

Extracellular Vesicles: A Review of Their Therapeutic Potentials, Sources, Biodistribution, and Administration Routes

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Abstract: Extracellular vesicles (EVs) participate in intercellular communication and play an essential role in physiological and pathological processes. In recent years, EVs have garnered significant attention as cell-free therapeutic alternatives, vectors for drug and gene delivery, biomarkers for disease diagnosis and prognosis, vaccine development, and nutraceuticals. The biodistribution of EVs critically influences their efficacy and toxicity. Therefore, this review aims to discuss the main factors influencing the biodistribution of unmodified EVs, highlighting their distribution patterns, advantages, limitations, and applications under different routes of administration. In addition, we provide a comprehensive discussion of the currently available sources of EVs and summarize the current status of the therapeutic potentials of EVs. By optimizing administration routes and selecting appropriate EV sources, we aim to offer valuable insights to enhance the delivery efficiency and therapeutic efficacy of EVs to target tissues.

Keywords: extracellular vesicles, administration routes, biodistribution, drug delivery

Introduction

Extracellular vesicles (EVs) are lipid bilayer vesicles secreted by seemingly all cells. Based on their biogenesis, EVs are primarily classified as exosomes and microvesicles. Moreover, specific cellular processes, such as cell migration and programmed cell death, generate migrasomes and apoptotic bodies, which are also classified as EVs.^{1,2} According to the MISEV 2023 guidelines, vesicles are recommended to be classified into extracellular vesicles, synthetic vesicles, and artificial cell-derived vesicles. The guidelines further advise against using nomenclature related to biogenesis, including terms like exosomes and microvesicles.² Therefore, in this review, all types of vesicles will be collectively referred to as EVs.

EVs were first reported in 1946 when researchers observed coagulation-promoting particles derived from platelets in plasma.³ Initially, EVs were thought to be a way for cells to dispose of their “garbage”.⁴ With the ongoing advancement in research, EVs have been found to carry various proteins, nucleic acids, and lipids derived from their parental cells, playing a crucial role in intercellular communication. Presently, EVs are believed to interact with receptor cells through three mechanisms: direct receptor interaction to trigger downstream signaling cascades, plasma membrane fusion to release their contents into the cytoplasm, and internalization (Figure 1).^{5,6} Among these, EV internalization is the most extensively reported mechanism, occurring via multiple pathways including clathrin-mediated endocytosis, lipid raft-mediated endocytosis, caveolin-mediated endocytosis, phagocytosis, and micropinocytosis. However, there remains no

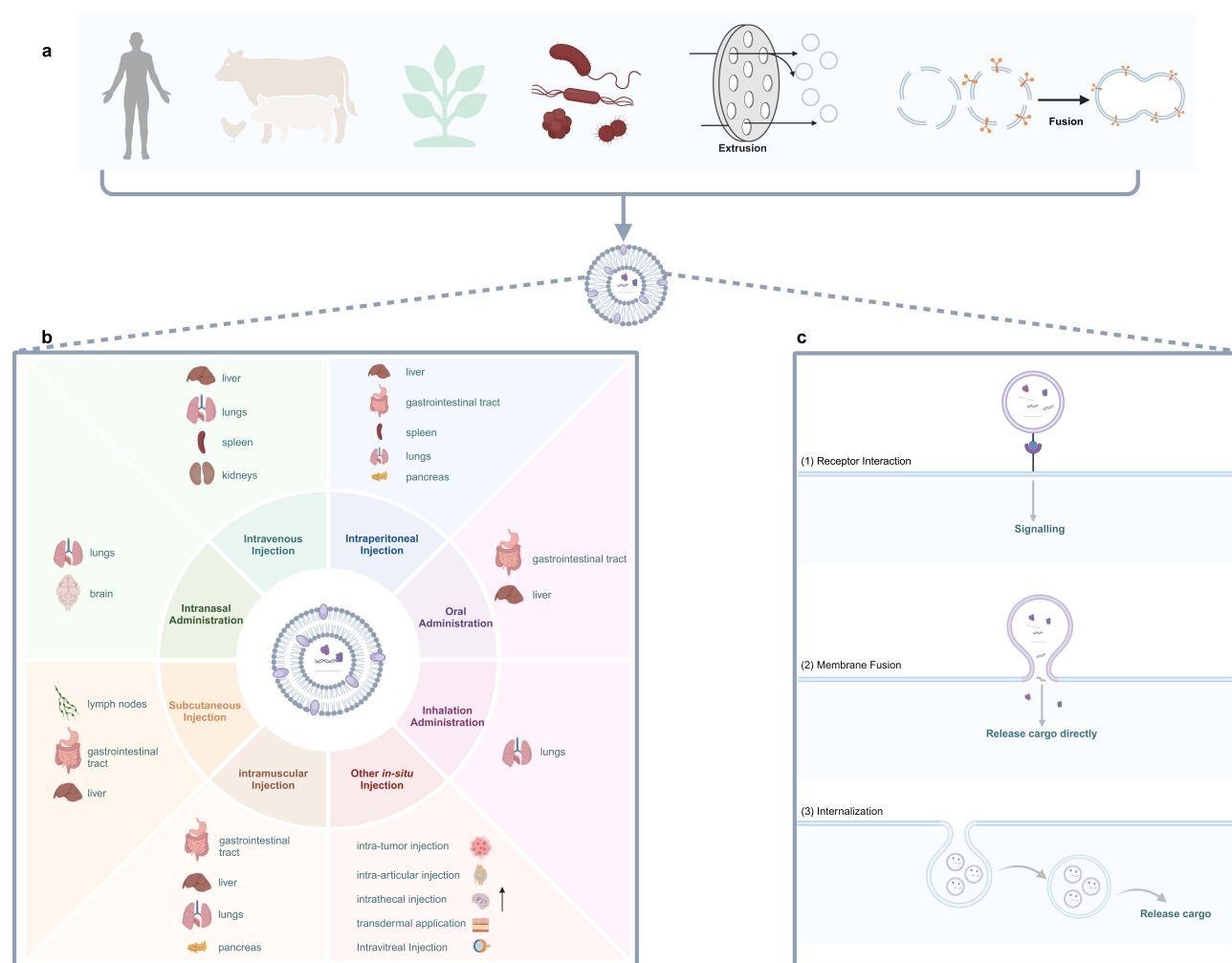


Figure 1 Biodistribution and cellular uptake of EVs in organisms (a) EVs originate from diverse sources, including animals, plants, microorganisms, artificial cell-derived vesicles, and synthetic vesicles. (b) The biodistribution profiles of EVs vary depending on the route of administration. (c) Receptor cells employ various mechanisms for the uptake of EVs. Created in BioRender. Su, X. (2025) <https://BioRender.com/t381645>.

consensus on the predominant mechanism governing EV uptake and content delivery. Furthermore, some researchers have suggested that the EV uptake pathway may depend more on the type of receptor cell than on the characteristics of the EVs themselves.⁷ Nonetheless, identifying the precise mechanism of EV-receptor cell interactions has profound implications for advancing therapeutic applications of EVs.

Based on their biological activities, EVs play an important role in the physiological and pathological processes of organisms. Furthermore, EVs are distinguished by their intrinsic stability, low immunogenicity, biocompatibility, extended half-life, and superior ability to traverse biological barriers.^{8,9} Consequently, EVs have recently become a focal point of intensive research as cell-free therapeutic alternatives, drug and gene delivery vectors, biomarkers for disease diagnosis and prognosis, vaccine development platforms, and nutraceutical carriers. The efficacy and toxicity profiles of EVs, whether utilized as drug delivery systems or functional entities, are profoundly influenced by their biodistribution characteristics.¹⁰ A significant amount of research has focused on engineering modifications to enhance the circulation stability and tissue-targeting capabilities of EVs.¹¹ However, limited attention has been devoted to investigating how various EV sources, delivery routes, and other conditions influence tissue accumulation and therapeutic efficacy. For instance, intravenously administered EVs derived from mesenchymal stem cells (MSCs) exhibited significant accumulation in the liver, making them a promising option for liver-targeted therapies.¹² In contrast, intranasally delivered MSC-derived EVs predominantly accumulated in the lungs and showed greater distribution in

the brain compared to intravenous administration.¹³ Intranasal delivery thus represents a viable route for enhancing the targeting of EVs to central nervous system disorders. This review discusses the main factors influencing the biodistribution of unmodified EVs, emphasizing their distribution and applications across various administration routes, including intravenous, intraperitoneal, oral, intranasal, transdermal, inhalation, intramuscular injection, and in situ administration (Figure 1). This information serves as a valuable reference for researchers selecting appropriate EV delivery routes based on their specific research goals. Furthermore, as the sources of EVs are becoming increasingly diversified, a comprehensive summary of the characteristics of EVs from various sources remains lacking. We provide a comprehensive discussion of current EV sources, highlighting the advantages and limitations of each, including underexplored EVs such as plant exosome-like nanovesicles (PELNVs), bacterial outer membrane vesicles (OMVs), and EV mimetics (EVMs). In addition to targeted modification, the selection of suitable EV sources and optimization of administration routes appear to help enhance the therapeutic potential of EVs.

Therapeutic Potentials of EVs

EVs as Cell-Free Therapeutic Alternatives

Stem cells, particularly mesenchymal stem cells (MSCs), have emerged as a cornerstone in the field of regenerative medicine. However, certain studies have indicated potential challenges with stem cell applications, including graft rejection after allogeneic transplantation¹⁴ and the risk of microvascular occlusion.¹⁵ EVs inherit numerous bioactive molecules from their parent cells, thereby exhibiting comparable therapeutic potential.¹⁶ For instance, both intra-articular injection of bone marrow-derived MSCs (BM-MSCs) and BM-MSC-derived EVs have been shown to mitigate cartilage destruction and subchondral bone remodeling in a rat model of osteoarthritis.¹⁷ Compared to their parent cells, EVs offer distinct advantages such as lower immunogenicity, non-tumorigenicity, and the ability to cross biological barriers.^{18,19} Additionally, research has demonstrated that the size characteristics of EVs enable secure systemic delivery via various routes without inducing embolism.¹⁹

To date, numerous clinical trials have validated the efficacy and safety of EVs. Small EVs derived from human placental MSCs have significantly reduced mortality rates and extended survival times in the intervention groups, suggesting a promising therapeutic strategy for severe COVID-19 patients.²⁰ For chronic kidney disease patients, EVs from human umbilical cord MSCs have been shown to decrease plasma TNF- α levels while increasing TGF- β 1 and IL-10 levels, thereby safely mitigating inflammatory immune responses and improving renal function.²¹ Platelet-derived EVs (pEVs) activate the ERK and Akt signaling pathways in recipient cells, promoting fibroblast proliferation and migration in vitro, as well as angiogenesis. A clinical trial has confirmed the safety of allogeneic pEVs in promoting wound healing in healthy individuals, indicating potential applications for patients with delayed or impaired wound healing.²² Moreover, research on the efficacy and safety of EVs in treating various systemic diseases, including graft-versus-host disease,²³ chemotherapy-induced alopecia,²⁴ and Alzheimer's disease,²⁵ has shown promising results in clinical trials. Despite being in its nascent stages, clinical research on EVs has revealed significant advantages and substantial potential for disease treatment and health promotion.

EVs as Important Carriers for Drug Delivery

Currently, clinically utilized therapeutic agents, including methotrexate, paclitaxel, doxorubicin, and curcumin, face problems such as inadequate water solubility, rapid clearance, and poor bioavailability.^{26,27} Moreover, non-specific uptake by healthy cells and tissues leads to widespread distribution of these cytotoxic agents throughout the body rather than targeted delivery to tumors.²⁸ The issues of low targeting specificity and high toxicity associated with chemotherapeutic drugs are interrelated and challenging to address. MicroRNAs (miRNAs) play a crucial role in human growth and development, regulating various physiological and pathological processes. However, naked miRNAs and other gene therapy agents exhibit poor stability, short circulation half-life, and inadequate cellular uptake due to their susceptibility to degradation by nucleases in vivo. To overcome these limitations, nanoparticle delivery systems, including polymer nanoparticles, liposomes, and EVs, have been extensively developed to improve the pharmacokinetic and pharmacodynamic properties of therapeutic drugs, proteins, and nucleic acids. Since the FDA approved the first anticancer nano-drug,

Doxil[®], in 1995, liposomal products have undergone rapid development.²⁹ However, several challenges have limited their clinical application, including poor long-term stability, high toxicity, drug leakage, and rapid clearance from circulation.³⁰ For instance, liposomes can induce acute hypersensitivity reactions known as complement activation-related pseudoallergy (CARPA). Reportedly, 2–45% of patients experience infusion-related hypersensitivity reactions to liposomal treatments.³¹ Similarly, polymer nanoparticles also face challenges such as insufficient biocompatibility and toxicity.³² Utilizing natural carrier systems represents an effective strategy to address challenges in therapeutic drug delivery.

Compared with liposomes and synthetic polymer nanoparticles, EVs exhibit superior biocompatibility, stability, circulation half-life, and lower toxicity due to their endogenous nature.^{28,33} A study comparing liver injury indicators in mice pre-treated with grapefruit-derived nano vectors (GNVs) versus liposomes revealed significantly higher levels of alanine aminotransferase and aspartate aminotransferase in the liposome-treated group compared to those treated with GNVs.²⁸ Furthermore, EVs demonstrate better inherent targeting capabilities compared to liposomes and polymer nanoparticles. Research has shown that when co-incubated with neurons, macrophage-derived EVs accumulated significantly more in neurons compared to polymeric nanoparticles and liposomes.³⁴ Owing to the tight junctions between endothelial cells and the selective permeability of the blood-brain barrier (BBB), 100% of large molecule drugs and over 98% of small molecule drugs are unable to cross the BBB, thereby significantly limiting their therapeutic efficacy for brain diseases.³⁵ Recent studies have demonstrated that EVs can traverse the BBB primarily through endocytosis,⁹ highlighting their considerable potential in the treatment of brain disorders. For instance, compared to free catalase (CAT), EVs loaded with CAT have been shown to effectively mitigate oxidative stress and enhance neuronal survival in a Parkinson's disease mouse model.³⁴ Furthermore, as natural nano-carriers, EVs carry bioactive molecules from their parent cells, which are advantageous for therapeutic applications. For instance, intestinal macrophages exhibited a preferential uptake of grapefruit-derived nanovesicles (GDN), leading to the upregulation of heme oxygenase-1 (HO-1) expression and the inhibition of TNF- α and IL-1 β production. Compared with free methotrexate (MTX), MTX-loaded GDN significantly reduced MTX toxicity and improved DSS-induced colitis in mice.³⁶

EVs as Biomarkers for Disease Diagnosis and Prognosis

Early diagnosis and prognosis monitoring of diseases are critical for reducing mortality and mitigating disease progression. EVs are present in various biological fluids, carrying bioactive molecules from parent cells and exhibiting distinct expression patterns during physiological and pathological processes. Furthermore, EVs have been identified in all bodily fluids, including blood, urine, saliva, and malignant ascites. They are considered promising biomarkers for disease diagnosis and prognostic monitoring in central nervous system disorders, oncology, cardiovascular diseases, and gastrointestinal conditions.³⁷ Chatterjee et al demonstrated that plasma EVs contain measurable levels of TAR DNA-binding protein 43 (TDP-43) and full-length tau. The quantification and integration of plasma EV TDP-43 levels along with the ratio of 3R/4R tau isoforms could significantly enhance the molecular diagnosis of frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). This represents a novel biomarker for identifying the molecular pathology associated with FTD and ALS.³⁸ CD63, vimentin, and the epithelial cell adhesion molecule on plasma EVs from osteosarcoma patients can be utilized in a rapid diagnostic classification model for osteosarcoma. This model demonstrates a sensitivity of 100%, specificity of 90%, and overall accuracy of 95%.³⁹ Jiang et al developed an effective urinary EVs-based prostate score (EPS) model for the Chinese population by utilizing urine-derived EVs and integrating the gene expressions of FOXA1, PCA3, and KLK3. This model demonstrates significantly higher diagnostic accuracy for clinically significant prostate cancer compared to prostate-specific antigen.⁴⁰ The dual biomarker panel, comprising two small RNAs (tRNA-GlyGCC-5 and sRESE) derived from salivary EVs, effectively distinguishes patients with esophageal squamous cell carcinoma from healthy controls with a specificity of 90.5% and an accuracy of 94.2%. Additionally, this biomarker panel can predict overall survival and assess the potential benefit of adjuvant therapy for patients before surgery.⁴¹ Furthermore, EVs derived from gut microbiota have emerged as novel biomarkers for the diagnosis of gastrointestinal diseases.⁴²

EVs for Tumor Immunotherapy and Vaccine Development

Cancer continues to pose a significant and lethal health challenge annually, and tumor immunotherapy has emerged as a promising new strategy for cancer treatment. EVs carry diverse components from their parental cells, including antigens, which renders them potential candidates for tumor immunotherapy. Specifically, numerous studies have demonstrated that dendritic cells (DCs)- and tumor-derived EVs exhibit high expression levels of tumor markers and major histocompatibility complex class I molecules (MHC I), thereby participating in antigen presentation and the stimulation of T cells. These mechanisms have been shown to elicit CD8⁺ T cell-dependent anti-tumor responses both in vitro and in vivo.⁴³ For instance, tumor-derived exosomes and α -galactosylceramide (α -GalCer)-pulsed DCs have been observed to robustly activate and expand tumor-specific cytotoxic T lymphocytes in a glioblastoma rat model, re-establishing immune surveillance and demonstrating more potent anti-tumor immune responses compared to tumor lysates.⁴⁴ However, concerns regarding the safety of tumor-derived EVs (TEVs) persist. Several studies have indicated that TEVs play a bidirectional role in tumor immune responses.⁴⁵

Furthermore, EVs can function as adjuvants to potentiate adaptive immune responses elicited by vaccines. A recent investigation demonstrated that ginseng-derived EV-like particles (G-EVLPs), when co-administered with autologous tumor antigens via membrane fusion, served as effective adjuvants. Specifically, G-EVLPs augmented dendritic cell phagocytosis of tumor antigens, activated tumor-specific cytotoxic T lymphocytes (CTLs), and conferred long-term immune protection.⁴⁶

Pathogenic microorganisms-derived EVs similarly contain a variety of antigens and pathogen-associated molecular patterns (PAMPs), which confer vaccine-like properties.⁴⁷ For instance, studies have compared the efficacy of *Mycobacterium tuberculosis*-derived EVs with that of standard BCG vaccination, demonstrating comparable protective effects against *Mycobacterium tuberculosis* infection.⁴⁸ Additionally, Li et al utilized a novel lysin-based approach to enhance the production of outer membrane vesicles (OMVs) from *Acinetobacter baumannii*. In a mouse model of pneumonia and bacteremia, three intramuscular injections of these OMVs resulted in significant protection against *A. baumannii* infection.⁴⁹

EVs as Nutraceuticals

Nutraceuticals have garnered significant attention in recent years. Milk-derived EVs (M-EVs), which possess physiological functions including immunomodulation, maintenance of intestinal and bone homeostasis, and promotion of neural development, represent excellent multifunctional components.⁵⁰ The processing methods used in milk powder production can influence the composition and integrity of these vesicles. Therefore, supplementing M-EVs into infant formula can help prevent necrotizing enterocolitis and promote neonatal brain development.⁵⁰ Similarly, given their role in maintaining intestinal and bone homeostasis, M-EVs can be incorporated into functional foods to inhibit osteoporosis and alleviate intestinal inflammation.

Biodistribution of EVs

Elucidating the biodistribution of EVs is critical for their successful clinical application. Extensive research has demonstrated that, due to the activity of the mononuclear phagocyte system, less than 5% of small EVs remain in circulation within five minutes post-intravenous administration.⁵¹ These EVs are rapidly distributed to various organs, with predominant accumulation observed in the liver, lungs, spleen, gastrointestinal tract, and kidneys.^{51,52} Apart from tissue-targeted EVs generated through genetic engineering or chemical modification, the biodistribution of EVs primarily varies based on their size, cellular source, and route of administration. Numerous studies have shown that small EVs predominantly accumulate in the liver, whereas large EVs exhibit a higher propensity for pulmonary accumulation. Small EVs achieve peak concentrations in the liver and kidneys within one hour post-intravenous administration, while peak levels in the lungs and spleen are observed at later time points. Conversely, large EVs attain maximum concentration in the lungs during the first hour post-administration, with increased hepatic accumulation as lung levels decline.⁵¹ Furthermore, researchers utilized asymmetric flow field-flow fractionation to isolate two subpopulations of exosomes from B16-F10-derived vesicles: large exosome vesicles (90–120 nm) and small exosome vesicles (60–80 nm). It was found that large exosome vesicles exhibited lymph node tropism following intravenous injection.⁵³

Furthermore, it has been demonstrated that the protein corona, dosage, and isolation methods of EVs also influence their natural biodistribution. Studies have shown that nanoparticles can adhere to proteins in biological fluids to form a “protein corona”, which impacts their *in vivo* stability, targeting efficacy, cytotoxicity, and immunotoxicity.⁵⁴ Recent research has identified differences in the protein corona of EVs isolated from 2D versus 3D culture conditions, indicating that EVs derived from 3D cultures enhance hepatocyte uptake and hepatic aggregation.⁵⁵ Additionally, the dose of EVs has been shown to affect their distribution patterns. For instance, intravenous administration of three different doses of HEK293T EVs revealed a decrease in relative hepatic signal with increasing EV dose, potentially due to saturation of the mononuclear phagocyte system.¹⁰ Moreover, isolation methods also affect biodistribution patterns. EVs isolated using ultrafiltration combined with size-exclusion liquid chromatography exhibited significantly reduced lung accumulation compared to those isolated by traditional ultracentrifugation following intravenous administration.⁵⁶

Among these factors, particular attention will be given to the source and the administration route, which will be elaborated upon in the subsequent sections.

Sources of Vesicles

The Sources of Vesicles Significantly Influence Their Biodistribution

Natural EVs, as bioactive components, exhibit surface receptors that are identical to those of their parent cells. Extensive research has demonstrated that EVs derived from different cellular sources display distinct tropisms. For instance, a study compared the biodistribution of intravenously administered EVs from C2C12 myoblasts, B16F10 melanoma cells, and primary immature bone marrow-derived dendritic cells (DCs). The results indicated that while all EVs showed significant accumulation in the liver, lungs, spleen, and gastrointestinal tract, C2C12-derived EVs exhibited particularly high accumulation in the liver, B16F10-derived EVs accumulated more prominently in the gastrointestinal tract, and DC-derived EVs were preferentially distributed in the spleen.¹⁰ Under the same intravenous injection conditions, macrophage-derived EVs exhibited significantly greater accumulation in the liver compared to EVs from mesenchymal stem cells (MSCs) and the melanoma cell line (A375). No significant differences were observed in the distribution of these EVs in the spleen, lung, kidney, muscle, or bone.⁵⁷ The organ-specific distribution of EVs may be influenced by the membrane components of their parental cells, which partially explains the higher accumulation of macrophage-derived EVs in macrophage-rich tissues such as the liver. Similarly, under identical inhalation administration conditions, human lung spheroid cell (LSC)-derived EVs demonstrated a more pronounced distribution in the lung parenchyma 24 hours post-administration compared to EVs from HEK293T cells. Additionally, LSC-derived EVs appeared later in the liver, spleen, and kidneys and exhibited prolonged retention in the lungs.⁵⁸ Tumor-derived EVs exhibit a preferential homing tendency to the organs from which their parental cells originate or to sites of tumor metastasis. This phenomenon may be attributed to differential integrin expression profiles.^{59,60} These findings suggest that in optimizing EVs as drug delivery systems, cell sources can be selectively chosen based on the therapeutic target tissues and the inherent tropism of EVs from different origins for personalized regulation, thereby enhancing accumulation in target tissues. However, the precise mechanisms underlying the intrinsic tropism of EVs derived from endogenous cell sources remain unclear, and the interactions between EVs and receptor cell surfaces warrant further investigation.

Currently Available Vesicle Sources

EVs originate from a diverse array of sources, with virtually any cell type in nature serving as a potential source. These sources encompass animal cells (such as stem cells, tumor cells, immune cells, etc.), plant cells, and microorganisms. However, EVs naturally secreted by cells often exhibit limitations, particularly in terms of low productivity. To address these challenges, researchers have developed extracellular vesicle mimetics (EVMs), including artificial cell-derived vesicles and synthetic vesicles, which play crucial roles in drug delivery applications. Vesicles from different origins possess distinct advantages and disadvantages (Table 1), allowing researchers to select the most suitable cell sources based on their specific research objectives and requirements.

Table 1 The Advantages and Disadvantages of Different Sources of EVs

	Human Sources	Milk Sources	Plant Sources	Microbial Sources	ACDVs	SVs
Advantages	Endogenous function Low immunogenicity Intrinsic targeting capability	Bioactive function Well cross-species tolerance Easy availability Stability against digestion	Bioactive function High safety; free of zoonotic or human pathogens Easy availability Gastrointestinal stability	Stimulate cellular and humoral immunity	Endogenous function Large-scale production Intrinsic targeting capability	Endogenous function Easy surface modification Intrinsic targeting capability High drug loading efficiency
Disadvantages	Cancer-stimulating risk Limited yields	Risk of accelerated cancer metastasis Inadequate mucus penetration capability	Plant toxin transfer Time-consuming in the separation and purification process	Lack of safety due to factors such as carrying virulence factors	Contained non-exosome substances	Complexity of production

Abbreviations: ACDVs, artificial cell-derived vesicles; SVs, synthetic vesicles.

Animal Sources

Human cell-derived EVs, including those from macrophages, dendritic cells (DCs), neutrophils, B cells, T cells, neurons, oligodendrocytes, tumor cells, and stem cells, have garnered significant attention in clinical applications.⁶¹ For instance, EVs derived from monocytes and macrophages inherit the surface components of their parent cells, enabling them to target inflammation sites while evading rapid clearance by the mononuclear phagocyte system, thereby extending their circulation half-life and making them ideal drug carriers for inflammatory diseases.⁶² Additionally, EVs from dendritic cells play a crucial role in antigen presentation and T-cell stimulation, with research demonstrating their capacity to transfer peptide-MHC complexes to other DCs, thereby enhancing tumor rejection.⁶³ Consequently, these EVs have been extensively investigated for vaccine development and immunotherapy. Neutrophil-derived EVs exhibit chemotactic properties similar to those of neutrophils themselves. In a glioma mouse model, neutrophil-derived EVs loaded with doxorubicin effectively targeted inflamed brain tumors and inhibited tumor growth.⁶⁴

Given that tumor-derived EVs (TEVs) exhibit a tropism for their parental cells,⁶⁵ they represent promising candidates as vectors for targeted drug delivery. Compared to EVs derived from HeLa cells, those isolated from HT1080 cells demonstrated a preferential fusion with their parental cancer cells in vitro and exhibited greater accumulation in tumor tissues within a subcutaneous HT1080 tumor-bearing nude mouse model.⁵⁹ In a murine model involving multiple tumor cell lines inoculated into the pleural cavity, TEVs induced by UVB and loaded with methotrexate significantly reduced pleural tumor burden, the number of pleural lesions, and effusion volume compared to empty TEVs and free methotrexate. These TEVs demonstrated potential efficacy and safety in a clinical trial involving 11 patients with malignant pleural effusion secondary to advanced lung cancer.⁶⁶ Moreover, TEVs carry a diverse array of proteins and nucleic acids, including antigens from parental tumor cells, which are essential for disease diagnosis and tumor immunotherapy. However, the high heterogeneity of TEVs and the bidirectional tumor immune response raise significant safety concerns, thereby limiting their broader application.

Among all human-derived cells, mesenchymal stem cells (MSCs) are considered the most promising source of EVs. This is attributed to their diverse origins (including umbilical cord, bone marrow, and adipose tissue) and their immunomodulatory and regenerative capacities in various diseases.⁶⁵ Consequently, MSC-derived EVs represent a viable alternative to cell-based therapies for tissue repair and regeneration. During in vitro passage culture of mesenchymal stem cells (MSCs), both the proliferative and differentiation capacities of MSCs diminish, leading to

reduced expansion potential.^{67,68} Research has demonstrated that EVs from senescent MSCs and those from young MSCs exhibit distinct effects. For instance, EVs from senescent MSCs can elevate senescence markers in young cells, whereas EVs from young MSCs have the opposite effect.⁶⁹ Numerous studies have explored methods to reverse MSC senescence during expansion, including intrinsic factors such as targeting signaling pathways, small molecule therapies, epigenetic regulation, and extrinsic factors like using paracrine factors to modulate the external environment and altering culture conditions to rejuvenate senescent MSCs.⁷⁰ Notably, induced pluripotent stem cells (iPSCs) have garnered significant interest due to their unlimited proliferation capacity. Unlike UC-MSCs, which lose their MSC characteristics by the 15th passage, MSCs derived from iPSCs (iMSCs) maintain their MSC properties without chromosomal abnormalities. Furthermore, somatic cells obtained from the patient can be utilized to generate induced pluripotent stem cells (iPSCs), thereby circumventing ethical concerns and mitigating the risk of immune rejection.⁷¹ In a mouse model of ischemic stroke, human urine-derived iPSC-derived small EVs (sEVs) specifically targeted the peri-infarct region, leading to the upregulation of neuroprotective genes and the downregulation of inflammation-related genes. Notably, BDNF-loaded sEVs derived from human urine-induced iPSCs demonstrated more pronounced effects on enhancing functional recovery, reducing infarct volume, promoting neurogenesis and angiogenesis, and controlling inflammation.⁷² However, EVs derived from iMSCs still face the conventional challenges associated with the clinical translation of EVs, and there is an urgent need to enhance their yield. Yuan et al demonstrated a significant increase in the yield of EVs derived from mesenchymal stem cells (MSCs) through three-dimensional dynamic culture, which not only boosted their immunomodulatory potential but also presented a promising approach for enhancing the yield of EVs from MSCs.⁷³ It remains to be explored whether this method can similarly improve both the yield and functionality of EVs derived from iMSCs, making it a worthwhile area for further investigation.

In addition to human-derived EVs, EVs from other animal sources, particularly milk-derived EVs (M-EVs), have garnered significant attention from researchers. This interest is driven by their ease of availability, safety, stability against digestion, and compatibility with tissue absorption.^{50,74} Moreover, M-EVs exhibit immunomodulatory properties, contribute to the maintenance of intestinal and bone homeostasis, support neurodevelopment, and possess antifibrotic and anticancer activities, making them suitable for functional products and oral delivery systems.⁵⁰ For example, orally administered paclitaxel-loaded M-EVs exhibited significantly enhanced tumor growth suppression and demonstrated substantially reduced systemic toxicity and immunotoxicity compared to intraperitoneal administration of paclitaxel.⁷⁵ Notably, Samuel et al observed that oral administration of M-EVs reduced the primary tumor burden in mouse models of breast and pancreatic cancer while simultaneously accelerating tumor metastasis.⁷⁶ Nevertheless, several studies have demonstrated that natural M-EVs lose approximately 85% of their protein content in simulated gastric environments and encounter significant challenges posed by the mucus-epithelial barrier.⁷⁷ To address these issues, researchers have employed surface modifications using hydrophilic and zwitterionic peptides to enhance the stability and intestinal epithelial absorption of M-EVs.⁷⁷ Further investigation is required to elucidate the potential and mechanisms of M-EVs in crossing gastrointestinal barriers. Engineering EVs to improve their gastrointestinal stability is essential for the realization of oral EV-based drug delivery systems. Furthermore, EVs derived from various animal sources have been extensively investigated. Notably, EVs isolated from oyster mantle have demonstrated the ability to promote osteoblast differentiation while simultaneously inhibiting osteoclast differentiation. This dual effect has been shown to effectively alleviate osteoporosis in ovariectomized mice following oral administration.⁷⁸

Plant Sources

Plant Exosome-like Nanovesicles (PELNVs) were initially identified in plant cells in 1967 but have received limited attention until recently.⁷⁹ Over the past decade, significant advancements have been made in understanding their diverse biological effects, including anti-tumor, immunomodulatory, and regenerative properties, positioning PELNVs as promising therapeutic agents. For instance, ginger-derived PELNVs have demonstrated anticancer potential by reducing cyclin D1 mRNA levels in a mouse model of colorectal cancer.⁸⁰ Turmeric-derived PELNVs have alleviated colitis through the regulation of antioxidant gene HO-1 and pro-inflammatory cytokines such as IL-6, IL-1 β , and TNF- α , showcasing their anti-inflammatory and antioxidant capabilities.⁸¹ Wheat-derived PELNVs have promoted skin regeneration and angiogenesis.⁸² Compared to animal-derived EVs, PELNVs offer several advantages: they are readily

available from a wide range of sources (eg, carrot, lemon, citrus, ginger, grapefruit, wheat, and broccoli), enabling large-scale production at lower economic costs. Moreover, PELNVs exhibit lower toxicity and higher safety profiles, avoiding potential risks associated with animal-derived EVs, such as tumor stimulation and zoonotic or human pathogens.³³ Studies have also shown that PELNVs can withstand harsh gastrointestinal environments, resisting digestive enzymes (eg, pepsin, trypsin, and bile extract solution) and strong gastric acid conditions, highlighting their potential for oral drug delivery.³⁰ For example, turmeric-derived EVs remained stable in simulated gastric (pH ~2.0) and intestinal (pH ~6.5) solutions, accelerating colitis remission when administered orally.⁸¹ However, it is important to address potential risks, such as the transmission of plant toxins like trichosanthin and allergens, which could pose challenges for clinical translation.³³ As emerging therapeutic agents, limited information is available regarding the specific mechanisms of biogenesis, release, and function of PELNVs, which poses significant challenges to their clinical translation. Moreover, the isolation and purification processes for PELNVs, which are considerably more time-consuming compared to those of EVs derived from cell cultures, represent a critical area requiring substantial improvement for large-scale production.³³

Bacterial and Other Microbial Sources

The bacterial outer membrane vesicles (OMVs) play a crucial role in mediating both bacteria-bacteria and bacteria-host interactions. These interactions encompass modulating host immune responses, enhancing antibiotic resistance, and influencing bacterial pathogenicity.⁸³ The cargo transported by OMVs, which includes proteins and nucleic acids, can reflect the physiological or pathological states of the host. Consequently, OMVs serve as potential sources of specific biomarkers for various diseases, including both tumor and non-tumor conditions such as psychiatric disorders, abdominal obesity, allergic rhinitis, periodontitis, and atopic dermatitis.⁸⁴ Furthermore, OMVs carry a diverse array of pathogen-associated antigens, effectively eliciting robust cellular and humoral immune responses, which positions them as promising candidates for vaccine development. For instance, Jiang et al engineered a novel SARS-CoV-2 vaccine by conjugating the mammalian cell culture-derived Spike receptor-binding domain (RBD) to EVs derived from *Salmonella typhimurium*. This intranasally administered vaccine induced neutralizing antibodies against both the wild-type and Delta variants, providing effective protection against SARS-CoV-2.⁸⁵ The immunosuppressive tumor microenvironment has posed a significant challenge to tumor immunotherapy. Researchers have leveraged the immunostimulatory properties of OMVs to reprogram the tumor microenvironment by inducing M1 polarization of macrophages.^{86,87} However, the intrinsic immunotoxicity associated with OMVs derived from bacteria has considerably impeded their clinical application. A recent study addressed this issue by constructing attenuated OMVs through gene editing. This approach eliminated the inflammatory response triggered by flagellin and lipopolysaccharide on the OMVs surface while preserving its immunostimulatory function, thereby presenting a promising strategy to enhance the therapeutic potential of OMVs.⁸⁸ Recently, research into fungal and parasitic EVs has expanded. However, concerns regarding their safety and therapeutic efficacy have been raised due to factors such as the potential carriage of virulence factors.⁸³

Artificial Cell-Derived Vesicles (ACDVs)

Artificial cell-derived vesicles (ACDVs) are predominantly generated through continuous cell extrusion, enabling large-scale production with stability, distribution, and immune compatibility that closely resemble those of natural EVs.⁶⁵ Fan et al have demonstrated high-yield production of EV mimetics by extruding human mesenchymal stem cells (hMSCs) through polycarbonate membrane filters with progressively decreasing pore sizes. Compared to naturally derived hMSC-EVs, these extruded EV mimetics exhibit comparable characteristics and biological internalization capabilities, while demonstrating enhanced osteogenic effects.⁸⁹ However, the researchers also observed that EV mimetics contain various non-exosomal components, including GM-130, cytochrome c, and calnexin. In a recent study, immunosuppressive microvesicle mimetics (MVMs) were developed by extruding endotoxin-resistant dendritic cells and conjugating α CD3 antibodies to the vesicles to specifically target T cells. The engineered MVMs demonstrated a significant capacity to induce regulatory T cells (Tregs) and polarize anti-inflammatory M2 macrophages, leading to rapid and efficient immunosuppression in both sepsis and rheumatoid arthritis (RA) models. These findings suggest that MVMs may represent a promising therapeutic option for both acute and chronic inflammatory diseases.⁹⁰

Synthetic Vesicles (SVs)

Natural EVs exhibit low drug loading efficiency,⁹¹ while liposomes do not possess the endogenous functions and low immunogenicity characteristics of natural EVs. Scientists have addressed these limitations by fusing the membranes of EVs and liposomes to construct hybrid vesicles that possess low immunogenicity, high drug loading efficiency, ease of surface modification, inherent targeting capabilities, and enhanced stability.⁹² Goh et al pioneered the fusion of EVs with liposomes to create EXOPLEX, which showed doxorubicin hydrochloride loading efficiencies exceeding 65%. Compared to liposomes or EVs alone, EXOPLEX exhibits superior in vitro cytotoxic effects and is anticipated to represent a new generation of engineered vesicles for efficient drug delivery.⁹³ Moreover, tumor-derived EVs, known for their ability to target parental cancer cells,⁵⁹ have been extensively studied for fusion with liposomes, emerging as promising tools for precise tumor targeting.^{94,95} In a recent study, Li et al developed hybrid vesicles by fusing hematopoietic stem and progenitor cell (HSPC) membranes with liposomes to specifically target the bone marrow. In a mouse model of leukemia, these hybrid vesicles facilitated targeted delivery of cytarabine to the bone marrow via the hyaluronan-CD44 axis, leading to a significant reduction in leukemic stem cell burden.⁹⁶ Furthermore, the integration of thermosensitive, photosensitive, and pH-sensitive liposomes endowed the hybrid vesicles with controlled release capabilities, thereby enhancing drug delivery efficiency and mitigating adverse effects. For instance, synthetic vesicles generated by fusing macrophage-derived EVs with pH-sensitive liposomes enhanced the release of encapsulated doxorubicin in acidic microenvironments, thereby improving drug delivery to acidic tumor environments.⁴² However, the practical application of these hybrid vesicles is currently constrained by the complexity of their fabrication process and the challenges associated with large-scale production.

Administration Routes of EVs

The Administration Routes of EVs Influence Their Biodistribution

EVs can be administered through various routes including intravenous, intraperitoneal, oral, intranasal, subcutaneous, or local administration (eg, intratumoral, intra-articular). Numerous studies have demonstrated that different administration routes significantly influence the biodistribution of EVs (Figure 2). For instance, Wiklander et al compared tissue distribution 24 hours post-administration of HEK293T-derived EVs via intravenous, intraperitoneal, and subcutaneous injections. Their findings indicated that intravenously administered EVs accumulated more in the liver and spleen, whereas intraperitoneally and subcutaneously administered EVs exhibited less accumulation in these organs and a significantly greater presence in the gastrointestinal tract and pancreas.¹⁰ Manca et al reported that orally administered milk-derived EVs (M-EVs) predominantly accumulated in the intestines and liver, while intravenously administered M-EVs were mainly distributed to the liver and spleen.⁹⁷ Twenty-four hours post-intravenous injection, mesenchymal stem cell (MSC)-derived EVs were predominantly localized in the liver, spleen, and lung. In contrast, EVs administered via nasal delivery primarily accumulated in the lung, with reduced hepatic accumulation.¹³ In an additional study, nebulized inhalation of MSC-derived EVs resulted in the predominant distribution in the lung and stomach after 24 hours.⁹⁸ These studies suggest that optimizing routes of administration to enhance tissue distribution and improve EV delivery efficiency is a viable strategy. In the subsequent section, we provide a detailed summary and comparison of the various administration routes and their corresponding distribution profiles in EV applications.

The Biodistribution and Applications of EVs via Diverse Administration Routes

Intravenous Injection

Intravenous injection remains the predominant method for delivering EVs. This route allows EVs to directly enter the circulatory system, thereby enabling precise regulation of their concentration and dosage within the bloodstream. Following intravenous administration, EVs predominantly accumulate in organs such as the liver, lungs, spleen, and kidneys.⁵¹ Most studies investigating EV biodistribution have relied on imaging techniques. Vos et al conducted proteomic analysis to examine the presence of EV-derived proteins in various tissues at 1-hour and 24-hour intervals post-injection of adipose-derived mesenchymal stem cell EVs. Their findings indicated that 24 hours post-injection, EV-derived proteins were primarily localized in the liver, corroborating previous observations made using imaging methodologies.¹²

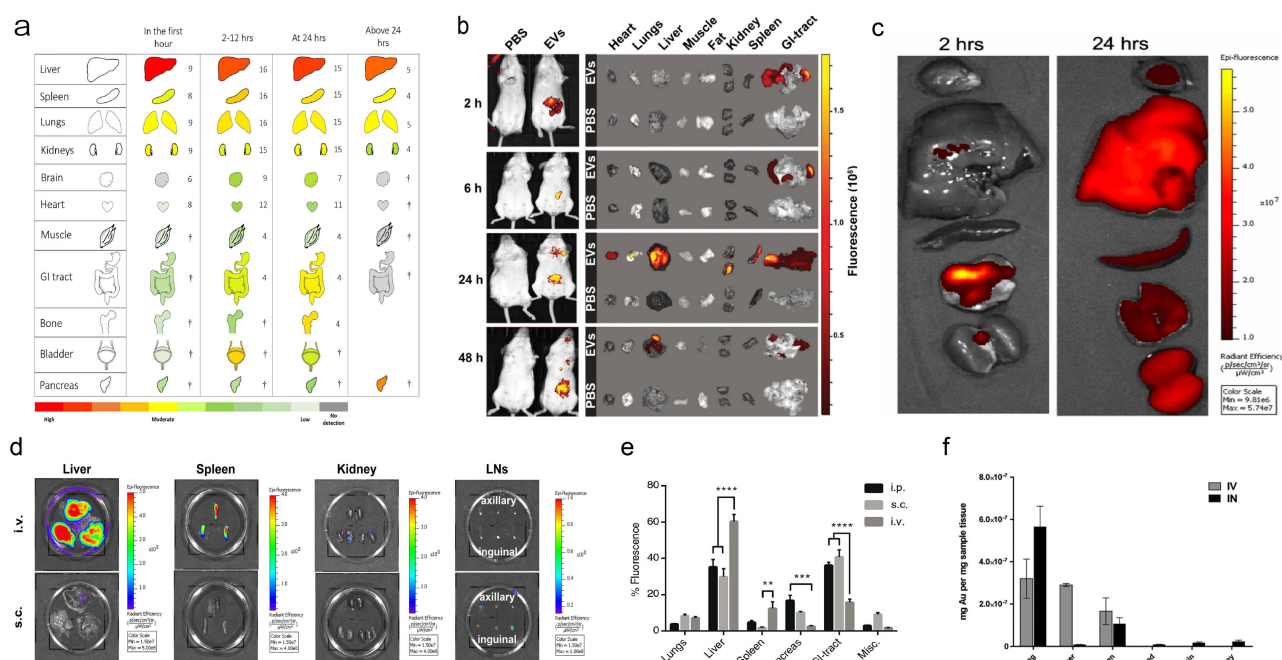


Figure 2 Representative examples of the biodistribution of EVs via various administration routes (a) Ex vivo organ distribution of small EVs following intravenous injection, summarized from 36 studies. The numbers adjacent to each organ indicate the number of studies for that organ, with organs marked by † represented in three or fewer studies. Reprinted with permission from Kang M, Jordan V, Blenkiron C, Chamley LV. Biodistribution of extracellular vesicles following administration into animals: a systematic review. *J Extracell Vesicles*. 2021;10(8):e12085. Copyright 2021, John Wiley and Sons.⁵¹ (b) In vivo imaging of mice post oral administration of milk-derived EVs. Reprinted with permission from Samuel M, Fonseka P, Sanwlani R, et al. Oral administration of bovine milk-derived extracellular vesicles induces senescence in the primary tumor but accelerates cancer metastasis. *Nat Commun*. 2021;12(1):3950. Copyright 2021, Springer Nature.⁷⁶ (c) Organ distribution after inhalation delivery of human lung spheroid cell (LSC)-derived EVs. Reprinted with permission from Wang Z, Hu S, Popowski KD, et al. Inhalation of ACE2-expressing lung exosomes provides prophylactic protection against SARS-CoV-2. *Nat Commun*. 2024;15(1):2236. Copyright 2024, Springer Nature.⁵⁸ (d) Organ distribution of melanoma-derived small EVs following subcutaneous and intravenous injections. Reprinted from *Journal of Controlled Release*, Sakurai Y, Ohtani A, Nakayama Y, et al. Logistics and distribution of small extracellular vesicles from the subcutaneous space to the lymphatic system. *J Control Release*. 2023;361:77–86. Copyright 2023, with permission from Elsevier.⁹⁹ (e) Percentage of fluorescence signals in various organs after intraperitoneal, subcutaneous, and intravenous injections of HEK293T EVs. Misc.=brain, heart, kidneys and quadriceps muscle. N=3–4. **represents $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$. Reprinted with permission from Wiklander OPB, Nordin JZ, O’Loughlin A, et al, Extracellular vesicle in vivo biodistribution is determined by cell source, route of administration and targeting. *J Extracell Vesicles*. 2015;4(1):26316. © 2015 Oscar P.B. Wiklander et al.¹⁰ (f) Quantitative comparison of MSC-derived small EVs delivered intranasally versus intravenously. Reprinted with permission from Betzer O, Perets N, Angel A, et al, In vivo neuroimaging of exosomes using gold nanoparticles. *ACS Nano*. 2017;11(11):10883–10893. Copyright 2017, American Chemical Society.¹³

EVs administered via intravenous injection have diverse applications across a range of conditions, including metabolic disorders, ischemic events (such as stroke), osteoarticular pathologies, and oncological diseases, among others. Sun et al demonstrated that the intravenous administration of EVs derived from human mesenchymal stem cells (MSCs) reversed peripheral insulin resistance by restoring the phosphorylation of insulin receptor substrate 1 and protein kinase B. Concurrently, this treatment alleviated pancreatic β -cell damage, leading to a reduction in blood glucose levels in rats with type 2 diabetes.¹⁰⁰ In the rat model of doxorubicin and trastuzumab-induced cardiotoxicity, intravenous administration of EVs derived from human cardiac-resident mesenchymal progenitor cells (CPCs) significantly attenuated myocardial oxidative stress and cardiotoxicity. This effect was attributed to the high enrichment of miR-146a-5p within the vesicles.¹⁰¹ As circulating EVs have demonstrated the ability to cross the blood-brain barrier (BBB), intravenously administered EVs are extensively utilized for drug delivery and as functional components in cerebral ischemia, glioma, and other neurological applications. For instance, researchers developed a curcumin delivery vehicle derived from bone marrow mesenchymal stem cell (MSC)-derived EVs conjugated with c(RGDyK), targeting the ischemic region of the brain. Following intravenous injection, this construct significantly reduced the levels of pro-inflammatory factors (TNF- α , IL-6, and IL-1 β), thereby inhibiting post-ischemic brain inflammation more effectively than free curcumin.¹⁰² In the context of osteoarticular diseases, Ma et al developed hybrid EVs by fusing M2 macrophage-derived EVs with bone marrow mesenchymal stem cell-derived EVs (M2-BMSCs-Exos). Intravenous administration of M2-BMSCs-Exos targeted areas of osteolysis following joint replacement surgery, thereby inhibiting M1 polarization and promoting M2 polarization. This approach represents a promising strategy for potentially extending the lifespan of prosthetic implants in the future.⁶²

Nonetheless, a substantial body of research demonstrates that EVs exhibit a markedly short half-life following intravenous administration.⁵¹ To address this limitation, researchers have employed strategies such as coating nanoparticles with polyethylene glycol (PEG). Additionally, incorporating molecules associated with the “don’t eat me” signal (eg, CD47, CD31, CD24) into the EV membrane has been explored. These modifications aim to reduce clearance by the mononuclear phagocyte system, thereby prolonging circulation half-life and enhancing the delivery efficiency of EVs to target tissues.⁵²

Intraperitoneal Injection

Intraperitoneal injection leverages the extensive abdominal vasculature, providing a large absorption area and bypassing first-pass metabolism in the gastrointestinal tract. This route also accommodates larger drug volumes, making it a subject of extensive research.¹⁰³ For instance, studies have shown that milk-derived EVs loaded with a suboptimal dose of the anticancer drug WFA, when administered intraperitoneally to mice bearing human lung cancer (A549) xenografts, exhibited superior tumor inhibition compared to free WFA.¹⁰⁴ Another study proposed that intraperitoneal injection represents a promising therapeutic option for obesity, as it can effectively deliver EVs to visceral adipose tissue, in addition to the liver, lungs, and spleen.¹⁰⁵ According to the research by Wiklander et al, EVs administered via intraperitoneal injection exhibited comparable accumulation in the gastrointestinal tract to that observed in the liver, followed by the pancreas, indicating that this route of administration could be efficacious for treating gastrointestinal diseases.¹⁰ For example, intraperitoneal injection of adipose-derived mesenchymal stem cells (MSCs) has been shown to alleviate symptoms of dextran sulfate sodium-induced acute colitis by reducing inflammatory factor levels and modulating the Treg population.¹⁰⁶

Oral Administration

Oral administration remains the preferred route for treating various diseases owing to its cost-effectiveness, ease of use, and high patient compliance. However, the presence of enzyme degradation and strong acidic conditions in the gastrointestinal tract, along with mucosal barriers, markedly diminishes the bioavailability of orally administered drugs.^{107,108} Extensive evidence indicates that EVs derived from diverse sources such as plants, milk, intestinal epithelial cells, and bacteria can endure harsh gastrointestinal environments. This resilience positions EVs as promising candidates for oral drug delivery systems (Table 2). For example, EVs derived from human milk (M-EVs) have been shown to withstand simulated gastric and pancreatic digestion.¹⁰⁸ Additionally, curcumin-loaded M-EVs exhibit resistance to degradation by human digestive enzymes.¹⁰⁹ Researchers initially labeled M-EVs with the lipophilic near-infrared fluorescent dye DiR. They observed that the highest fluorescence signal in the bloodstream was detected at 24 hours post-administration and persisted in circulation until day 6. Furthermore, ex vivo organ imaging conducted on day 4 after oral administration revealed a relatively uniform distribution of EVs across multiple organs, including the liver, lungs, kidneys, pancreas, spleen, ovaries, colon, and brain. This contrasts with the predominant liver accumulation observed following intravenous injection.¹⁰⁴ EVs exhibit widespread distribution across multiple organs, offering a more cost-

Table 2 Typical Examples of Oral Delivery of EVs in Recent years

Source of EVs	Recipient Animal	Distribution of EVs	Mechanism	Therapeutic Effect	Ref
Oyster mantles	Healthy mice	3h, 6h, 12h, 24h Femur, liver (rare in the lungs, spleen, kidneys, and heart)	Activate PI3K/Akt/ β -catenin pathway in osteoblasts and inhibit NF- κ B pathway in osteoclasts	Alleviate osteoporosis	[78]
Robinia pseudoacacia L. flower	Hypoxic mice	6h, 16h, 24h Whole GIT (gradually wane after 16 h)	Inhibit HIF-1 α - and HIF-2 α -mediated lipid peroxidation	Attenuates gastric and small intestinal mucosal ferroptosis caused by hypoxia	[110]
Portulaca oleracea L	Colitis mice	3h, 6h, 12h, and 24h Increase at 6 h and decrease by 24 h in the colon (rare in lungs, liver, spleen, kidneys, and heart)	Promote the expansion of double-positive CD4+CD8+ T cells	Mitigate DSS-induced colitis	[112]

(Continued)

Table 2 (Continued).

Source of EVs	Recipient Animal	Distribution of EVs	Mechanism	Therapeutic Effect	Ref
Tea leaves	Mice bearing 4T1 tumors	6h: GIT, liver, spleen 12h: liver, spleen, GIT, tumor 24h: liver, spleen, GIT, kidneys, tumor 48h: liver, kidneys, tumor, spleen	Promote apoptosis and regulate microbiota	Retard breast tumor growth	[113]
Turmeric	Colitis mice	6h, 24h GIT	Regulate the expression of the pro-inflammatory cytokines and antioxidant gene	Alleviate colitis in mice	[81]
Milk	Healthy mice	2, 6h: GIT 24h: GIT, liver, spleen, kidney, lungs, heart 48h: liver and GIT	Induce cellular senescence and epithelial-to-mesenchymal transition	Induce senescence in the primary tumor but accelerate cancer metastasis	[76]
Milk	Healthy mice	4h Colon, stomach	Neutralize TNF- α in the colon	Alleviate ulcerative colitis in mice	[111]
Milk	DSS-induced ulcerative colitis mice	12h colon	Regulate the gut immunity and reshape the gut microbiota	Alleviate ulcerative colitis	[114]
Milk	Healthy mice	24h Intestine, liver	NA	NA	[97]
Acinetobacter baumannii	A mouse model of intestinal ETEC infection	1h, 12h, 36h Intestine	Exert passive and active bactericidal effects through drug release and bacterial invasion	Effectively protect against mouse intestinal bacterial infection	[115]

Abbreviations: GIT, gastrointestinal tract; ETEC, enterotoxigenic *Escherichia coli*; DSS, dextran sulfate sodium; UC, ulcerative colitis.

effective and simpler alternative to intravenous administration, which has garnered significant attention from researchers. A previous study demonstrated that M-EVs were predominantly localized in the intestines and liver 24 hours post-oral administration, whereas intravenously administered M-EVs were primarily confined to the liver and spleen.⁹⁷ Furthermore, subsequent studies have consistently observed the accumulation of EVs within the gastrointestinal tract following oral administration.^{110,111} Oral administration of EVs has demonstrated superior intestinal distribution compared to intravenous administration and holds the potential for enhancing the targeting of intestinal diseases.

Since Munagala et al first demonstrated in 2016 that milk-derived EVs (M-EVs) are promising carriers for chemotherapeutic drugs and can be used for oral administration,¹⁰⁴ researchers have been increasingly motivated to explore the potential of M-EVs in oral drug delivery. For instance, paclitaxel (PAC) exhibits limited water solubility, which restricts its oral bioavailability. Nevertheless, the oral administration of M-EVs loaded with PAC demonstrates superior anti-tumor efficacy compared to intraperitoneal administration of a lower dose of free PAC. Additionally, it exhibits reduced systemic and immune toxicity relative to intravenous administration of a lower dose.⁷⁵ Similarly, oral administration of curcumin-loaded M-EVs resulted in 3–5 times higher curcumin levels in various organs and stronger inhibition of cervical tumor xenografts compared to free curcumin.¹¹⁶ A recent study demonstrated that VHHm3F-loaded M-EVs accumulated predominantly in the colon after four hours of oral administration, enhancing VHHm3F activity and alleviating inflammation in mice with acute ulcerative colitis compared to free VHHm3F.¹¹¹ Notably, oral administration of insulin-loaded M-EVs showed better and more sustained hypoglycemic effects in type 1 diabetic rats compared to subcutaneous insulin injection.¹¹⁷

Plant-derived EVs, owing to their abundant availability, low immunogenicity, edibility, and inherent physiological effects, have garnered significant attention as oral drug delivery systems. Various plant sources have been explored, including ginger,⁸⁰ turmeric,⁸¹ broccoli,¹¹⁸ garlic,¹¹⁹ grapefruit,³⁶ tea flowers,¹²⁰ and *Robinia pseudoacacia* L. flowers.¹¹⁰ For instance, Wang et al demonstrated that the antioxidant properties of *Robinia pseudoacacia* L. flower exosome-like nanoparticles (RFELNs) led to the inhibition of HIF-1 α and HIF-2 α -mediated peroxidation, thereby mitigating hypoxia-

induced gastric and small intestinal mucosal damage following oral administration. Moreover, RFELNs were observed to accumulate predominantly in the gastrointestinal tract at 6, 16, and 24 hours post-administration, positioning them as promising candidates for natural drugs targeting this system.¹¹⁰ Beyond gastrointestinal applications, oral administration of garlic-derived EVs has shown preferential uptake by microglia in mouse models of high-fat diet-induced obesity. These EVs effectively inhibited brain inflammation through the IDO1-mediated AHR pathway and the c-Myc-mediated c-GAS/STING inflammatory pathway, reversing obesity and insulin resistance induced by a high-fat diet.¹¹⁹ Additionally, a comparative study on tea leaf-derived exosome-like nanotherapeutics (TLNTs) revealed comparable anti-tumor efficacy between oral and intravenous administration routes, with no hepatorenal toxicity observed in oral TLNTs compared to intravenous administration.¹¹³

Intestinal epithelial cell-derived EVs (IEC-EVs) have been investigated to enhance the oral bioavailability of drugs. Compared with free curcumin, curcumin-loaded IEC-EVs significantly improved cellular uptake and intestinal permeability in a Caco-2 cell model, demonstrating superior performance over M-EVs.¹²¹ Additionally, bacterial outer membrane vesicles (OMVs) have been explored for oral drug delivery applications. For instance, Huang et al developed a novel antibiotic delivery system utilizing the drug efflux resistance mechanism of OMVs. Compared with free antibiotics, OMV-encapsulated antibiotics exhibited prolonged retention in the intestine for at least 36 hours, enhanced bactericidal efficacy in a murine model of enterotoxigenic *Escherichia coli*-induced intestinal infection, and mitigated adverse effects on gut microbiota.¹¹⁵

Intranasal Administration

A substantial body of research demonstrates that intranasal administration of EVs significantly enhances their delivery to the brain. Intranasal drug delivery primarily facilitates the transport of drugs across a single epithelial cell layer into the systemic circulation or bypasses the blood-brain barrier via the olfactory and trigeminal nerves, allowing direct entry into the brain.^{34,122} Zhuang et al observed diffuse fluorescently labeled EVs in the brain within 30 minutes post-intranasal administration, indicating rapid translocation of EVs to the brain.¹²³ Betzer et al found that mesenchymal stem cell (MSC)-derived EVs predominantly accumulated in the lungs 24 hours after intranasal administration, whereas intravenously administered EVs were mainly localized in the liver, lungs, and spleen. Notably, intranasal administration of MSC-derived EVs exhibited superior brain accumulation at both 1 and 24 hours compared to intravenous administration.¹³ Consistent with previous findings, Wang et al demonstrated that DiR-labeled grapefruit-derived EVs delivered intranasally were primarily distributed to the lungs and brain and remained stable in the brain.²⁸ Intranasal delivery of EVs offers advantages such as rapid onset, non-invasiveness, and the ability to bypass hepatic and intestinal metabolism,¹²⁴ thereby providing high bioavailability. This makes it a promising route for treating central nervous system (CNS) diseases (Table 3), including brain inflammatory diseases,¹²³ brain tumors,¹²⁵ cerebral ischemia,⁷² Parkinson's disease,¹²⁶ Alzheimer's disease,¹²⁷ and spinal cord injuries.¹²⁸

For instance, intranasally administered EVs were observed to be absorbed by microglia. EVs loaded with curcumin effectively inhibited the progression of lipopolysaccharide-induced brain inflammation and myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis. Additionally, EVs carrying a Stat3 inhibitor significantly inhibited the growth of brain tumors.¹²³ Notably, no particles larger than exosomes (30–100 nm), specifically those in the range of 500 nm to 1 μ m, were detected within the brain, suggesting that EV particle size is a critical determinant for translocation into the brain via intranasal administration. In a separate study, researchers utilized folic acid ligand-modified grapefruit-derived nanovesicles (GNVs) to target folate receptor-positive GL-26 brain tumors. miR17-loaded GNVs were rapidly transported to the brain and effectively inhibited tumor growth.¹²⁵ Selective targeting of the peri-infarct region was observed in a mouse model of cerebral ischemia 24 hours following intranasal administration of human urine-derived iPSC-derived small EVs (sEVs) or HEK293 cell-derived sEVs, as compared to minimal signals detected in other organs such as the heart, lung, liver, spleen, and kidney. Furthermore, sEVs loaded with brain-derived neurotrophic factor (BDNF) were found to upregulate neuroprotective genes, downregulate inflammatory genes post-administration, and activate BDNF/TrkB signaling pathways in ischemic stroke models. Consequently, these sEVs significantly improved functional outcomes, reduced infarct volume, promoted neurogenesis and angiogenesis, and enhanced synaptic plasticity and fiber preservation.⁷² Intranasal administration of EVs has garnered significant attention

Table 3 Typical Examples of Intranasal Delivery of EVs in Recent years

Source of EVs	Recipient Animal	Distribution of EVs	Mechanism	Therapeutic Effect	Ref
iPSC-derived MSCs	<i>SOD1^{G93A}</i> transgenic mice	24h Widely distributed in the brain and spinal cord	Inhibit neuroinflammation and overactivation of the complement and coagulation cascades	Delay the progression of amyotrophic lateral sclerosis	[129]
UC-MSCs	Contusive SCI mice	7day Kidney, spinal cord lesion area, liver, and stomach	Stabilize the BSCB via the miR-501-5p/MLCK axis	Promote functional recovery	[130]
Astrocytes	Healthy mice	4h, 24h Brain, liver, kidney	Decrease the expression of miR-106b in astrocytes	Ameliorate morphine-mediated primary ciliogenesis and prevent morphine tolerance	[131]
iPSC-induced MSCs	tMCAO mice	24h Peri-infarct region of the brain > heart, lung, liver, spleen, and kidney	Upregulation of neuroprotection-related genes and downregulation of inflammation-related genes	Alleviate the symptoms of cerebral ischemia	[72]
BM-MSCs	SCI rat	24h Spinal cord > spleen, lungs, liver, kidneys, brain, and heart	Attenuate the expression of PTEN in the injured spinal cord region	Significantly elicit functional recovery	[128]
Neural stem cells	GBM mice	48day Brain, lung	Anti-miRNA-21 and miRNA-100 sensitize GBM cells to temozolomide	Tumor regression and improved overall survival	[132]
MSCs	Healthy mice	24h Lungs > spleen > kidneys, brain, and liver	NA	NA	[13]

Abbreviations: sEVs, small extracellular vesicles; iPSC, induced pluripotent stem cell; MSCs, mesenchymal stem cells; SCI, spinal cord injury; UC-MSCs, umbilical cord derived mesenchymal stem cells; BSCB, Blood-Spinal Cord Barrier; tMCAO, transient middle cerebral artery occlusion; BM-MSCs, bone marrow derived mesenchymal stem cells; GBM, Glioblastomas; NA, not analyzed.

from researchers studying neurodegenerative diseases such as Parkinson's disease^{34,126} and Alzheimer's disease.¹²⁷ Peng et al developed self-oriented nanocarriers termed PR-EXO/PP@Cur, which integrate bone marrow MSC-derived EVs with curcumin. These PR-EXO/PP@Cur nanocarriers were engineered to enhance membrane translocation and target diseased dopaminergic neurons following intranasal administration. This approach significantly improved motor and coordination abilities in Parkinson's disease model mice by reducing α -synuclein aggregates, promoting neuronal function, and mitigating neuroinflammation.¹²⁶ Another study on Parkinson's disease demonstrated that macrophage-derived EVs accumulated more extensively in brain tissue four hours post-intranasal administration compared to intravenous injection. Intranasal delivery of catalase-loaded EVs markedly reduced microglial activation in 6-OHDA-intoxicated mice, offering robust neuroprotection, whereas catalase alone failed to reduce inflammation in Parkinson's disease mice.³⁴ Furthermore, EVs can traverse the blood-brain barrier (BBB) to reach the spinal cord region via intranasal administration. Guo et al demonstrated that intranasal delivery of MSC-EVs loaded with phosphatase and tensin homolog (PTEN) siRNA reduced PTEN expression in damaged spinal cord regions, promoted axonal growth and neovascularization, mitigated neuroinflammation and gliosis, thereby significantly enhancing functional recovery in rats with complete spinal cord injury.¹²⁸ This approach appears promising as a clinical option for promoting recovery in patients with spinal cord injuries.

In addition to central nervous system diseases, intranasal EV delivery provides a suitable route for respiratory diseases due to lung accumulation. Intranasal administration of macrophage-derived EVs loaded with paclitaxel to Lewis lung carcinoma mice demonstrated significant anti-tumor efficacy, with $97.9 \pm 2.0\%$ of the EVs colocalizing with lung metastases.¹³³

However, intranasal delivery is constrained by the mucosal layer and mucociliary clearance mechanisms, resulting in suboptimal absorption efficiency.¹²⁴ To address this limitation, integrating EVs with advanced biomaterials to prolong nasal retention time appears promising. Wang et al developed a gel system comprising a glutamine amide derivative and

benzaldehyde ligands conjugated with endothelial cell-derived EVs for intranasal administration. This hydrogel forms a viscous liquid that coats the nasal mucosa, facilitating prolonged contact between EVs and the nasal epithelium, thereby extending retention time and enhancing delivery efficiency. In a murine model of myocardial ischemia/reperfusion injury, this system exhibited superior anti-inflammatory properties and protection of endothelial function compared to EVs alone.¹³⁴ Additionally, Sukumar et al suggested that reducing respiratory rate can minimize intranasal nanoparticle accumulation in the respiratory tract when targeting brain diseases. Under deep anesthesia, a slower respiratory rate increased the retention duration of nanoparticles in the nasal mucosa and improved their absorption efficacy relative to light anesthesia.¹³⁵

Subcutaneous Injection

Subcutaneous injection, due to its operational simplicity and potential for patient self-administration, has garnered significant interest in EV delivery. However, the biodistribution of subcutaneously administered EVs exhibits variability. In early studies, Wiklander et al performed an *ex vivo* analysis of the biodistribution of DiR-labeled EVs derived from HEK293T cells 24 hours post subcutaneous injection. The findings revealed that EVs were predominantly localized in the gastrointestinal tract, with a secondary accumulation observed in the liver. Notably, the distribution of EVs in lymph nodes was not evaluated.¹⁰ Other studies have reported that a substantial proportion of EVs were delivered to lymph nodes. Royo et al observed considerable accumulation of 124I-labeled MLP29-derived EVs in both proximal and distal lymph nodes 72 hours after subcutaneous injection via the hoof.¹³⁶ Yu et al assessed the organ distribution of melanoma-derived small EVs (sEVs) following either tail vein intravenous or tail skin subcutaneous injection. Their findings indicated that subcutaneously injected sEVs consistently accumulated primarily in lymph nodes at 6, 24, 48, and 168 hours, whereas intravenously injected sEVs were mainly distributed to the liver and spleen.⁹⁹ To verify that lymphatic delivery of subcutaneously injected sEVs is a common phenomenon, the researchers examined the distribution of sEVs derived from additional cell lines, including melanoma YUMM1.7, mouse mammary carcinoma 4T1, macrophages, and lymphatic endothelial cells. Consistent results were observed across these different sources. The researchers confirmed that subcutaneously injected sEVs preferentially accumulate in lymph nodes via the lymphatic system, primarily regulated by surface membrane proteins.⁹⁹ These findings suggest that targeting the lymphatic system with subcutaneous sEV delivery is feasible, particularly for immunotherapy applications, similar to liposome-based approaches. For instance, lipid-coated magnetite micelles have been shown to efficiently deliver antigens (OVA) and TLR9 agonists (CpG) to lymph nodes via subcutaneous injection, thereby enhancing antigen-specific immune responses.¹³⁷ Furthermore, subcutaneous injection of sEVs has been widely utilized in dermatology. Subcutaneous administration of human umbilical cord blood-derived sEVs into skin wounds in mice reduced scar width and promoted angiogenesis, accelerating wound healing.¹³⁸ Notably, another study reported that mice treated with subcutaneously injected sEVs from adipose-derived MSCs exhibited slower wound healing compared to the intravenous group.¹³⁹

Nevertheless, conventional subcutaneous administration necessitates frequent drug dosing due to the limited retention duration of EVs within the body. Microneedles have attracted considerable attention for the subcutaneous delivery of EVs owing to their high penetration efficiency, minimal pain, reduced infection risk, and absence of psychological burden.¹⁴⁰ For instance, Yang et al engineered a detachable microneedle patch composed of keratin derived from hair to co-deliver human bone marrow MSC-derived EVs and the small-molecule drug UK5099. This approach demonstrated superior efficacy in promoting hair growth compared to subcutaneous injections of EVs or topical application of UK5099.¹⁴¹

Inhalation Administration

Inhalation is a widely investigated route of drug delivery due to the lungs' extensive vascular network, large surface area, and relatively permeable mucosal barrier.¹⁴² Moreover, drugs absorbed into the pulmonary circulation bypass first-pass metabolism by entering the systemic circulation directly via the pulmonary veins. Common devices for inhalation administration include nebulizers, pressurized metered-dose inhalers (pMDIs), and dry powder inhalers (DPIs). While nebulizers are cost-effective, they may compromise the integrity of lipid-based nanocarriers, leading to leakage of encapsulated molecules. In contrast, DPIs offer portability and enhanced stability of the dry powder formulation.¹⁴³ Similar to intranasal delivery, mucus in the airway lining can clear drug particles. However, inhalation administration's

noninvasive nature and ability to achieve high lung deposition make it suitable for delivering EVs for respiratory diseases.¹⁴⁴ In a recent study, Wang et al observed that human lung spheroidal cell-derived EVs (LSC-Exo) expressing ACE2 were deposited throughout the lungs at three distinct time points (2 hours, 4 hours, and 24 hours) following nebulized inhalation. These inhaled LSC-Exo bound to and neutralized SARS-CoV-2, leading to a significant reduction in lung inflammation and viral load, thereby effectively preventing SARS-CoV-2 infection.⁵⁸ Dinh et al demonstrated that nebulized inhalation of LSC-derived secretome and EVs could re-establish normal alveolar structure, and reduce collagen deposition and myofibroblast proliferation in a model of bleomycin- and silica-induced fibrosis, thus restoring lung function. In another study,¹⁴⁵ Khaled et al developed dry powders of B-cell-derived EVs loaded with miR-335 using thin-film freeze-drying technology. This formulation can down-regulate SOX4 overexpression in breast cancer and holds potential for treating primary lung cancers and lung metastases associated with SOX4 overexpression.¹⁴³

Intra-Tumor Injection

The vascular system of tumors exhibits a higher propensity for leakage compared to healthy blood vessels, which facilitates the penetration of nanoparticles into the tumor stroma and results in significant retention within tumor tissue. Several studies have demonstrated that direct injection of EVs into tumors leads to superior retention compared to intravenous administration, which is associated with rapid clearance from the circulation.⁵¹ A clinical trial involving 20 patients with end-stage extrahepatic cholangiocarcinoma and malignant biliary obstruction administered methotrexate-loaded tumor-derived EVs via percutaneous transhepatic biliary drainage directly into the tumor lumen. This intra-tumoral injection of methotrexate-loaded EVs not only directly destroyed tumor cells but also recruited a substantial number of neutrophils, thereby triggering neutrophil-mediated anti-tumor activity. Consequently, five cases experienced relief from obstruction, including one case with remission lasting up to 5 months.¹⁴⁶ Although intra-tumoral EV injection reduces toxicity to non-target tissues and enhances tumor clearance, this method is more invasive and less acceptable to patients compared to systemic administration, particularly for deep-seated tumors.¹⁴⁷

In addition, Sakurai et al observed a substantial accumulation of B16F0-derived small EVs (sEVs) in the lymph nodes following intra-tumoral injection, which was significantly higher compared to that in the liver and spleen. This suggests that lymphatic transit may be a prevalent phenomenon after intra-tumoral administration of sEVs, potentially offering a promising strategy for preventing or treating lymph node metastasis.⁹⁹

Intramuscular Injection

Intramuscular injection has been utilized as a delivery method for EVs. For instance, intramuscular administration of EVs derived from iMSCs significantly promoted microvessel formation in ischemic limbs of mice by upregulating the expression of angiogenesis-related molecules, thereby alleviating limb ischemia.⁷¹ In a follow-up study, researchers investigated the biodistribution of intramuscularly injected EVs derived from skeletal muscle 24 hours post-injection. The results indicated substantial accumulation in the gastrointestinal tract and liver, followed by the pancreas and lungs, with minimal presence in the kidneys, spleen, muscle, heart, and brain. Compared to intravenous administration at the same dose, intramuscular injection of EVs exhibited reduced distribution in the liver and spleen.¹⁴⁸

Other in-situ Injection

Intra-Articular Injection

Intra-articular local injection represents a promising approach to enhance the accumulation of EVs at lesion sites in osteoarticular diseases. Zhang et al demonstrated that intra-articular administration of EVs derived from bone marrow mesenchymal stem cells (BMSCs) promoted macrophage polarization from the M1 to M2 phenotype, thereby mitigating synovial inflammation and cartilage damage, and slowing the progression of osteoarthritis.¹⁴⁹ In a follow-up study, Jin et al corroborated these findings, showing that both BMSCs and their EVs, when administered intra-articularly, reduced cartilage destruction and subchondral bone remodeling in an osteoarthritis rat model. This was accompanied by a decrease in chondrocyte senescence and apoptosis, potentially elucidating their therapeutic mechanisms.¹⁷

Intrathecal Injection

Intrathecal injections, despite being highly invasive and associated with poor patient compliance, have been investigated

for the delivery of EVs in neurological disorders due to their capacity to enhance drug concentration in the cerebrospinal fluid. For instance, Lu et al demonstrated that intrathecal administration of EVs derived from human placental MSCs alleviated neuropathic pain in a spared nerve injury mouse model via the miR-26a-5p/Wnt5a pathway.¹⁵⁰

Transdermal Application

Transdermal administration of EVs has demonstrated considerable potential in dermatology. By bypassing first-pass metabolism, EVs can enhance bioavailability at the target site, thereby reducing dosing frequency and minimizing adverse effects. Zhang et al investigated the impact of topically applied Oil-in-Water Emulsions containing mesenchymal stem cell (MSC) exosomes on imiquimod (IMQ)-induced psoriasis in mice. Their study revealed significant inhibition of complement activation in the stratum corneum and a reduction in IL-17 release.¹⁵¹ However, EVs were predominantly confined to the stratum corneum, with limited penetration into the epidermis. Hydrogels, which are superior carriers for transdermal drug delivery, have been utilized in conjunction with EVs for topical applications. Shiekh et al developed synthetic hydrogel dressings loaded with adipose-derived stem cell EVs embedded in antioxidant polyurethane (PUAO), facilitating continuous oxygen release. This approach mitigated oxidative stress, promoted angiogenesis, enhanced collagen deposition, and accelerated re-epithelialization, leading to faster diabetic wound healing within two weeks.¹⁵²

Intrathoracic Injection

A study investigated the intrapleural administration of EVs. In healthy mice, tumor-derived EVs injected intrapleurally were predominantly distributed to the lungs, liver, and spleen, suggesting the potential of this route for delivering EVs in lung-related conditions.⁶⁶ In a mouse model of lung cancer-induced pleural effusion, intrapleurally administered EVs primarily accumulated in the lungs as well as in tumors, with prolonged retention in pleural cavity tumors compared to intravenous administration. A subsequent clinical trial demonstrated that intrapleural injection of methotrexate-loaded autologous tumor-derived EVs in patients with malignant pleural effusion effectively eliminated tumor cells and significantly reduced pleural effusion, with only grade 1 or 2 toxicity observed. This approach offers a promising new direction for symptom management in patients with advanced malignant pleural effusion.⁶⁶

Intravitreal Injection

Pathological angiogenesis is a hallmark of various vision-threatening disorders. Tian et al developed regulatory T cell-derived EVs conjugated with anti-VEGF antibodies, which significantly prolonged the intraocular retention time of anti-VEGF antibodies following intravitreal injection. This approach effectively reduced choroidal neovascularization in a laser-induced choroidal neovascularization mouse model, presenting a promising therapeutic strategy for ocular neovascularization.¹⁵³ However, Xue et al investigated the distribution of endothelial cell-derived EVs conjugated with the anti-angiogenic peptide KV11 (EXOKV11) via both intravitreal and retro-orbital injection routes. The FITC signal colocalized with retinal vessels 12 hours post-retro-orbital injection, whereas intravitreal injection did not show significant co-localization.¹⁵⁴ To enhance intraocular EV retention, Bao et al engineered degradable polymeric microcapsules loaded with bone marrow mesenchymal stem cells (MSCs), mimicking the natural release of cellular EVs. Intravitreal injection in a retinal ischemia/reperfusion injury mouse model demonstrated that microcapsules released EVs over a period of one month, while free EVs nearly disappeared by day 7 and MSC signals nearly disappeared by day 14.¹⁵⁵ Notably, intravitreal injection carries risks such as retinal detachment, retinal hemorrhage, and endophthalmitis,¹⁵⁶ whereas retro-orbital injection is less invasive and poses fewer risks.¹⁵⁷

Furthermore, localized EV delivery to the myocardium,¹⁵⁸ kidneys,¹⁵⁹ and prostate¹⁶⁰ has been investigated due to its high retention in target tissues and reduced toxicity in non-target tissues. However, given that local delivery to deep tissues is more complex and invasive compared to systemic administration, researchers tend to favor high-dose systemic delivery to achieve the desired therapeutic outcomes.

Conclusions and Future Directions

In summary, EVs play crucial roles in both physiological and pathological processes. They serve as biomarkers for disease diagnosis and prognosis, as well as functional components in disease treatment. Owing to their natural origin,

inherent stability, low toxicity, superior biocompatibility, and efficient biological membrane penetration—qualities that surpass those of liposomes and polymer particles—EVs have emerged as novel and exceptional nano-carriers for effective drug delivery. It is of paramount importance to elucidate the biodistribution of EVs within the body to enhance their therapeutic efficacy and minimize adverse effects. Genetic engineering and chemical modifications can improve organ targeting, thereby overcoming the rapid clearance of natural EVs and their high retention in tissues such as the liver.¹⁶¹ However, notable variations in biodistribution among EVs derived from different sources and administered via different routes underscore the necessity of optimizing delivery methods and selecting appropriate EV sources to improve delivery efficiency based on target tissues. As research into EVs continues to advance, investigators are increasingly focusing on more cost-effective and accessible sources, such as plant- and milk-derived EVs, which warrant further exploration of their physiological impacts. EV mimetics can mitigate large-scale production challenges to a significant extent. Moreover, synthetic vesicles derived from liposome fusion acquire enhanced controlled-release properties, thereby improving delivery efficiency.⁴² Additionally, the integration of EVs with advanced biomaterials for localized delivery represents an exciting frontier. This approach can prolong EV retention in target tissues, enhance therapeutic efficacy, and reduce the frequency of administration.

However, the *in vivo* biodistribution of EVs derived from various sources remains inadequately explored. A substantial body of research is urgently required to elucidate the distribution patterns of EVs from different origins and under varying administration methods. Moreover, multiple studies have confirmed that the distribution patterns of the same type of EVs differ between normal conditions and disease models. This inherent tropism can be partially attributed to EVs inheriting membrane components and biological activities from their parental cells. Presently, studies have adopted diverse combinations of EV sources, sizes, delivery methods, and disease models, leading to variations in the observed biodistribution of EVs. When researchers aim to enhance the therapeutic efficacy of EVs by optimizing their source and delivery method, they must consider these multiple factors to avoid overgeneralization. Given the growing body of research on the distribution patterns of EVs in the future, elucidating the distinct distribution profiles of EVs from the same source across various disease models and delivery routes holds significant guiding value for advancing the clinical application of EVs. Notably, due to the small size of EVs, precise *in vivo* tracking is complex, driving the development of various tracking methods.¹⁶² However, it has been demonstrated that genetically modifying EVs for imaging purposes can alter their natural organ distribution. For instance, the fusion of CD63 with NanoLuc luciferase has been shown to increase EV accumulation specifically in the lungs.¹⁶³ Therefore, the careful selection of an appropriate EV tracing method is crucial for ensuring the scientific validity of EV biodistribution studies.

Furthermore, given that we are still in the exploratory phase of EVs, there remain significant gaps in our understanding of their mechanisms of biogenesis, uptake, and function, as well as the heterogeneity of EVs. Ensuring the homogeneity and reproducibility of therapeutic effects necessitates controlling the source of EVs and developing standardized methods for separation and characterization to enhance exosome yield and purity.¹⁶⁴ According to the Minimal Information for Studies of Extracellular Vesicles 2023 (MISEV2023) guidelines, recognized separation techniques include differential (ultra)centrifugation, density gradients or cushions, size exclusion chromatography, fluid flow-based separation, and charge or molecular recognition-based separations. However, each of these methods has inherent limitations and may not be optimal.¹⁶⁵ Furthermore, research has indicated that varying separation and purification methodologies can influence the biodistribution of EVs.⁵⁶ Addressing these limitations or exploring novel approaches is of paramount importance. Characterization of EVs presents unique challenges due to their diverse sources, small size, and purity concerns; consequently, no single method can comprehensively characterize all EVs. Currently, numerous advanced methodologies at the single-vesicle level have been devised to address the challenge of heterogeneity, including digital single extracellular vesicle flow cytometry. However, such high-resolution analyses tend to be time-consuming.¹⁶⁵ MISEV2023 recommends employing multiple complementary techniques concurrently.² As research on EVs advances, standardized protocols for EV isolation and characterization are urgently required to facilitate clinical translation. Additionally, with the increasing selectivity of EV sources and the proliferation of clinical studies, ethical concerns regarding engineered EVs, such as those modified through genetic means, have arisen. Therefore, it is imperative to establish guidelines and implement oversight for these emerging issues.¹⁶⁶

Despite the numerous challenges that the clinical application of EVs continues to encounter, EVs undoubtedly represent a highly promising frontier in nanomedicine, providing new hope across various domains of clinical practice.

Data Sharing Statement

No data were used for the research described in the article.

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Disclosure

The authors declare no conflicts of interest.

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