeply penetrate into the internal regions of pancreatic tumors. Leveraging this homing effect, the first-line chemotherapeutic regimen for pancreatic cancer, nab-paclitaxel in combination with gemcitabine, has demonstrated enhanced antitumor efficacy when assisted by BMSC-Exos. One study employed an endogenous drug-loading strategy by treating BM-MSCs directly with 2000 ng/mL PTX, prompting the cells to secrete PTX-carrying vesicles via the paracrine system. These vesicles, largely composed of exosomes, showed significant anti-proliferative activity against pancreatic cancer CFPAC-1 cells.[109] This combination also demonstrates efficacy against breast cancer by effectively inhibiting the proliferation of MDA-MB-231 breast cancer cells.[63] However, this combination also exhibited cytotoxicity toward normal breast epithelial cells MCF-10A. This raises the concern that the internalization of BMSC-Exos by normal cells may compromise the targeting specificity of the drug delivery system and lead to side effects, particularly when these cells take up PTX-loaded BMSC-Exos and are subsequently exposed to the toxicity of PTX. Nonetheless, this phenomenon is not solely detrimental.

A study on hepatocellular carcinoma demonstrated that exosome-based delivery systems exert divergent effects in tumor cells versus normal cells. Using electroporation, norcantharidin (NCTD) was loaded into BMSC-Exos (NCTD@BMSC-Exos) (Figure 3).[110] Interactions between BMSC-Exos and liver-specific proteins CD29 and CD44 facilitate targeted homing of the exosomes to hepatic tissue.[111] As a result, NCTD@BMSC-Exos exhibited significantly stronger antitumor effects than free NCTD, effectively suppressing HepG2 proliferation. Importantly, that study evaluated the impact of NCTD@BMSC-Exos on normal hepatocytes. NCTD@BMSC-Exos caused less impairment to

L02 cell proliferation and morphology, and more effectively reduced reactive oxygen species (ROS), a marker of oxidative damage, compared to free NCTD. Furthermore, BMSC-Exos alone were found to significantly enhance the survival of L02 cells in a model of $H_2O_2$-induced liver injury, indicating that the inherent tissue-repair capacity of BMSC-Exos can effectively mitigate the toxicity of loaded drugs toward normal tissues.

### Parkinson's Disease

Due to their inherent capability to traverse multiple biological barriers, BMSC-Exos have been extensively investigated for Parkinson's disease (PD) treatment, a progressive neurodegenerative disorder. BMSC-Exos carry abundant miRNAs, with miR-188-3p,[112] miR-106b,[113] and miR-133b[111] demonstrating significant therapeutic potential in facilitating substantia nigra neuron regeneration, mitigating neuronal apoptosis, and promoting axonal sprouting in PD models, respectively. However, BMSC-Exos exhibit limited efficacy in eliminating α-synuclein aggregates, the neurotoxic species responsible for dopaminergic neuron degeneration in the midbrain substant nigra. These persistent aggregates induce microglial activation and subsequent pro-inflammatory cytokine secretion, ultimately leading to neuronal dysfunction and death.[114] Cur, a neuroprotective small-molecule drug proven to inhibit α-synuclein aggregation, was loaded into BMSC-Exos to construct a nano-delivery system (Figure 3). When administered intranasally in a mouse model of PD, this system achieved excellent enrichment within the brain. BMSC-Exos can selectively target brain regions affected by neurodegeneration or neurodevelopmental disorders. Together with Cur, they act synergistically to clear α-synuclein and reduce neurotoxicity, promote axonal growth and neuronal functional recovery, alleviate neuroinflammation, and ultimately exert therapeutic effects against PD.[114]

## Adipose-Derived Mesenchymal Stem Cells-Derived Exosomes

### Rheumatoid Arthritis

Adipose-derived mesenchymal stem cells (ADSCs), a type of MSC isolated from adipose tissue, possess self-renewal capacity, multi-lineage differentiation potential, and potent immunomodulatory properties, making them a promising cell-based therapy for rheumatoid arthritis (RA).[115] ADSC-derived exosomes (ADSC-Exos), which retain these immunomodulatory capabilities, have also been applied in the treatment of RA while avoiding the potential tumorigenic risks associated with whole-cell administration.[115] Previous studies have explored the use of ADSC-Exos loaded with Cur and ICA for treating RA-associated synovial fibroblast proliferation and synovial inflammation.[33] The high susceptibility of Cur to hydrolysis results in a significantly faster degradation rate in PBS and plasma for free Cur compared to its ADSC-Exos-loaded form (Cur@ADSC-Exos).[116] ADSC-Exos offer protective effects for Cur, enhancing its stability in aqueous solutions. However, this does not alter the release rate of Cur@ADSC-Exos in acidic microenvironments. In terms of bioactivity, results demonstrate that Cur@ADSC-Exos inhibit the proliferation and promote apoptosis of synovial cells (HIG-82) more effectively than free Cur. This is mainly due to Cur@ADSC-Exos ability to downregulate the expression of anti-apoptotic proteins IAP1 and IAP2, a downregulation that is not directly mediated by ADSC-Exos.[33] Instead, it is the protective role of exosomes that enhances the bioactivity of Cur, thus improving its bioavailability. Regarding RA synovial inflammatory marker expression, both ADSC-Exos and free Cur exhibited downregulation of TNF-α, IL-6, Prostaglandin E2 (PGE2), and MMP1 genes in HIG-82 cells, with Cur@ADSC-Exos showing a more pronounced effect. These findings suggest a synergistic inhibitory effect of ADSC-Exos combined with Cur on RA synovial inflammation. Additionally, the combination of ADSC-Exos and natural small-molecule compounds modulates RA synovial inflammation through immune cell regulation. A multifunctional drug delivery system composed of ADSC-Exos and ICA (ICA@ADSC-Exos) targets active macrophages in collagen-induced arthritis rat synovial tissue, regulating the polarization of macrophages from M1 to M2 and inhibiting glycolysis to suppress arthritis (Figure 3). ADSC-Exos can be internalized by macrophages during co-culture, influencing macrophage polarization phenotypes.[117] Research suggests that exosomes secreted by adipocytes from obese mice promote inflammatory polarization of macrophages by increasing miR-155, miR-34a, TNF-α, and IL-6.[118–120] Conversely, ADSC-Exos derived from lean mice carry active STAT3, which promotes M2 polarization in macrophages by increasing arginase-1 expression, thus suppressing inflammation in macrophages.[121] Both ADSC-Exos and ICA inhibit gene transcription of the TLR4/Myd88/NF-κB classic inflammatory signaling pathway in macrophages.[122] The ICA@ADSC-Exos system enhances this inhibitory effect, further reducing the

inflammatory response. Additionally, ICA@ADSC-Exos reduces glycolysis in M1 macrophages by inhibiting the ERK1/2/HIF-1α/GLUT1 pathway, facilitating the repolarization of M1 macrophages into the M2 phenotype.[123] As a carrier for ICA, ADSC-Exos overcome the compound's poor solubility and low bioavailability, thereby enhancing ICA distribution across various tissues.

### Tumor

ADSC-Exos have emerged as a promising drug delivery vehicle due to their innate targeting capability specifically toward tumor. Thymoquinone (Tq), a natural bioactive compound derived from Nigella sativa, exhibits antitumor activity by influencing multiple biological processes such as tumor cell migration, invasion, and epigenetic modifications.[124] However, its pharmacokinetic profile is suboptimal, characterized by slow absorption and rapid elimination.[125] These limitations stem from the inherent hydrophobicity and poor membrane permeability of Tq, which lead to low solubility, limited bioavailability, and fast biotransformation. To overcome these challenges, Tq has been encapsulated into ADSC-Exos (Tq@ADSC-Exos), achieving a loading efficiency of 57%.[126] This exosomal formulation not only enhances the delivery efficiency of Tq but also reduces its cytotoxicity toward normal cells. Furthermore, Tq@ADSC-Exos exhibit sustained release properties against MCF7 breast cancer cells over 24 h, thereby prolonging the drug's therapeutic effect (Figure 3).[126]

The targeting capability of ADSC-Exos originates from their innate affinity for the breast cancer microenvironment, which is shaped by active crosstalk between adipocytes and tumor cells. In obesity-associated breast cancer, adipocytes secrete factors such as leptin, adiponectin, and IL-6, which may be accompanied by exosomes, to promote tumor progression.[127,128] Breast cancer cells, in turn, exploit these adipocyte-derived lipids and signaling molecules to support their growth through metabolic reprogramming via fatty acid oxidation, thereby enhancing invasiveness.[129] Importantly, ADSC-Exos utilize the same intercellular communication pathways as adipocyte-derived factors, which allows them to be efficiently taken up by breast cancer cells that are dependent on these signals.[130] It incarnates the potential of ADSC-Exos as a targeted delivery system for anticancer agents.

Engineering exosomes has shown great potential in improving drug loading efficiency and targeting specificity for natural compounds. In one study, ADSC-Exos were fused with folate-modified liposomes to create a hybrid exosome system (ELP) for the delivery of PTX.[131] This engineered system demonstrated superior drug loading capacity and stability compared to naive exosomes, and it was more efficiently internalized by various tumor cell lines (CT26, B16, A2780), resulting in significantly enhanced apoptosis. In a CT26 colon cancer mouse model, ELP not only augmented antitumor immune responses, such as promoting M1 macrophage polarization, dendritic cells (DCs) activation, CD4+ and CD8+ T cell infiltration, and suppressing regulatory T cells (Tregs), but also sensitized the tumor microenvironment to chemotherapeutic agents, thereby improving efficacy and reducing immune escape.[131] ADSC-Exos can serve not only as efficient nanocarriers for breast cancer therapy but also as immune-modulatory agents capable of remodeling the tumor microenvironment, offering a novel strategy to overcome chemoresistance and improve immunotherapy in "cold" tumors.[132]

### Acute Liver Injury

ADSC-Exos have been demonstrated to exert beneficial therapeutic effects on liver diseases.[133,134] Quercetin (Que), a natural flavonoid compound, exhibits both antioxidant and anti-senescence activities and can downregulate multiple inflammatory factors associated with the senescence-associated secretory phenotype (SASP).[135,136] In one study, vitamin A-modified ADSC-Exos were utilized to deliver Que (Que@ADSC-Exos) for the treatment of a mouse model of acute liver injury induced by $CCl_4$, which involves strong oxidative stress (Figure 3).[137] The results showed that Que@ADSC-Exos significantly improved liver function markers (reducing alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (γ-GT)) and increased albumin levels. Vitamin A, serving as an engineering modification on ADSC-Exos, confers enhanced hepatocyte-targeting ability due to its innate liver tropism. In terms of the mechanism, oxidative stress can induce premature cellular senescence in tissues. Que@ADSC-Exos effectively downregulated the expression of senescence markers (such as β-galactosidase), senescence-related genes (P16 and P21), and SASP factors (including IL-6, Ccl2, and Cxcl24) in liver tissue. ADSC-Exos

retain the regenerative capacity of MSCs, thereby contributing to the repair of rapid senescence-like responses in cells.[134,137] Thus, ADSC-Exos represent a more desirable drug delivery vehicle for the treatment of liver injury compared to exosomes from other sources.

### Umbilical Cord Mesenchymal Stem Cell-Derived Exosomes

Umbilical cord-MSCs (UCMSCs), compared to other types of MSCs, are easier to obtain through non-invasive methods (umbilical cord following newborn birth), exhibit high proliferative capacity, and have lower tumorigenicity.[138] Current research on UCMSC-derived exosomes (UCMSC-Exos) loaded with natural small-molecule compounds mainly focuses on cancer therapy. Studies have shown that UCMSC-Exos, ADSC-Exos, and BMSC-Exos can be rapidly internalized by glioblastoma cells (U87MG), but UCMSC-Exos and BMSC-Exos significantly induce cell cycle arrest in the subG1 phase, which is associated with apoptosis, while ADSC-Exos promote U87MG cells' progression into the proliferative S and G2/M phases.[139] These findings suggest that MSCs, which can migrate to tumor regions and differentiate into fibroblasts and perivascular cells, not only provide structural support for tumor growth but also promote tumor vascularization. Therefore, the potential tumorigenicity of MSCs should be considered when selecting MSC-Exos for therapeutic purposes. In this study, the researchers also loaded UCMSC-Exos with vincristine (Vin@UCMSC-Exos), which enhanced the apoptosis-inducing effect on U87MG cells (apoptosis rate of 40% with Vin@UCMSC-Exos) compared to free vincristine (inducing apoptosis at 20% with 20 ng vincristine) (Figure 3). When using exosomes to deliver the same natural small-molecule compounds, UCMSC-Exos demonstrated a stronger pro-apoptotic effect on various cancer cell lines compared to exosomes derived from normal cells. A study demonstrated that UCMSC-Exos from four different donors loaded with PTX exhibited stronger cytotoxicity against A549 lung cancer cells, SK-OV-3 cells, and MDA-hyb1 cells compared to human umbilical vein endothelial cells (HuVECs) loaded with PTX.[140] Exosomes derived from Wharton's jelly MSCs of UC tissue loaded with PTX (PTX@UCMSC-Exos) increased the expression of apoptosis-related proteins (Caspase-3, Caspase-9, Bax) in cervical cancer HeLa cells, while suppressing the expression of epithelial-mesenchymal transition-related factors (TGF-β, SMAD, Slug, Snail) (Figure 3).[141] The aforementioned studies indicate that UCMSC-Exos in the context of natural small-molecule compounds loading have been primarily studied for cancer therapy, with the loaded compounds being well-established anti-cancer drugs. Due to the inherent bioactivity of UCMSC-Exos, they can arrest the growth cycle of various tumor cells, thereby inhibiting tumor progression.

### Placental Mesenchymal Stem Cell-Derived Exosomes

Mesenchymal stem cell-derived exosomes isolated from placental chorionic trophoblasts (PLMSC-Exos) demonstrate significant anti-inflammatory properties through TLR4-mediated modulation of NF-κB/MAPK and PI3K signaling pathways,[142] exhibiting therapeutic potential in mitigating acute lung and liver injuries.[143,144] Recent investigations have explored their application as nanocarriers for Ber, an is quinoline alkaloid derived from *Coptis chinensis* Franch., in treating murine models of ulcerative colitis.[145] The Ber@PLMSC-Exos formulation markedly enhanced Ber bioavailability while effectively suppressing pro-inflammatory mediators including IL-6, TNF-α, PGE2, and nitric oxide in colonic tissues. This combinatorial therapy established a low-inflammatory microenvironment characterized by reduced M1 macrophage polarization and enhanced M2 differentiation. Mechanistic studies revealed that Ber@PLMSC-Exos administration significantly inhibited phosphorylation of key MAPK pathway components (ERK, JNK, and p38), thereby restoring intestinal barrier integrity and maintaining structural homeostasis of colonic epithelial cells (Figure 3). Compared with UCMSC-Exos, fewer studies have investigated PLMSC-Exos for natural compound delivery. This disparity may stem from technical challenges in tissue processing and MSCs isolation, as placental tissue exhibits greater anatomical complexity compared to umbilical cord specimens, despite both being classified as perinatal medical waste.

## Macrophages-Derived Exosomes

### Tumor

MφExos represent a natural delivery vehicle for anticancer therapeutics.[65] MφExos achieve targeted drug delivery through interaction between surface protein LFA1 on the exosomes and ICAM-1 expressed on tumor cells. The acidic

nature of the tumor microenvironment enhances the fusion efficiency of exosomes with target cells, promoting preferential accumulation of drug-loaded exosomes in tumor tissues.[146] In a study evaluating MφExos loaded with paclitaxel (PTX@MφExos) for the treatment of triple-negative breast cancer and lung cancer, PTX@MφExos administered via intravenous, intraperitoneal, or intratumoral injection effectively accumulated in MDA-MB-231 xenograft tumors in nude mice.[65] In contrast, liposome-formulated PTX was retained in tumor tissue only when administered intratumorally. The delivery advantages of PTX@MφExos include resistance to P-glycoprotein-mediated efflux (with a significantly increased drug resistance reversal index) and enhanced metabolic stability in the circulation.[66] Surface modification strategies can further improve targeting specificity. For instance, PTX@MφExos functionalized with aminoethylanisamide, a high-affinity ligand for the sigma receptor overexpressed in non-small cell lung cancer cells, significantly suppressed the growth of primary lung tumors and metastatic lesions (Figure 4).[147]

TP, a natural antitumor compound from *Tripterygium wilfordii*, faces limitations due to organotoxicity and poor solubility. TNF-related apoptosis-inducing ligand (TRAIL)-modified MφExos (TRAIL-TP@MφExos) overcome these challenges by targeting death receptor 5 (DR5), highly expressed in melanoma cells.[34] TRAIL binding induces DR5 trimerization, activating the caspase-8/Bid pathway, while TP synergistically triggers mitochondrial apoptosis (via Bax/Bak activation and cytochrome c release). This dual mechanism culminates in caspase cascade-mediated melanoma cell apoptosis and downregulation of NF-κB-dependent survival signals (Figure 4).[34]

The MφExos system can serve as a drug delivery vehicle to transport therapeutic agents across the BBB for glioma treatment. Cur demonstrates remarkable antitumor activity against gliomas while exhibiting a more favorable safety profile compared to other natural pharmaceutical active ingredients.[148,149] However, the therapeutic application of Cur is restricted by its poor BBB permeability and limited tumor targeting. To overcome these limitations, researchers

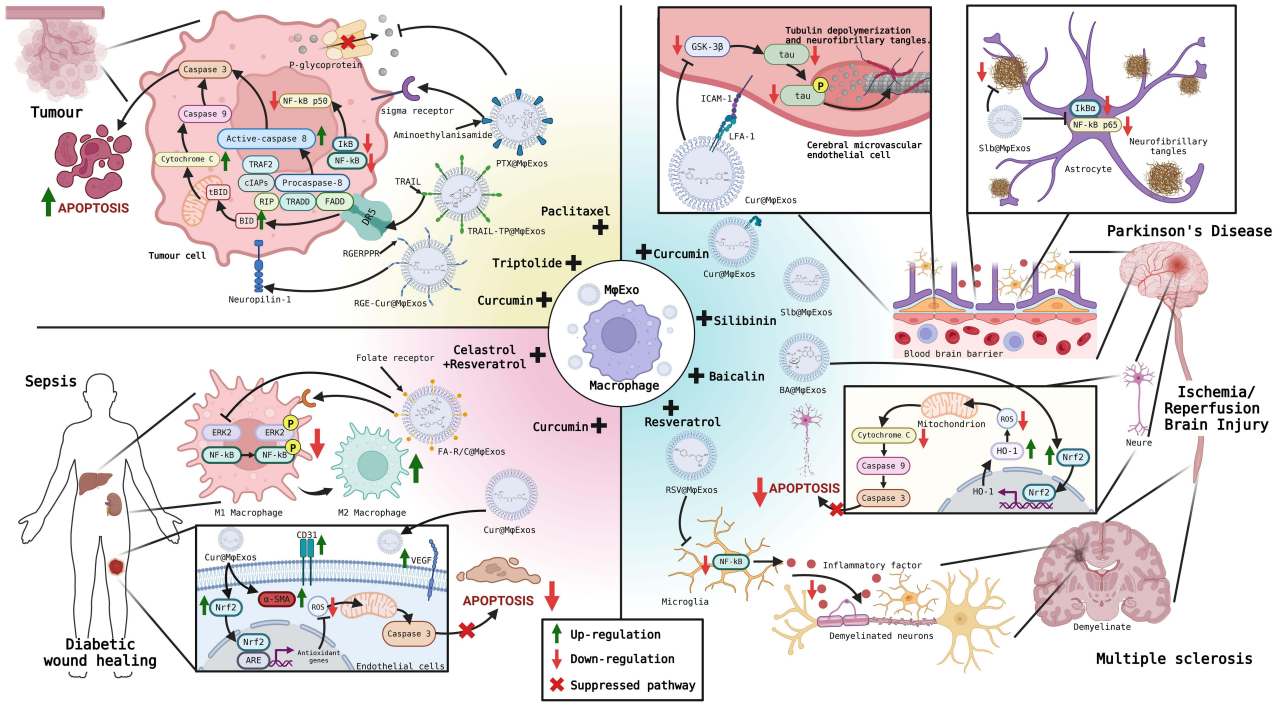


**Figure 4** Molecular Mechanisms of MφExos Loaded with Natural small-molecule compounds in Disease Treatment. MφExos loaded with paclitaxel, triptolide, or curcumin inhibit P-glycoprotein-mediated drug efflux, activate mitochondrial apoptosis pathways, and enhance tumor cell apoptosis. In neurological disorders such as Parkinson's disease, cerebral ischemia-reperfusion injury, and multiple sclerosis, MφExos carrying curcumin, silibinin, baicalin, or resveratrol reduce neurofibrillary tangle-associated astrocyte inflammation, attenuate neuronal apoptosis induced by mitochondrial oxidative stress, and suppress microglial inflammation-driven demyelination. For inflammatory conditions including sepsis and diabetic wound healing, MφExos co-loaded with celastrol and resveratrol or curcumin block macrophage polarization toward the M1 phenotype and diminish endothelial cell apoptosis caused by mitochondrial oxidative stress. The green colored upward arrow indicates an increase, the red colored downward arrow indicates a decrease, the red colored "X" symbol indicates inhibition or suppression of the corresponding pathway, and the black colored plus sign represents the drugs carried by the exosomes. Created in BioRender. Xiaomeng, Z. (2025) https://BioRender.com/ c39rlg4.
**Abbreviation**: MφExos, Exosomes derived from macrophages.

developed an engineered exosome complex (RGE-Cur@MφExos/SPION) by co-encapsulating Cur with biocompatible superparamagnetic iron oxide nanoparticles (SPIONs) for magnetic hyperthermia, while surface-modifying the exosomes with RGERPPR peptide (RGE) to target neuropilin-1 (NRP-1), a receptor overexpressed in glioma cells and associated vascular endothelium (Figure 4). Experimental data revealed that U251 human glioma cells with high NRP-1 expression exhibited significantly higher cellular uptake of RGE-Cur@MφExos/SPION compared to Bel-7404 human hepatoma cells with low NRP-1 expression, accompanied by substantial inhibition of U251 cell viability.[150] Furthermore, in vivo studies demonstrated that this engineered delivery system effectively improved survival rates and markedly suppressed tumor growth in glioma-bearing mice.[150]

Currently, MφExos used as delivery vehicles are predominantly derived from the RAW264.7 cell line. However, the phenotypic heterogeneity of macrophages endows their secreted exosomes with distinct bioactivities, thereby differentially influencing tumor progression.[151] For instance, exosomes from classically activated (M1) macrophages exhibit high expression and delivery of miR-29a-3p, which suppresses melanoma proliferation.[152] In contrast, M2 macrophage-derived exosomes can inhibit tumor immunogenicity via apolipoprotein E and activate the PI3K/Akt pathway to promote metastasis.[153] Therefore, selecting macrophage polarization states to source exosomes with desired functions represents a promising strategy.

## Alzheimer's Disease

MφExos exhibit broad applications in central nervous system (CNS) diseases, including Alzheimer's disease (AD), ischemic brain injury, cerebral ischemia-reperfusion injury, multiple sclerosis, and spinal cord injury (SCI). Drug delivery across the BBB remains a major therapeutic challenge, as only small molecules (<400 Da) with high lipophilicity typically achieve BBB penetration.[154] A critical mechanism enabling MφExos transmigration through the BBB involves the interaction between lymphocyte function-associated antigen-1 (LFA-1) expressed on exosomal membranes and ICAM-1 on endothelial cells.[155]

MφExos loaded with Cur (Cur@MφExos) significantly enhanced Cur solubility from 1.8 μg/mL to 18.5 μg/mL while reducing plasma degradation rates at 2 h from 60% to 20%.[156] Pharmacokinetic analyses revealed that free Cur reached a peak plasma concentration (Cmax) of 0.52 μg/mL at 5 min post-administration, followed by rapid clearance. In contrast, Cur@MφExos achieved a higher Cmax (0.91 μg/mL) at 2 h, demonstrating superior bioavailability, reduced metabolic rates, lower systemic clearance, and enhanced brain targeting, with an increase in cerebral accumulation compared to free Cur[156] Blocking experiments using ICAM-1 antibodies with hCMEC/D3 human cerebral microvascular endothelial cells reduced Cur@MφExos transmigration efficiency from 60% to 40%, confirming the pivotal role of LFA-1/ICAM-1 pairing in facilitating BBB penetration (Figure 4).[156] From a functional perspective, hyperphosphorylated Tau protein detaches from microtubules and aggregates into neurofibrillary tangles, contributing to cognitive deficits.[155] MφExos attenuates Tau hyperphosphorylation, suppresses aberrant microglial activation, and reduces microglial density in brain tissues, thereby mitigating neuroinflammation and improving cognitive impairment.[157] To enhance therapeutic efficacy, synergistic treatment combining MφExos with Cur was developed.[158] Cur@MφExos suppresses Tau hyperphosphorylation by activating AKT-mediated phosphorylation of glycogen synthase kinase-3β (GSK-3β) at Ser9, thereby inhibiting GSK-3β activity (Figure 4).[156]

In a parallel study, MφExos loaded with silibinin (Slb@MφExos) demonstrated comparable therapeutic effects.[159] Amyloid β-protein (Aβ)1–42 aggregates form amyloid plaques that disrupt neuronal signaling and induce neurodegeneration.[160] Slb@MφExos effectively inhibits Aβ1-42 aggregation in astrocytes and enhances silibinin bioavailability, BBB permeability, and hippocampal accumulation.[159,161] This formulation attenuates Aβ-induced astrocytic inflammation by suppressing glial fibrillary acidic protein (GFAP) overexpression, thereby deactivating astrocytes[159] Consequently, Slb@MφExos delays phosphorylation of P65 and IκBα in the NF-κB pathway, curbs inflammatory cascades, protects neurons, and ameliorates cognitive deficits in AD models (Figure 4).[159]

## Ischemia/Reperfusion Brain Injury

The treatment of ischemia/reperfusion brain injury is hindered by the BBB and inefficient drug delivery to ischemic regions.[162] Studies investigating MφExos for targeted delivery of baicalin (BA) (BA@MφExos) and Cur

(Cur@MφExos) revealed enhanced therapeutic outcomes. In transient/permanent middle cerebral artery occlusion (tMCAO/pMCAO) models, BA@MφExos achieved higher cerebral accumulation compared to free BA, respectively. This formulation significantly suppressed ROS generation by activating the Nrf2/HO-1 pathway, thereby attenuating neuronal damage (Figure 4).[163] In cerebral focal ischemia, accumulated ROS triggers mitochondrial-mediated apoptosis and disrupts BBB integrity via degradation of tight junction proteins.[164] Mechanistic studies further demonstrated that MφExos intrinsically scavenges ROS and blocks ROS-induced mitochondrial apoptotic pathways. In Cur@MφExos-treated groups, drug concentration in ischemic brain regions was higher than in non-ischemic areas. This targeted delivery inhibited cytochrome c release and caspase-9/caspase-3 activation, markedly reducing apoptosis-related protein expression.[165] Notably, Cur@MφExos exhibited a improvement in cerebral delivery efficiency over free Cur, synergizing with its antioxidant effects to significantly reduce infarct volume.[165]

### Multiple Sclerosis

Multiple sclerosis (MS), an inflammatory demyelinating disease of the CNS, progresses through a pro-inflammatory cytokine storm driven by aberrant microglial activation.[166] Studies demonstrate that MφExos not only selectively suppresses microglia-mediated inflammatory responses but also exhibits pronounced CNS tropism. Compared to exosomes derived from DCs or T lymphocytes (DC-Exos, T-Exo), MφExos showed the highest accumulation in the brain and spinal cord at any time point within 24 h post-administration, with an increase in uptake efficiency by BV2 microglial cells.[167] Leveraging these properties, researchers engineered MφExos loaded with the natural polyphenol resveratrol (RSV) (RSV@MφExos), which exerts potent anti-inflammatory effects by inhibiting the NF-κB pathway (Figure 4). In MS animal models, RSV@MφExos achieved sustained intracerebral drug release over 48 h, significantly reducing inflammatory cell infiltration and demyelination.[167,168]

### Spinal Cord Injury

Following SCI, aberrant activation of M1-polarized macrophages/microglia exacerbates inflammatory responses, making targeted modulation of their polarization states a critical therapeutic strategy.[169] Studies demonstrate that M2Exos not only retain the anti-inflammatory properties of their parental cells but also induce phenotypic switching from M1 to M2 macrophages/microglia. Furthermore, M2Exos serve as natural delivery vehicles for anti-inflammatory agents such as Ber (Ber@M2Exos).[31,86] Mechanistic investigations reveal that Ber@M2Exos simultaneously regulates macrophage polarization and apoptotic pathways. Specifically, it downregulates M1 markers iNOS while upregulating M2 markers CD206, thereby reversing pro-inflammatory phenotypes. Concurrently, Ber@M2Exos suppresses the expression of key apoptotic proteins, including caspase-9, caspase-8, cleaved caspase-3, and the BAX/Bcl-2 ratio.[86] This dual mechanism significantly reduces neuronal apoptosis, ameliorates pathological progression in SCI, and enhances functional motor recovery.[86]

### Sepsis

Sepsis, a systemic inflammatory response syndrome, is characterized by excessive secretion of pro-inflammatory cytokines and subsequent multi-organ failure, representing a critical therapeutic challenge.[170] Macrophages play a central role in the pathogenesis of sepsis.[171] Leveraging the overexpression of folate receptors (FA) on activated macrophages, researchers developed FA-modified MφExos (FA-MφExos) co-loaded with RSV and celastrol (Cel) (FA-R/C@MφExos).[172,173] This delivery system achieved dual-drug sustained release over 48 h: RSV inhibits NF-κB/ERK phosphorylation to mitigate cytokine storms, while Cel synergistically modulates immune homeostasis.[174] Experimental validation confirmed that FA-R/C@MφExos markedly reduces LPS-induced iNOS expression in macrophages, promoting M1-to-M2 phenotypic switching (Figure 4). Furthermore, FA-R/C@MφExos suppresses the expression of phosphorylated pNF-κB and pERK2, effectively blocking NF-κB and ERK2 activation to attenuate inflammatory cascades.[173] Concurrently, it inhibits apoptosis-related pathways, ultimately alleviating multi-organ damage.[173] This study underscores the necessity of combining exosomal carriers with natural small-molecule compounds to achieve functional complementarity in sepsis therapy.

### Diabetic Wound Healing

Delayed wound healing, a hallmark complication of diabetes, is closely associated with persistent inflammatory responses and impaired angiogenesis.[175,176] MφExos have demonstrated dual capabilities in suppressing inflammation while promoting angiogenesis and tissue regeneration.[177] Cur, a natural compound, exhibits anti-inflammatory, hypoglycemic, endothelial-repairing, and pro-angiogenic properties.[178] Studies have reported using Cur@MφExos to evaluate their synergistic therapeutic potential in diabetic wound healing using rat models.[179] Cur@MφExos not only significantly delayed Cur degradation in PBS and plasma but also ameliorated mitochondrial dysfunction-induced endothelial apoptosis by suppressing high glucose-induced ROS accumulation. Concurrently, it activated the Nrf2/ARE pathway to enhance anti-inflammatory and antioxidant defenses.[179] In diabetic rat models, Cur@MφExos promoted the proliferation and migration of human umbilical vein endothelial cells, upregulated the expression of vascular endothelial growth factor (VEGF), α-smooth muscle actin (α-SMA), and platelet-endothelial cell adhesion molecule CD31 in wound tissues, thereby driving neovascularization (Figure 4).[179] Histological analyses revealed well-organized collagen fiber alignment and complete epithelial regeneration in treated wounds, confirming that Cur@MφExos accelerates healing through multi-target regulatory mechanisms.[179]

In current drug delivery system research, the utilization of MφExos as carriers for natural small-molecule compounds primarily hinges on their targeting specificity, drug-loading optimization capabilities, and inherent bioactivity. Regarding targeting mechanisms: (1) MφExos achieve BBB penetration and tumor targeting via surface protein interactions (LFA-1 binding to endothelial ICAM-1); (2) their targeting precision is further enhanced through ligand modifications, such as RGD peptides for targeting the NRP-1 receptor in gliomas, AA for sigma receptor targeting, and TRAIL for DR5 receptor binding; (3) MφExos targeting also benefits from tumor acidic microenvironments that promote exosome-cell fusion and preferential tissue distribution post-systemic administration (tumor tissue uptake efficiency significantly exceeds that of healthy tissues). In terms of drug-loading advantages, MφExos markedly improve the solubility and stability of hydrophobic drugs (Cur, PTX) while prolonging drug action through sustained-release properties. Their loading efficiency surpasses that of conventional liposomes and enables circumvention of tumor resistance mechanisms (inhibition of P-glycoprotein-mediated drug efflux). From a bioactivity perspective, MφExos inherently exhibit anti-inflammatory and immunomodulatory functions, synergistically enhancing therapeutic efficacy when combined with cargo drugs.

## Microglia-Derived Exosomes

### Spinal Cord Injury

Apoptosis-induced secondary damage is a critical factor limiting the potential for neurological recovery following SCI.[180] While RSV exhibits dual neuroprotective effects via anti-inflammatory and pro-autophagy mechanisms, its clinical application is hindered by poor plasma stability and limited BBB penetrability.[181] Microglia, as the resident immune cells of the central nervous system, play a pivotal role in maintaining neural homeostasis through their secreted exosomes (MG-Exos). Studies demonstrate that microglial exosomes loaded with RSV (RSV@MG-Exos) not only reduce its 2 h plasma degradation rate from 60% to 30% but also significantly enhance its accumulation in target tissues such as the brain and spinal cord.[182] Mechanistically, RSV@MG-Exos activates the autophagy pathway by inducing PI3K phosphorylation, thereby suppressing spinal neuronal apoptosis and ultimately promoting motor functional recovery in injured rats.[182]

### Alzheimer's Disease

The neuroinflammatory progression of AD is closely linked to microglial phenotypic imbalance.[183] Research indicates that Ber and palmatine (Pal) from Huanglian Jiedu Decoction markedly reverse the Aβ25-35-induced imbalance in the sphingosine-1-phosphate (S1P)/ceramide ratio in microglia, restoring lipid metabolic homeostasis and attenuating inflammatory responses.[184] When Ber and Pal were loaded into MG-Exos (B/P@MG-Exos), drug uptake by microglia increased substantially both in vitro and in vivo. This strategy synergistically amplified the therapeutic effects of Ber and Pal, elevated neuronal density in the cerebral cortex and hippocampus, and enhanced cognitive performance and neuroprotection.[184]

When utilizing MG-Exos as drug delivery vehicles, researchers prioritize their targeting specificity toward parental microglia in CNS disorders, which enhances the plasma solubility and BBB permeability of poorly soluble therapeutics. This strategy concurrently exploits MG-Exos' intrinsic capacity to modulate microglial polarization, synergistically suppressing neuroinflammation in combination with cargo drugs.

## Dendritic Cell-Derived Exosomes

DCs, as pivotal antigen-presenting cells in the immune system, dynamically regulate the balance between T cells activation and immune tolerance through surface-expressed MHC I/II molecules and T cells co-stimulatory markers CD80/CD86.[185,186] TP, an immunosuppressive agent, targets DC maturation and trafficking, suppresses LPS-induced chemokine secretion, blocks DC-mediated recruitment of neutrophils and T cells, and reprograms T cells differentiation by inducing tolerogenic DC phenotypes.[187,188] DCs-derived exosomes (DC-Exos) serving as a delivery vehicle for TP (TP@DC-Exos) leverage their surface molecules (MHC I/II, CD80/CD86) to form specific interaction networks with TCR-mimicking receptors and CD28 family receptors on DCs, thereby constituting a targeted delivery system.[189,190] In vivo biodistribution studies revealed preferential accumulation of intravenously administered TP@DC-Exos in DC-rich secondary lymphoid organs (lymph nodes/spleen).[189] This high targeting specificity translated to superior therapeutic outcomes in colitis mice: TP@DC-Exos (tail vein injection) significantly reduced colonic expression of inflammatory cytokines (IL-2, IL-6, TNF-α) compared to free TP, altering T-cell distribution and differentiation.[189] Colon tissues exhibited decreased infiltration of $CD3^+$ and $CD4^+$ T cells alongside increased proportions of Foxp3+ Treg cells. Mechanistically, this occurs through TP@DC-Exos -mediated downregulation of DC surface co-stimulatory markers (CD80, CD86), ICAM-1, and MHC I, thereby inhibiting DC-dependent T cells activation. Additionally, apoptosis assays suggested that TP@DC-Exos may induce DC apoptosis, selectively depleting pro-inflammatory DC subsets. Comparable therapeutic benefits were observed in RA models, where TP@DC-Exos suppressed arthritis index progression and alleviated joint inflammation.[189] Critically, no significant toxicities were observed in major organs, as DC-Exos effectively concentrated TP in target cells while minimizing off-target distribution and systemic toxicity.[189] These findings robustly validate the dual value of DC-Exos as natural targeting carriers in amplifying drug efficacy while minimizing systemic toxicity.

## Fibroblasts Cell-Derived Exosomes

The proliferation of fibroblast-like synoviocytes (FLS) drives synovial tissue thickening and the release of inflammatory mediators, exacerbating inflammatory responses in RA. To address this, hRAF-Exos from MH7A cells were loaded with TP via electroporation, enabling targeted delivery to synovial FLS. Owing to the superior cellular uptake efficiency and favorable safety profile of hRAF-Exos, this strategy prolonged TP retention in particular regions while minimizing off-target organ toxicity.[68] Therapeutically, TP@hRAF-Exos upregulated the expression of apoptotic factors (caspase-9, caspase-3, Bax), inducing FLS apoptosis, which alleviated joint swelling in RA rats, reduced serum levels of TNF-α, PGE2, and rheumatoid factor, and diminished inflammatory cell infiltration in joints.[68]

## T Lymphocyte Cell-Derived Exosomes

T lymphocyte cell-derived exosomes (T-Exos) exhibit unique targeting advantages as Cur delivery vehicles for encephalitic disease therapy, owing to their capacity to traverse the BBB.[191] Given the central role of microglia in neuroinflammation, studies confirm that Cur@T-Exos is selectively internalized by immature myeloid cells, with brain-resident macrophages (microglia) serving as primary target cells.[64] In LPS-stimulated RAW264.7 macrophages and septic shock models, Cur@T-Exos outperformed free Cur in suppressing pro-inflammatory cytokines such as IL-6 and TNF-α. Notably, while intranasal administration bypasses the BBB, conventional formulations typically achieve less than 15% of peak drug concentrations in the brain.[192] T-Exos enhance nose-to-brain delivery efficiency, enabling Cur to reach peak brain concentrations within 1 h post-administration, with detectable levels persisting in the olfactory bulb for up to 12 h. Mechanistic analyses revealed that over 60% of Cur@T-Exos is specifically internalized by microglia, significantly reducing activated microglial populations and IL-1β secretion. This targeted therapeutic efficacy was validated in myelin

oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis models, underscoring the broad applicability of this strategy.[191]

## Tumor Cell-Derived Exosomes

Tumor-derived exosomes (Tumor-Exos) possess unique advantages in regulating macrophage polarization.[193] A hybrid delivery system, formed by fusing exosomes from human non-small cell lung cancer H1299 cells (H1299-Exos) with liposomes, was developed to co-deliver rhein (Rhe) and tanshinone IIA (TIIA), natural anti-inflammatory components derived from *Rheum palmatum* and *Salvia miltiorrhiza* (Lipo-R/T@H1299-Exos), for sepsis treatment.[194] These compounds exhibit potent anti-inflammatory and anti-apoptotic properties.[195,196] This system achieved significantly enhanced macrophage uptake under LPS stimulation via scavenger receptor SCARA3-mediated recognition compared to conventional liposomes.[197] Although Lipo-R/T@H1299-Exos and standard liposome-loaded Rhe/TIIA exhibited comparable efficacy in suppressing macrophage inflammatory cytokine release, the hybrid system demonstrated significantly enhanced anti-apoptotic effects, thereby mitigating secondary infection risks caused by excessive immune cell depletion during sepsis. Furthermore, H1299-Exos-mediated targeted delivery amplified the anti-apoptotic actions of Rhe and TIIA while mitigating Rhe's high-dose toxicity and improving TIIA's bioavailability.

## Engineered Cell-Derived Exosomes

HEK293 and HEK293T, widely utilized as immortalized engineered cell lines in laboratories, have been established as versatile tool cells for diverse experimental applications following prolonged laboratory cultivation.[198] Exosomes derived from HEK293 and HEK293T (HEK-Exos/HEKT-Exos) exhibit stable physicochemical properties, positioning them as natural nanocarriers for novel drug delivery systems capable of membrane modification and therapeutic payload encapsulation.[199]

### Acute Lung Injury

The inflammatory progression of acute lung injury (ALI) is closely associated with the high expression of the receptor for advanced glycation end products (RAGE) in pulmonary tissues.[200] A targeted delivery system (RBP/Cur@HEK-Exos) was engineered by modifying HEK-Exos with the anti-inflammatory peptide RAGE-binding peptide (RBP) and co-loading Cur.[201] RBP competitively inhibits the interaction between advanced glycation end products (AGEs) and RAGE;[202] however, its strong positive charge predisposes it to mucosal layer entrapment and rapid clearance. HEK-Exos circumvent this limitation through their surfactant-like properties, which facilitate penetration of the respiratory mucosal barrier, protect RBP from mucosal clearance, and enhance targeted accumulation at ALI lesions while blocking RAGE-mediated inflammatory signaling.[203] Furthermore, HEK-Exos offer inherent advantages for delivering hydrophobic drugs like Cur, as the compound can infiltrate and stabilize within the exosomal lipid bilayer.[64] Experimental validation confirmed that RBP/Cur@HEK-Exos significantly suppresses LPS-induced inflammatory responses in RAW264.7 macrophages, with anti-inflammatory activity markedly surpassing that of free RBP/Cur mixtures, underscoring the indispensable role of HEK-Exos as delivery vehicles in this system.[201]

### Tumor

In a study on cervical cancer treatment, HEKT-Exos were utilized for the delivery of crocin (Cro) and Cur, the major active components derived from saffron and turmeric, both of which exhibit antitumor effects both in vitro and in vivo.[84,204,205] The results indicated that loading HEKT-Exos with Cro and Cur (Cro@HEKT-Exos/Cur@HEKT-Exos) via freeze-thaw cycles demonstrated superior drug delivery efficiency compared to ultrasound treatment.[84] Compared to free drug molecules, the intracellular levels of Cro and Cur were significantly increased in human papillomavirus E7-expressing TC-1 cells following delivery by Cro@HEKT-Exos/Cur@HEKT-Exos. Furthermore, Cro@HEKT-Exos/Cur@HEKT-Exos reduced the cytotoxicity of the free drugs to non-cancerous HEK-293T cells and promoted a shift in the Th1/Th2 immune balance toward Th1 (increased IFN-γ secretion), enhancing the immune response of cytotoxic T cells (increased Granzyme B secretion), thereby achieving tumor growth inhibition in mice bearing HPV E7-expressing TC-1 tumors.[84]

For engineered cell-derived exosomes, most research has focused on their role as drug carriers, with less emphasis on exploring their biological functions.

## Blood-Derived Exosomes

### Plasma-Derived Exosomes

Plasma-Exos represent a heterogeneous mixture of exosomes secreted by diverse cell types in circulating blood, predominantly originating from platelets, with additional contributions from immune cells, endothelial cells, and other tissue-derived exosomes. Plasma-Exos composition may dynamically reflect disease progression or immune status, as evidenced by elevated exosome particle counts and mitochondrial DNA copy numbers in chronic heart failure patients.[206] Consequently, Plasma-Exos serve as valuable biomarkers for clinical diagnosis and prognosis.[207] Traditional cell culture methods yield limited exosome quantities with prolonged timelines, whereas ultracentrifugation of abundant blood samples enables rapid, large-scale exosome isolation. Recent studies leveraged the BBB-penetrating capability of Plasma-Exos to deliver Que for AD therapy (Que@Plasma-Exos), overcoming Que's poor solubility and low bioavailability while enhancing its neuroprotective, antioxidant, and anti-inflammatory functions.[208] Results demonstrated that Que@Plasma-Exos increased Que accumulation by in the hippocampal region and in the cerebellum, mitigating cognitive dysfunction induced by hyperphosphorylated Tau in AD mice. This intervention also elevated NeuN-positive neuronal density in hippocampal CA1, CA3, and dentate gyrus subregions while preserving neuronal structural integrity. Notably, neuroprotection was mediated not only by Que but also by unloaded Plasma-Exos, highlighting their synergistic therapeutic roles. Mechanistically, Que@Plasma-Exos suppressed insoluble neurofibrillary tangle formation by reducing CDK5-mediated phosphorylation of Tau. These findings confirm Plasma-Exos as both delivery vehicles and active therapeutic components.

### Serum-Derived Exosomes

The reticulocytes release $10^{14}$ red blood cell vesicles daily during their maturation process, making Serum-derived exosomes (Serum-Exos) the main source of exosomes, far exceeding white blood cells ($4–10\times10^4$/μL) and platelets ($1–3\times10^5$/μL).[209,210] Serum-Exos exhibit intrinsic brain targeting ability and can penetrate the BBB, showing unique advantages in the treatment of neurological diseases.[211] Research on the delivery of the water-soluble natural small-molecule compounds salvianolic acid B (SAB) and cryptotanshinone (CPT), with anti-tumor and anti-angiogenesis properties, using Serum-Exos in combination with liposomes for membrane fusion targeting glioma, demonstrated that Serum-Exos can promote the uptake of drugs by glioma cells, extend the in vivo release time of drugs from 2 h to 6 h, and enhance transcytosis across the BBB.[212] This utilizes the high expression of transferrin receptors (TfR) in brain endothelial cells, which specifically bind with transferrin on Serum-Exos, and enter cells via a clathrin-mediated endocytosis pathway, escaping lysosomal degradation and undergoing transcellular transport into the brain parenchyma.[213] This enhancement in BBB permeability by SAB and CPT upregulated SHP-2 expression in cells, subsequently suppressing STAT3 signaling pathway phosphorylation and inducing glioma cell apoptosis, while also demonstrating significant inhibition of VEGF-induced angiogenesis.[212]

### Platelet-Derived Exosomes

Platelet-derived exosomes (Platelet-Exos) are natural nanoparticles released by platelets that can selectively target various sites of inflammation.[214] Platelet-Exos, through receptor-ligand binding mechanisms, carry CD40L, glycoprotein Ibα, αIIb and VI, and P-selectin, which can bind to activated/inflamed vascular walls, regulating immune cell differentiation and reducing the occurrence of inflammatory cytokine storms.[214,215] RA, as a chronic inflammatory autoimmune disease, is characterized by the activation of inflammatory immune cell infiltration (T cells, DCs, and macrophages) into the synovium, which is the cause of joint and cartilage destruction.[216] Ber, due to its ability to inhibit the inflammatory response in macrophages and DCs, has been used in RA treatment research.[217] By leveraging the hydrophobic properties of the drug, Ber was loaded into Platelet-Exos (Ber@Platelet-Exos). Ber@Platelet-Exos not only demonstrated sustained release properties but also exhibited targeting capability to inflammation sites, resulting in a high accumulation of Ber@Platelet-Exos at the inflamed joints in RA mice, significantly reducing the degree of M1

polarization and inducing apoptosis in bone marrow DCs.[218] Notably, in this study, Platelet-Exos demonstrated targeted delivery effects on inflammation, but did not suppress the pro-inflammatory response in macrophages and DCs by themselves. It was the synergy with Ber that resulted in significant improvement in joint lesions in RA mice.[218]

## Conclusion

Exosomes, as endogenous natural nanocarriers, can enhance the bioavailability of natural small-molecule compounds with poor water solubility, high susceptibility to hydrolysis, rapid metabolic clearance, or high toxicity, while reducing adverse effects. Their vesicular structure not only protects drugs from blood metabolism and elimination but also demonstrates significant advantages in transcellular transport (Figure 5). For physiological barriers like the BBB, which are challenging for drug penetration, exosomes achieve trans-BBB delivery through mechanisms such as macropinocytosis, lipid raft or nonspecific exosome-endothelial interactions, and receptor-mediated transcytosis (Figure 5).[54] Furthermore, the ability of exosomes derived from various cellular sources to inherit the biological activities of their parent cells represents a distinct advantage over synthetic nanocarriers. Examples include the regenerative capacity of MSC-Exos, the immunomodulatory effects of MφExos, T-Exos, and dendritic DC-Exos, the BBB permeability and anti-inflammatory properties of MG-Exos in brain diseases, and the synovial inflammation-targeting ability of RAF-Exos in RA. Tumor-Exos exhibit anti-apoptotic effects. These characteristics allow exosomes to serve dual roles as both drug carriers and therapeutic components. Researchers can select exosomes from specific cellular sources based on the intended therapeutic application. For instance, BMSC-Exos are often chosen for bone repair in orthopedic diseases, MφExos for inflammatory conditions, and MG-Exos for neuroinflammatory disorders within the central nervous system. Likewise, immune cell-derived exosomes such as DC-Exos or T-Exos may be utilized when immunomodulation is desired. Fully exploiting the inherent bioactivity of exosomes is key to leveraging their advantages over traditional

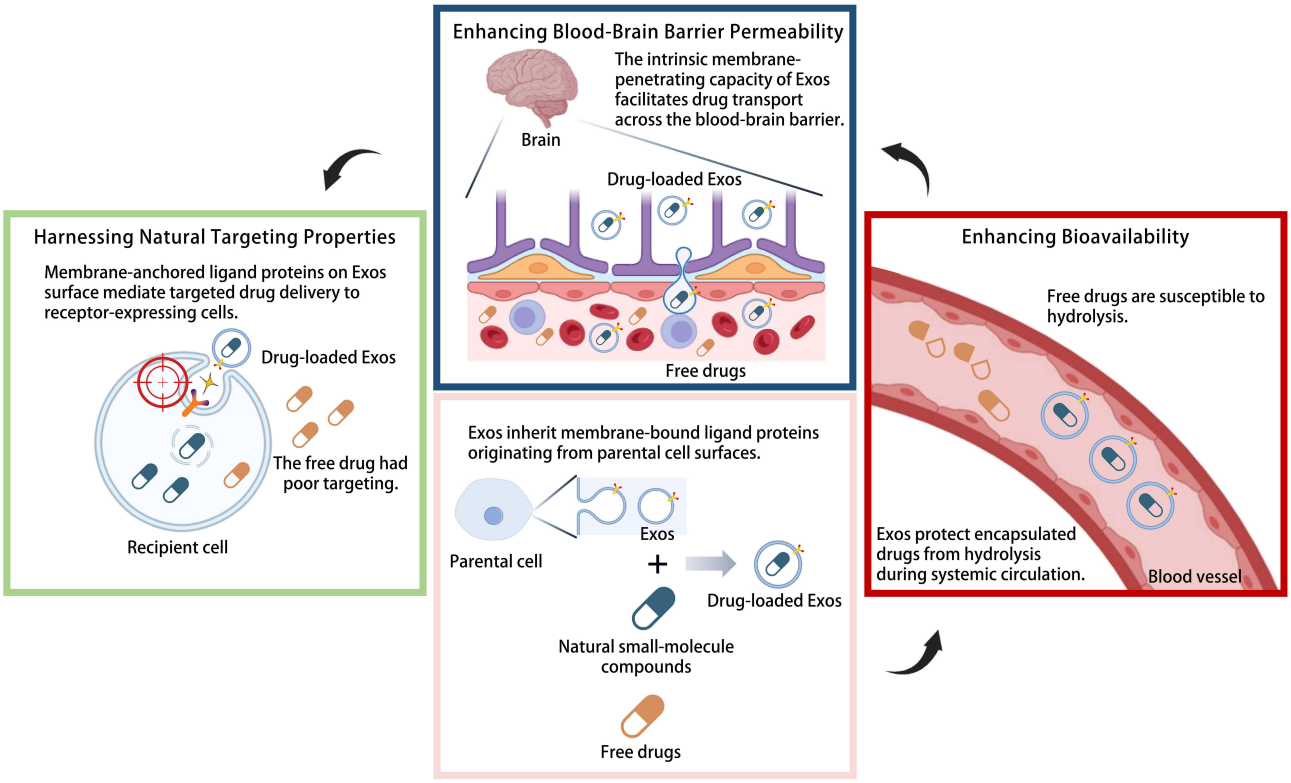


**Figure 5** Superiority of Exosomal Delivery Systems. Compared to free natural small-molecule compounds, exosomal encapsulation enhances drug bioavailability by improving systemic circulation stability, facilitating transmembrane transport, and enabling targeted delivery. Exosomes protect encapsulated drugs from degradation during blood circulation, leverage their intrinsic membrane-penetrating capacity to cross biological barriers such as the blood-brain barrier, and utilize surface-bound ligand proteins for receptor-specific cellular targeting. These combined properties ensure efficient drug delivery to intended sites while minimizing off-target effects. Created in BioRender. Xiaomeng, Z. (2025) https://BioRender.com/ gd7o0u1.
**Abbreviation**: Exos, Exosomes.


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synthetic nanocarriers, as it reduces both the complexity and cost associated with the design and production of delivery vehicles. However, current research comparing exosomes and conventional nanocarriers in the delivery of natural small-molecule compounds remains at an early stage, and further head-to-head comparisons under controlled conditions are needed to evaluate their relative performance in terms of delivery efficiency, targeting capability, and other critical metrics.

Exosome-based drug delivery still faces considerable limitations. The selection of appropriate drug loading methods remains a primary challenge. As this field is relatively novel, standardized protocols for loading and for calculating loading efficiency are lacking. Researchers often choose loading techniques (Probe Sonicator or Electroporator) and detection methods (high-performance liquid chromatography or spectrophotometry), based primarily on the equipment available in their laboratories. Furthermore, even when the same loading technique is employed, parameters such as carrier-to-drug concentration ratio, duration, intensity, and temperature vary significantly among studies, making it difficult to correlate these parameters with loading efficiency or to determine positive or negative effects through parallel comparison (Table S1). Thus, establishing a standardized methodology for loading natural small-molecule compounds into exosomes is crucial to advance research and promote clinical translation.

Another major limitation is that although exosomes have been observed to accumulate directionally in pathological areas such as tumors and inflamed tissues, their specific targeting mechanisms remain inadequately elucidated. This targeting ability appears to be highly dependent on both the exosome source and recipient cell type. For instance, as mentioned earlier, the targeting capability of MφExos may rely on interactions between surface ligands such as LFA-1 and VLA-4 and their cognate receptors ICAM-1 and VCAM-1 expressed on macrophages or endothelial cells.[65] In the absence of a clear mechanistic understanding, some researchers have turned to engineering approaches by modifying exosomal surfaces with ligands corresponding to known target receptors on specific cells. For example, MφExos modified with TRAIL ligands can bind specifically to DR5, which is highly expressed on melanoma cells.[34] Although such engineered exosomes are increasingly becoming mainstream in drug delivery research, they also introduce issues such as increased cost and manufacturing complexity, which may impede future clinical translation. A deeper understanding of the inherent targeting principles of different exosome sources in vivo would better inform the selection of disease-specific exosomes for therapeutic applications.

Finally, current research on exosome-based delivery of natural small-molecule compounds often lacks comprehensive assessment of potential issues such as immunogenicity of allogeneic exosomes, long-term biological accumulation, and adverse effects. Many studies primarily aim to address the inherent toxicity of free natural compounds and use side effects of the free drug as the control, without evaluating long-term safety after administration. Moreover, exosomes from different sources may exhibit varying drug metabolism profiles due to differences in the expression of anti-phagocytic molecules, potentially prolonging drug circulation time. However, this may also increase the risk of adverse reactions due to drug accumulation. Unfortunately, there is still insufficient experimental data to validate these concerns. Moreover, since storage conditions are critical for maintaining the integrity and function of exosomes[219] Current research has primarily focused on the short-term stability of drug-loaded exosomes in buffers such as PBS and solutions with different pH levels. Investigating long-term storage stability should represent a key focus for future research in the field of exosome therapeutics.

Future research on exosome-mediated delivery of natural small-molecule compounds should place greater emphasis on the physicochemical properties of the drug candidates and the characteristics of the target cells in the disease context to guide the selection of appropriate exosome types. A deeper investigation into the homing mechanisms of exosomes, along with harnessing their innate biological activities, may not only overcome the limitations of natural small-molecule compounds such as poor solubility rapid metabolism but also enhance therapeutic efficacy. Furthermore, establishing standardized drug-loading protocols and comprehensively evaluating the safety of these delivery systems are essential to advance exosome-based carriers from experimental tools to clinically viable therapeutic platforms.

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