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Review Paper

Exosomes From Biogenesis to Clinical Translation: Isolation Strategies, Heterogeneity, and Emerging Therapeutic Applications

Ashkan Dirbazian[1], Mohsen Chamanara[2], Mostafa Akbariqomi[3], Ali Saberian[4], Reza Heidari[5, 6*]

1. Cancer Epidemiology Research Center, AJA University of Medical Sciences, Tehran, Iran.
2. Toxicology Research Center, AJA University of Medical Sciences, Tehran, Iran.
3. Tissue Engineering and Regenerative Medicine Research Center, New Health Technologies Institute, Baqiyatallah University of Medical Sciences, Tehran, Iran.
4. Department of Food Science and Technology, ShK.C., Islamic Azad University, Shahrekord, Iran.
5. Infectious Diseases Research Center, Aja University of Medical Sciences, Tehran, Iran.
6. Medical Biotechnology Research Center, AJA University of Medical Sciences, Tehran, Iran.

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ABSTRACT

Exosomes are nanosized extracellular vesicles (EVs) (30–150 nm) secreted by virtually all cell types, acting as natural carriers of biomolecules, including proteins, lipids, and nucleic acids, to mediate intercellular communication. They participate in a wide range of physiological and pathological processes such as immune modulation, angiogenesis, tissue regeneration, tumor progression, infection propagation, and metabolic regulation. Their intrinsic biocompatibility, stability in biological fluids, and ability to cross biological barriers make them promising tools for diagnostics, targeted drug delivery, and regenerative medicine. The present study systematically reviews biological characteristics and functional roles of exosomes, emphasizing their dual behavior in health and disease. It further evaluates current and emerging methodologies for exosome isolation and purification, including conventional ultracentrifugation, size-exclusion chromatography (SEC), filtration, precipitation, and immunoaffinity approaches, as well as advanced microfluidic and hybrid multimodal systems. Particular attention is given to new commercial and integrated platforms combining tangential flow filtration (TFF) and SEC modules for good manufacturing practice (GMP)–compatible exosome production. Despite considerable progress, challenges remain regarding heterogeneity, yield optimization, and preservation of biological activity. Understanding how isolation methods influence exosome quality and functionality is essential for their safe and standardized clinical translation. This review highlights the need for integrated, high-purity, and reproducible workflows guided by the latest MISEV recommendations to unlock the full therapeutic and diagnostic potential of exosomes.

Keywords: Exosomes; Extracellular vesicles (EVs), Isolation techniques, Regenerative medicine

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*** Corresponding Author:**
Reza Heidari, Assistant Professor.
Address: *Medical Biotechnology Research Center, AJA University of Medical Sciences, Tehran, Iran.*
E-mail: *r_heidary@ajaums@ac.ir*

Highlights

- This study reviews exosome biology, molecular composition, and functional roles across physiological and pathological systems, emphasizing their diagnostic and therapeutic potential.

- This study reviews all major exosome isolation methods, from UC and chromatography to microfluidic, hybrid, and next-generation technologies

- There are emerging multimodal and high-purity workflows (e.g. TFFSEC, nanoparticle-based capture, label-free physicochemical methods) aligned with MISEV2023/2024 guidelines to support translational and clinical-grade exosome research.

Plain Language Summary

Exosomes are tiny particles released by cells that carry important biological information, helping cells communicate with each other throughout the body. Because they contain proteins, genetic material, and other molecules that reflect the condition of their parent cells, exosomes are becoming powerful tools for diagnosing diseases and delivering targeted treatments. This article investigates what exosomes are, how they form, and how they function in health and disease. It also reviews all major methods used to isolate exosomes from blood, urine, or laboratory samples, comparing their strengths and weaknesses. By summarizing both established and emerging technologies, the article helps readers understand how scientists can obtain highquality exosomes for research, regenerative medicine, and future therapeutic applications.

Introduction

Exosomes play a critical role in various biological and pathological processes [1]. Their compositions vary depending on the cell of origin; therefore, they regulate the expression of different genes. Exosomes also regulate important biological processes, including immune and inflammatory responses, pregnancy, tissue regeneration, blood coagulation, and angiogenesis. They contribute to pathological processes such as neurological disorder irregularities, cancer, infectious diseases, and cardiovascular diseases. In addition to their roles in both pathological and normal conditions, exosomes have received significant attention as a therapeutic measure in recent decades [2]. For example, exosomes are used as biomarkers for disease diagnosis, gene therapy, antigen presentation to activate the immune system (vaccines), and as vectors for the delivery of drugs and other various biological compounds [3]. The most significant therapeutic advantages of exosomes include the ability not to activate the immune system, ability to cross the blood-brain barrier (BBB), high stability in biological fluids, high specificity for binding to target cells, and the ability to engineer them for packaging various compounds [4]. The present study aimed to systematically review the biological characteristics and functional roles of exosomes, and evaluate current and emerging methodologies for exosome isolation and purification, including conventional ultracentrifugation (UC), size-exclusion chromatography (SEC), filtration, precipitation, and immunoaffinity approaches, as well as advanced microfluidic and hybrid multimodal systems.

Exosomes' features and benefits

Exosomes were discovered in 1983 by Pan and Johnstone, who reported that the release of transferrin into the extracellular space during the maturation of sheep reticulocytes was associated with the formation of various small vesicles. Exosomes are small vesicles with a topology similar to that of the plasma membrane, and are found in large numbers in a variety of, and perhaps all, eukaryotic cell fluids, including blood, urine, saliva, seminal fluid, serum, etc. Exosomes generally range in size from 30 to 150 nm, although larger sizes up to 200 nm have also been reported. Extracellular vesicle (EV) secretion in both prokaryotes and eukaryotes indicates a conserved evolutionary mechanism. EVs are typically categorized into exosomes, microvesicles (MVs), and apoptotic bodies (Figure 1). Apoptotic bodies are produced during the execution phase of apoptosis and may contain intranuclear components such as histones and DNA. Their size generally ranges from 1000 to 5000 nm. MVs, ranging from 100 to 1000 nm, are generated

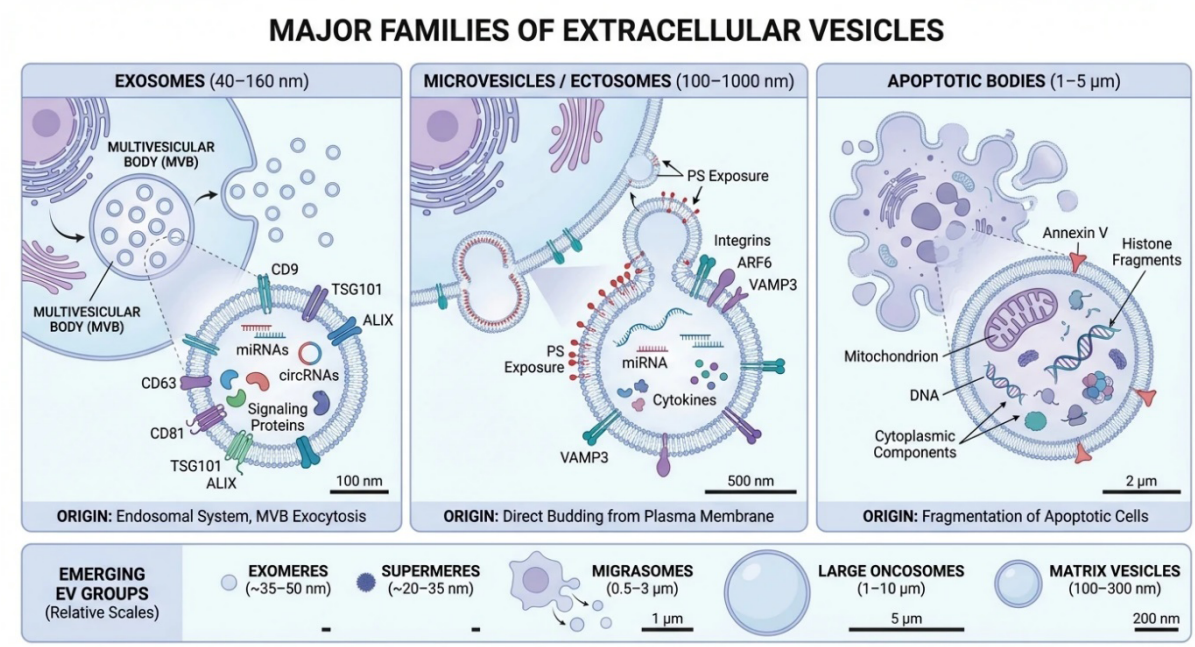

Figure 1. Classification of EVs (apoptotic bodies, microvesicles, and exosomes)

through outward budding and shedding of the plasma membrane. Exosomes, the smallest EV subtype (30–150 nm), are distinguished from MVs and apoptotic bodies by their endosomal biogenesis pathway, size profile, and characteristic surface markers [5, 6].

Exosomes' various components

Exosomes have different components depending on the cellular origin from which they are released [6]. As shown in Figure 2, they contain various proteins, including heat shock proteins (Hsp70 and Hsp90), membrane and adhesion transporters (GTPases and Annexins), and tetraspanins (CD9, CD63, CD81, and CD82). In addition to proteins, exosomes include lipids involved in cell transport, translatable messenger RNAs (mRNAs), and various small RNAs [4]. Among these small RNAs, microRNAs (miRNAs) are found in exosomes in greater proportion. Moreover, the ExoCarta database has been established for EVs, in which 8,000 proteins and 194 lipids associated with exosomes have been registered to date, and these numbers are increasing.

Exosomes' formation and secretion

Exosomes are vesicular particles formed through intracellular vesicle trafficking rather than by direct membrane shedding. Their production begins with endocytosis, during which specific regions of the plasma membrane invaginate to generate early endosomes. These early endosomes serve as dynamic sorting platforms that progressively mature into late endosomes while undergoing extensive remodeling of their protein and lipid composition [7, 8]. A key hallmark of exosome formation is the generation of intraluminal vesicles (ILVs) within the lumen of maturing endosomes. This process occurs through inward budding of the endosomal limiting membrane, resulting in the encapsulation of selected proteins, lipids, and nucleic acids into small, membrane-bound vesicles. When an endosome becomes enriched with numerous ILVs, it is referred to as a multivesicular body (MVB) [9]. The formation of ILVs within MVBs is regulated by multiple molecular machineries, including the ESCRT (endosomal sorting complex required for transport) system, tetraspanin-enriched microdomains, and ceramide-dependent pathways. These systems act in a coordinated manner to determine which molecules are incorporated into ILVs and subsequently into exosomes [10]. In the next stage, MVBs are trafficked toward the periphery of the cell, a process orchestrated by Rab family GTPases, cytoskeletal motors, and membranetethering factors. At this point, MVBs can follow one of two intracellular fates: degradation or secretion. Fusion of MVBs with lysosomes directs ILVs toward degradation, thereby participating in membrane turnover and proteostasis. Alternatively, a subset of MVBs is selectively guided toward the plasma membrane, where they undergo SNARE-mediated fusion with the cell surface. This fusion event releases the ILVs into the extracellular environment as exosomes [8, 11]. Overall, the process of exosome formation and secretion (Figure 3) constitutes a

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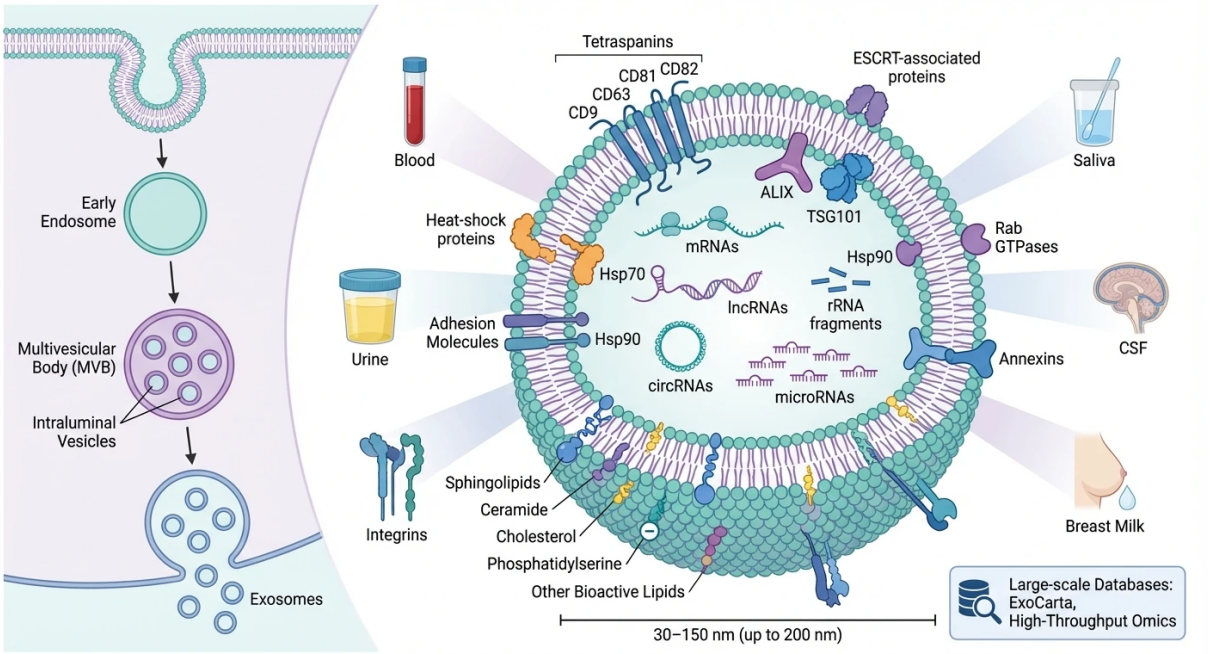

Figure 2. Schematic view of exosomes and their molecular architecture

Note: Illustrating nano-sized vesicles (30–150 nm) with a lipid bilayer, key surface markers (CD9, CD63, CD81, CD82), endosomal sorting complex required for transport system (ESCRT)-associated proteins (ALIX, TSG101), and internal cargo including mRNAs, lncRNAs, circRNAs, rRNA fragments, and abundant microRNAs. The diagram also highlights lipids (sphingolipids, ceramide, cholesterol, phosphatidylserine), Rab GTPases, annexins, and adhesion molecules, along with a simplified endosomal biogenesis pathway (early endosome → MVB → exosome release) and the presence of exosomes in multiple biological fluids.

multilayered and tightly regulated biological system that integrates endocytosis, intracellular trafficking, selective cargo loading, and exocytic release. This pathway not only defines the structural identity of exosomes but also underlies their functional roles in tissue homeostasis, immune modulation, regeneration, and disease progression.

Interaction of exosomes with target cells

Exosome–target cell interactions rely on coordinated molecular events that determine how exosomes deliver their cargo to recipient cells. This process begins with the encounter between exosomes and the plasma membrane of the target cell, where surface determinants such as tetraspanins, integrins, heat-shock proteins, and major histocompatibility complex (MHC) molecules define the specificity of recognition (Figure 4). These membrane components act as an identity code that guides which cells can interpret and respond to the exosomal signal [12].

The major interaction mechanism involves direct ligand–receptor engagement at the cell surface. In this pathway, exosomal membrane proteins bind to corresponding receptors on the recipient cell, initiating intracellular signaling without requiring internalization. Through interactions such as integrin binding or PD-L1/PD-1 engagement, exosomes can modulate adhesion, migration, immune activation, or transcriptional responses, functioning as highly stable, membrane-protected signaling units [13].

The second mechanism is direct membrane fusion, which occurs when the lipid composition and fusion machinery of both membranes are compatible. Fusion, often facilitated by fusogenic lipids or SNARE-like elements, allows the rapid release of exosomal cargo into the cytosol, granting immediate access to molecules such as microRNAs, transcription factors, or enzymes. This fast, cytosolic delivery route is particularly relevant in signaling contexts that require rapid cellular responses [14].

The third interaction mechanism involves endocytic uptake through clathrin-dependent or clathrin-independent mechanisms, including caveolin pathways, lipid rafts, phagocytosis, or macropinocytosis [15]. Internalized exosomes then enter the endosomal network, where they follow one of two major fates: transcytosis, enabling cargo transport across the cell and delivery to neighbor-

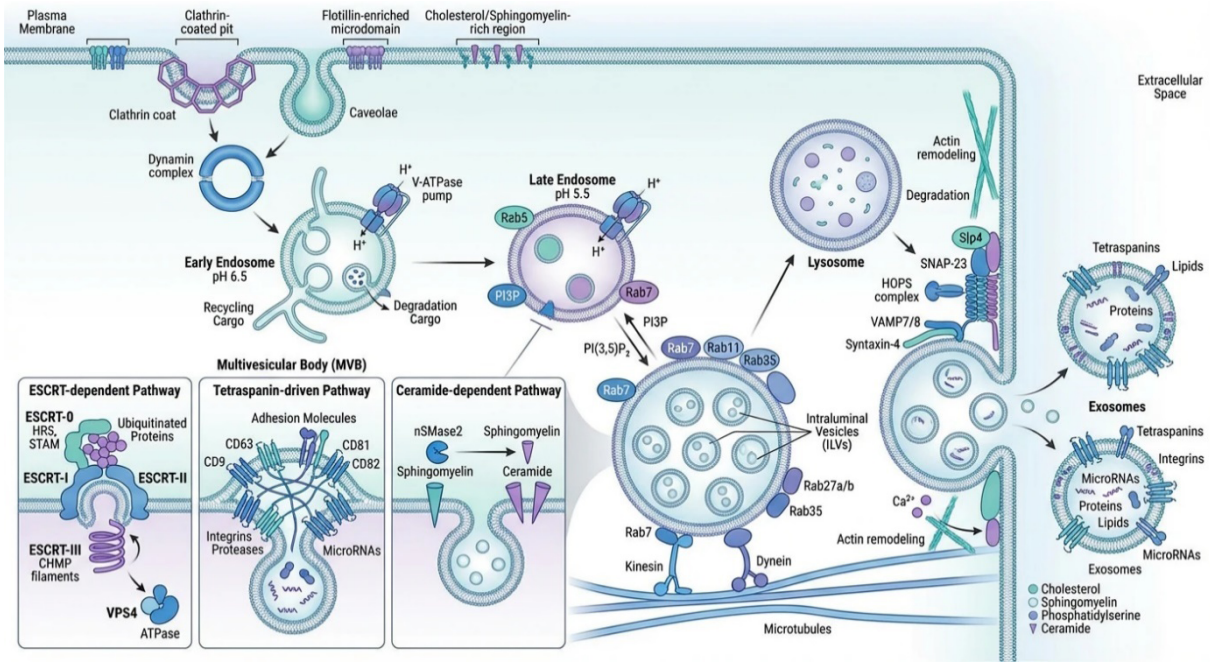

Figure 3. Schematic view of endosomal maturation, ILV biogenesis, and exosome secretion

Note: It illustrates the sequential formation of exosomes beginning with endocytic internalization at clathrin- or caveolin-enriched microdomains, followed by the conversion of early endosomes into late endosomes and the generation of ILVs via ESCRT complexes, tetraspanin microdomains, and ceramide-rich budding sites. The diagram further depicts the maturation of MVBs, their Rab-regulated trafficking along microtubules, and their fate decision between lysosomal degradation or SNARE-driven fusion with the plasma membrane, ultimately releasing exosomes into the extracellular space.

ing or distant tissues (such as endothelial transfer across the BBB) or progression toward lysosomal degradation [14]. In the latter fate, fusion of late endosomes with lysosomes results in enzymatic breakdown of exosomal components, regulating signal termination and resource recycling

Advantages of exosomes as carriers

Exosomes are one of the most promising biological carriers for drug and gene delivery owing to their unique physicochemical and immunological properties. As they are naturally released by nearly all cell types, exosomes can be produced in substantial quantities and present high stability in physiological fluids, maintaining their integrity far more efficiently than many artificial vesicles. Their nanoscale dimensions enable them to traverse biological barriers, including the BBBs, more effectively than larger lipid particles or viral vectors, making them particularly suitable for central nervous system (CNS)-directed therapeutics. In contrast to engineered viral vectors, exosomes carry minimal cytotoxicity risk and do not introduce viral components that might raise concerns about insertional mutagenesis or adaptive immune responses [16, 17].

Beyond biocompatibility, exosomes outperform many carrier systems in terms of targeting precision and cargo protection. Their surface is enriched with a diverse array of ligands, adhesion molecules, and receptor-binding proteins that allow cell-type-specific recognition, providing a level of targeting specificity that synthetic particles typically require chemical conjugation to achieve. The protective bilayer membrane of exosomes shields encapsulated molecules, whether small drugs, siRNAs, miRNAs, mRNAs, or CRISPR components, from enzymatic degradation, enhancing bioavailability compared with polymeric nanoparticles or lipid-based carriers that often face premature breakdown in circulation [4]. Moreover, exosomes can be engineered or loaded using advanced methods such as electroporation, sonication, or endogenous cargo programming, enabling versatile payload customization [18]. Their ability to be cryopreserved long-term without losing biological function further distinguishes them from liposomal and lipid nanoparticle (LNP) formulations, which often suffer from aggregation, leakage, or stability loss during storage. Overall, these features position exosomes as next-generation nanocarriers with superior safety, targeting capability, and translational potential compared with conventional delivery platforms.

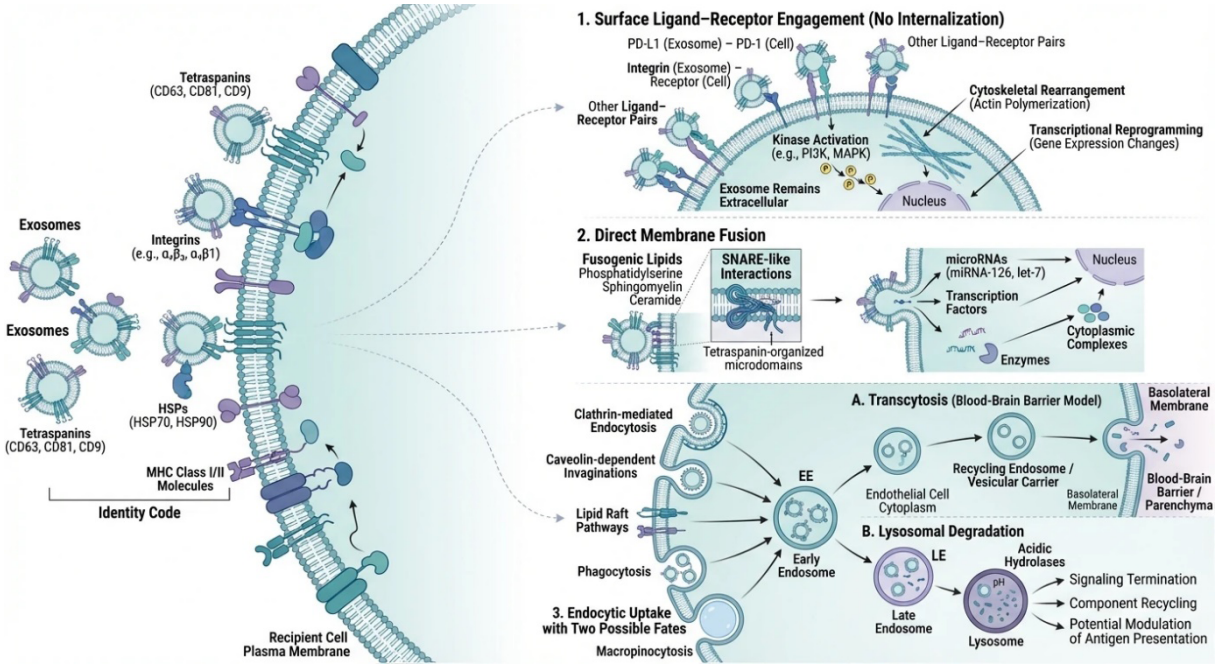

Figure 4. Schematic view of the mechanisms of exosome interaction with target cells

Abbreviations: HSP: Heat shock protein; MHC: Major histocompatibility complex; BBB: Blood–brain barrier.

Note: It illustrates the major mechanisms through which exosomes engage and deliver their molecular cargo to target cells.

Biological functions of exosomes

Exosomes participate in a wide spectrum of biological and pathological activities, functioning as specialized mediators of intercellular communication. Their molecular composition, shaped by the physiological state and identity of the donor cell, determines their specific roles within tissues. Historically, their first recognized biological function was the disposal of redundant proteins during cellular maturation, particularly in reticulocytes [19]. This expanded understanding positions exosomes not as cellular waste products, but as dynamic nanovesicles capable of orchestrating essential homeostatic and adaptive responses across multiple organ systems. Figure 5 illustrates the main biological functions of exosomes in six domains.

A major biological role of exosomes lies in immune regulation. Dendritic-cell- and B-cell–derived exosomes present MHC-I/II–bound peptides alongside co-stimulatory molecules, enabling T-cell activation and modulation of adaptive immunity. Conversely, exosomes from regulatory T cells or tumor cells carry immunosuppressive ligands such as PD-L1, FasL, and TGF-β, which attenuate cytotoxic T-cell responses and promote peripheral tolerance. These dual capacities allow exosomes to fine-tune inflammation, immune activation, and immune suppression in both healthy tissues and disease microenvironments [20, 21]. Exosomes also play central roles in the CNS, where they coordinate communication between neurons, astrocytes, oligodendrocytes, and microglia. Under stress, oligodendrocytes release exosomes enriched in protective enzymes, antioxidant molecules, and miRNAs that support axonal metabolism and prevent degeneration. Neural cell-derived exosomes contribute to synaptic remodeling by delivering activity-dependent molecules that influence dendritic spine structure and neurotransmission. In neurodegenerative diseases, however, exosomes may propagate pathological proteins such as misfolded tau or α-synuclein, facilitating disease spread across neural circuits [22, 23].

In tissue repair and regeneration, exosomes orchestrate multiple phases of healing by modulating inflammation, stimulating angiogenesis, and promoting cellular proliferation and differentiation. Mesenchymal stem cell (MSCs)–derived exosomes deliver pro-regenerative cargo, including VEGF, miR-21, and pro-survival factors, that enhance vascular growth and tissue remodeling in cardiac, renal, hepatic, and dermal injuries. In biomaterial engineering, exosome-incorporated scaffolds and hydrogels improve cellular recruitment, matrix deposition, and integration with host tissues, making exosomes a cornerstone of next-generation regenerative therapies

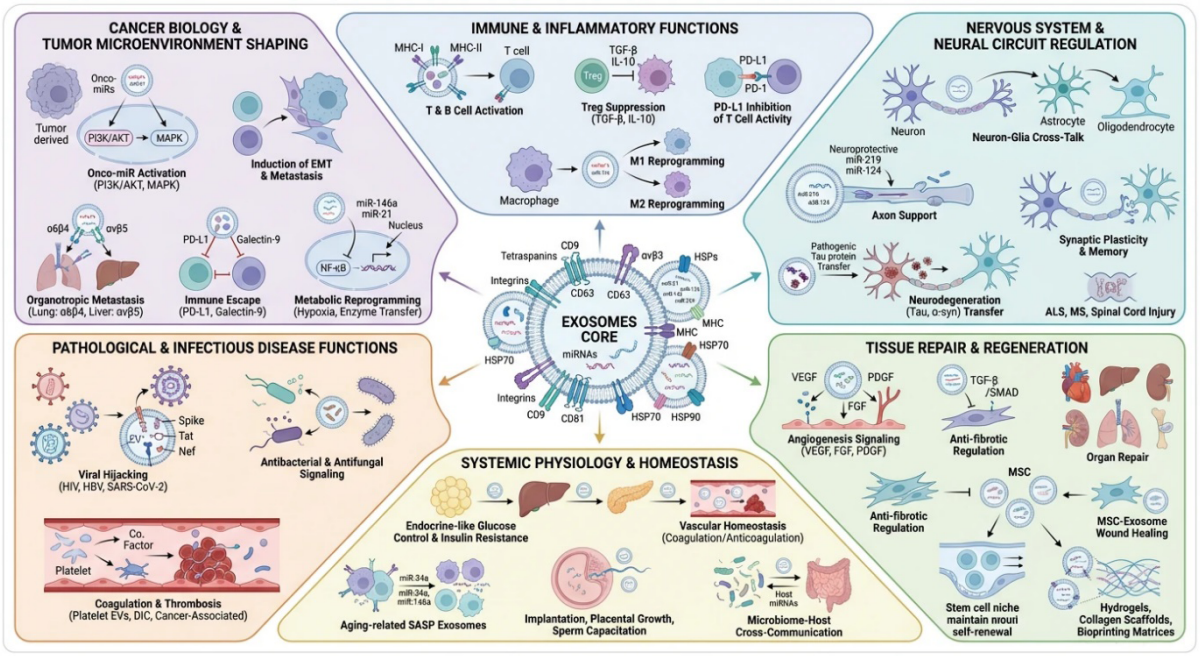

Figure 5. Schematic view of the major biological functions of exosomes

Note: This illustration depicts a central cluster of exosomes carrying characteristic surface markers (tetraspanins, integrins, MHC molecules) and regulatory cargo such as miRNAs and HSPs, surrounded by six major functional domains. Immune and inflammatory functions include antigen presentation, Tcell and Bcell activation, immunosuppression via Treg and tumor-derived exosomes, PDL1–mediated immune evasion, macrophage polarization, and modulation of NFκB–driven cytokine responses. Neural functions highlight neuron–glia communication, axonal support, neuroprotective miRNA transfer, regulation of neurogenesis and synaptic plasticity, and propagation of pathological proteins in neurodegeneration. Tissuerepair functions illustrate exosomemediated angiogenesis, antifibrotic signaling, multiorgan regeneration, MSCEVdriven wound repair, stemcell niche regulation, and interactions with regenerative biomaterials. Cancerrelated functions include delivery of oncomiRs activating PI3K/AKT and MAPK pathways, induction of EMT and metastasis, organotropic niche formation driven by integrin profiles, immunosuppressive ligand transfer, and metabolic reprogramming in hypoxic tumors. Systemic physiological roles encompass endocrinelike metabolic regulation, vascular homeostasis, reproductive communication, agingassociated SASP vesicles, and microbiome–host signaling. Pathological and infectious functions demonstrate viral hijacking of exosomal pathways, antimicrobial signaling, and contributions to coagulation and thrombosis. The integrated layout highlights directional routes from exosomes to each functional domain, emphasizing their central role in coordinating multilevel biological processes.

[24, 25]. Cancer progression is strongly influenced by exosome-mediated communication in the tumor microenvironment. Tumor-derived exosomes transfer oncogenic miRNAs, metabolic enzymes, and transcriptional regulators that support proliferation, survival, and therapy resistance. Their surface integrins determine organotropism and guide the preparation of pre-metastatic niches by reprogramming fibroblasts, endothelial cells, and immune populations. Additionally, exosomal PD-L1 and galectin family proteins suppress antitumor immune responses, allowing tumors to evade immune surveillance [26, 27].

Beyond pathological contexts, exosomes regulate essential systemic physiological processes. In metabolism, exosomes from adipocytes, hepatocytes, and pancreatic islet cells modulate insulin sensitivity, glucose homeostasis, and lipid handling. In reproduction, exosomes mediate embryo–endometrium communication, support trophoblast invasion, and influence gamete maturation. During aging, senescent cells release exosomes enriched in SASP-associated miRNAs and inflammatory mediators, transmitting aging signals to neighboring and distant tissues and contributing to organism-wide functional decline. Exosomes also play important roles in infectious diseases. Viruses such as HIV, HBV, and SARS-CoV-2 hijack the exosomal pathway to enhance immune evasion, enabling the protected transfer of viral proteins or RNAs. Conversely, immune cell-derived exosomes promote antiviral responses by transferring interferon-stimulating molecules or pathogen recognition components. In bacterial and fungal infections, exosomes help coordinate innate immunity through the delivery of antimicrobial peptides and inflammatory regulators [28, 29].

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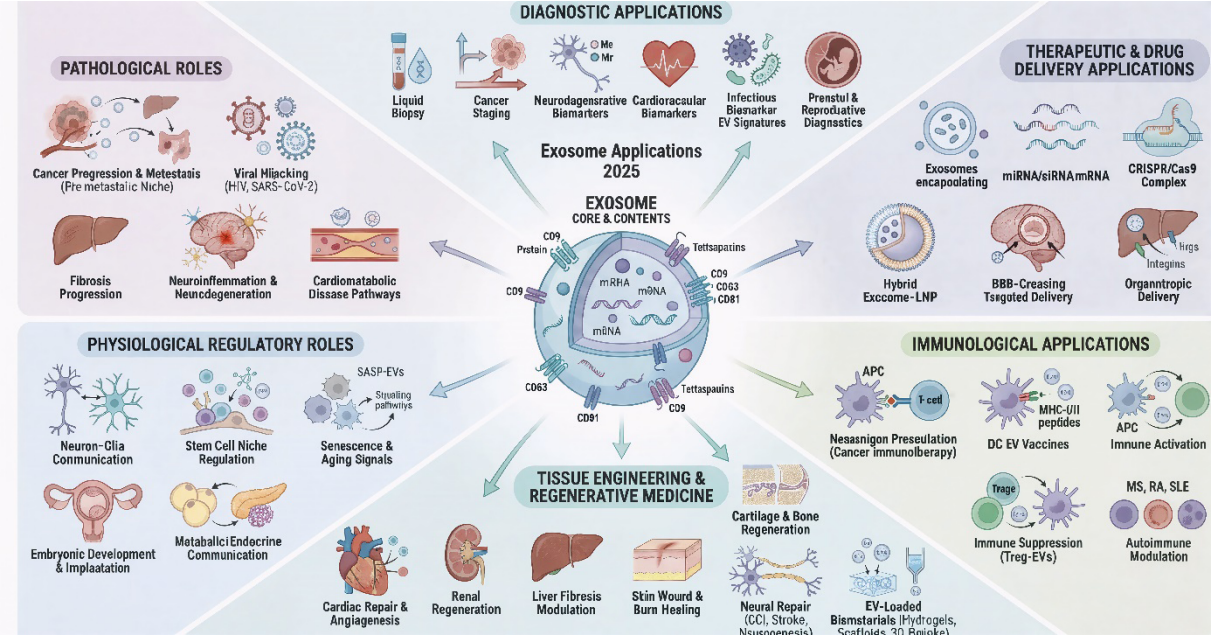

Figure 6. Schematic view of exosome applications in six domains

Note: At the center, the exosome structure and cargo (proteins, lipids, mRNA, miRNA, and tetraspanins such as CD9, CD63, and CD81) are highlighted, and their connections to diverse biological pathways and clinical applications are depicted.

Exosomes' biological applications

Exosomes are among the most versatile biological communication platforms, enabling a wide range of diagnostic, therapeutic, and regulatory applications across modern biomedicine. Unlike soluble cytokines or circulating free nucleic acids, exosomes preserve complex molecular combinations, including proteins, lipids, metabolites, and small or long RNAs, within a protective membrane, making them exceptionally informative for both real-time disease monitoring and early pathological detection. Consequently, the landscape of exosome applications now spans from bedside diagnostics to precision therapeutics and from organ repair to next-generation gene editing systems, positioning exosomes as foundational tools in the development of biologically intelligent therapies. Figure 6 illustrates exosome applications across six major domains of exosome biology: pathological roles, physiological regulatory functions, diagnostic applications, therapeutic and drug-delivery strategies, immunological functions, and tissue engineering/regenerative medicine.

Exosomes' diagnostic applications

Exosomes serve as rich, minimally invasive biomarkers across multiple clinical domains due to their stable lipid bilayers, cell-specific cargo profiles, and continuous release into biofluids. Liquid biopsy approaches increasingly utilize exosomal proteins, nucleic acids, and surface markers for early cancer detection, staging, and monitoring therapeutic resistance. In neurodegenerative disorders, neuronal cell-derived and glia-derived exosomes carry pathological species such as phosphorylated Tau, α-synuclein, and disease-specific miRNA signatures that enable pre-symptomatic detection. Cardiovascular biomarkers within EVs, including myocardialstress miRNAs and endothelial activation proteins, provide sensitive readouts of ischemia, heart failure, and vascular injury. Infectious diseases generate characteristic EV signatures that contain viral proteins, bacterial toxins, or fungal cell wall components, enabling rapid pathogen identification. Additionally, placental EVs in maternal circulation offer non-invasive windows into fetal development, chromosomal abnormalities, preeclampsia risk, and reproductive complications [30, 31].

Exosomes' therapeutic & drug delivery applications

Exosomes exhibit intrinsic therapeutic bioactivity, especially those derived from MSCs and immune cells, which modulate inflammation, enhance angiogenesis, and promote tissue repair. Their natural biocompatibility and ability to protect genetic cargo make them highly efficient nanocarriers for small molecules, chemotherapeutic agents, and nucleic acids, including miRNA, siRNA, mRNA, and antisense oligonucleotides. Engineered exo-

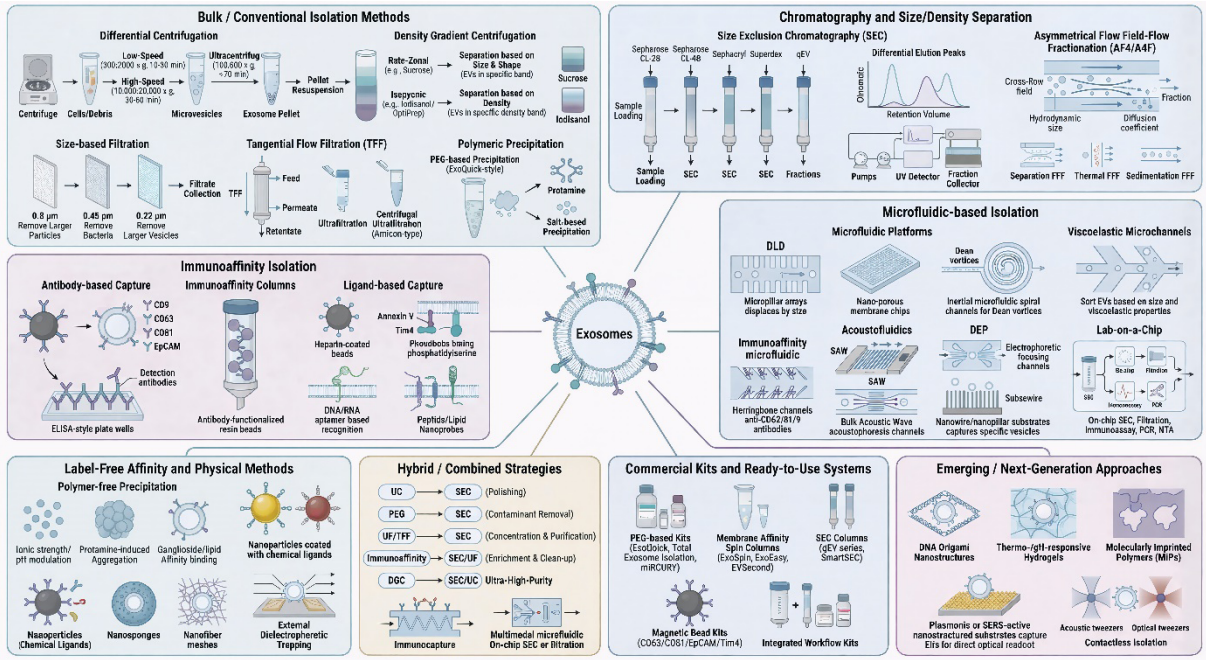

Figure 7. Schematic view of eight exosome isolation and extraction techniques, which cover the full spectrum of scalable, high-purity, and application-specific strategies for modern exosome research

Abbreviations: PEG: Polyethylene glycol; PBS: Phosphate-buffered saline; Exo: Exosome; g: Relative centrifugal force; AF4/A4F: Asymmetrical flow field-flow fractionation; MTA: Microfluidic total analysis; PCR: Polymerase chain reaction; EpCAM: Epithelial cell adhesion molecule; MIPs: Molecularly imprinted polymers; qEV: Quasi-size exclusion vesicle columns; MB: Magnetic beads; EVSecond, ExoChip, ExoSpin, ExoQuick, ExoEasy: Commercial EV Kits; DNA origami nanostructures: Self-assembled DNA-based nanostructures; SERS: Surface-enhanced raman spectroscopy.

somes further enable delivery of CRISPR/Cas systems, including exosome–LNP hybrid platforms, offering a safer and more targeted genomeediting route. Owing to their capacity to cross the BBB, exosomes enable precision delivery to neural tissues, while integrin-mediated organotropism supports targeted accumulation in specific organs such as the liver, lung, heart, or lymph nodes [32, 33].

Exosomes′ immunological applications

Exosomes act as potent immune modulators, with applications spanning activation, suppression, and antigen-specific immunotherapy. Cancer immunotherapy leverages exosomal neoantigens and dendritic cell–derived EV vaccines to prime cytotoxic Tcell responses and broaden tumor antigen recognition. EVs from antigen-presenting cells enriched in MHCI/II complexes promote robust immune activation in vaccination, infection models, and tumor settings. Conversely, regulatory T cell EVs and tolerogenic exosomes induce immunosuppression via TGF-β, IL-10, and checkpoint ligands, supporting transplant tolerance and the resolution of excessive inflammation. In autoimmune diseases (e.g. multiple sclerosis, rheumatoid arthritis, and systemic lupus erythematosus), engineered exosomes deliver tolerizing miRNAs or suppressive proteins to restore immune balance while reducing systemic toxicity [14].

Exosomes′ applications in tissue engineering/regenerative medicine

Exosomes participate actively in tissue repair by coordinating angiogenesis, modulating fibrosis, and directing lineagespecific differentiation. In cardiac injury, exosomal growth factors and cardioprotective miRNAs promote vascular regeneration and limit apoptosis. Renal and hepatic regeneration benefit from EVmediated modulation of inflammation and fibrosis, reprogramming injured parenchymal cells toward recovery. In skin wound healing and burn repair, exosomes enhance keratinocyte migration, collagen organization, and resolution of inflammatory phases. Osteogenic and chondrogenic EVs support bone and cartilage regeneration by delivering prodifferentiation signals and matrixregulating enzymes. Neural repair applications, including spinal cord injury, stroke, and remyelination, depend on exosomal transfer of neurotrophic factors and axonal growth signals. Integration of EVs into biomaterials such as hydrogels, scaffolds, and bioinks increases retention, targeted delivery, and sustained regenerative effect [34, 35].

Table 1. The EV isolation methods based on 13 performance and translational criteria

Method	Working Principle	Purity	Yield	Time	Sample Volume Capacity	Vesicle Integrity	Removal of Contaminants	Co-isolation Risk	Scalability	Cost	Suitability for Clinical-Grade Use	Key Limitations	Key Strengths
Differential centrifugation	Sedimentation by increasing g-forces	Low–moderate purity	Moderate–high yield	Long time	High volume	Moderate integrity	Weak contaminant removal	High co-isolation	High scalability	Low cost	Limited clinical suitability	Operator variability and poor reproducibility	Wide availability and foundational workflow
UC (100,000 g)	High-g small EV sedimentation	Moderate purity	High yield	Long time	High volume	Reduced integrity if high k-factor	Limited contaminant removal	High co-isolation	Moderate–high scalability	High cost	Limited clinical suitability	Protein/lipoprotein co-pelleting and aggregation	High yield and field standardization
DGC (Sucrose/Iodixanol)	Buoyant density separation	Very high purity	Moderate yield	Long time	Moderate volume	Excellent integrity	Excellent contaminant removal	Very low co-isolation	Moderate scalability	High cost	High clinical suitability	Labor intensity	Best purity and subpopulation resolution
Rate-zonal centrifugation	Sedimentation velocity	High purity	Moderate yield	Medium–long time	Moderate volume	Very good integrity	Good contaminant removal	Moderate co-isolation	Moderate scalability	High cost	Moderate clinical suitability	Gradient precision	Size-based EV subset separation
Isopycnic centrifugation	Equilibrium density separation	Very high purity	Low–moderate yield	Long time	Low–moderate volume	Excellent integrity	Excellent contaminant removal	Minimal co-isolation	Low–moderate scalability	Moderate cost	High clinical suitability	Complexity and slowness	Highest achievable purity
Sequential membrane filtration (0.8–0.22 µm)	Size exclusion	Low–moderate purity	High yield	Short time	Very high volume	Moderate integrity	Poor contaminant removal	High co-isolation	Very high scalability	Low cost	Limited clinical suitability	Fouling and deformation	Fast preprocessing
Track-etched membrane filtration	Cylindrical pore exclusion	Moderate purity	High yield	Short time	High volume	Better integrity	Moderate contaminant removal	High co-isolation	High scalability	Moderate cost	Moderate clinical suitability	Fouling	Improved size resolution
UF; Amicon-type	MWCO-based concentration	Low purity	High yield	Short time	Moderate volume	Moderate integrity	Poor removal	Very high co-isolation	Moderate scalability	Low cost	Low clinical suitability	Deformation and adsorption	Very fast concentration
TFF	Cross-flow filtration	Moderate–high purity	Very high yield	Medium time	Extremely high volume	Very good integrity	Moderate contaminant removal	Moderate co-isolation	Excellent scalability	High cost	High clinical suitability	Need for optimization	Best scalability
PEG-based precipitation	Polymer-induced aggregation	Very low purity	Very high yield	Short time	High volume	Good integrity	Very poor removal	Extremely high co-isolation	High scalability	Low cost	Not clinically acceptable	Co-precipitation of all soluble biomolecules	Fast and inexpensive
Commercial precipitation kits	Optimized PEG formulations	Low purity	Very high yield	Very short time	Low–moderate volume	Good integrity	Very poor removal	Extremely high co-isolation	Low–moderate scalability	High per-sample cost	No clinical suitability	High background noise	Standardized and easy
Other polymer/salt precipitation (Protamine-based)	Electrostatic aggregation	Low–moderate purity	High yield	Short time	Moderate volume	Good integrity	Poor removal	High co-isolation	High scalability	Low–moderate cost	Limited suitability	Non-specific aggregation	Slightly better selectivity than PEG

Exosomes′ pathological role

Exosomes are deeply implicated in disease progression, acting as vectors that amplify pathological signaling. In cancer, tumorderived EVs transport oncomiRs, proinvasive proteins, and integrin signatures that drive metastasis and generate premetastatic niches in distant organs. EVmediated communication contributes to fibrosis progression by reinforcing TGFβ and extracellularmatrix remodeling pathways across liver, kidney, lung, and cardiac tissues. Viral pathogens exploit the exosomal pathway to enhance infectivity, evade immunity, and distribute viral RNA or proteins, as observed in HIV and SARSCoV2. Neuroinflammatory and neurodegenerative conditions are exacerbated by EVs that propagate misfolded proteins, cytokines, and microglial activation signals. Cardiometabolic diseases similarly involve EVdriven endothelial injury, platelet activation, and systemic inflammatory imbalance [36].

Methods of exosome isolation

Various techniques have been developed for isolating exosomes from cell cultures or extracting them from different biological fluids, which are summarized in Table 1. Given the potential applications of exosomes in disease diagnosis and therapy, it is crucial to develop cost-effective, highly efficient isolation methods that yield exosomes with high purity and preserved biological functionality. These methods generally fall into eight categories (Figure 7) [2]:

- Bulk and conventional UC-based approaches;
- Chromatography and size/density-dependent separation techniques;
- Immunoaffinity-based isolation using antibodies, ligands, or aptamers;
- Microfluidic platforms integrating size-based, inertial, acoustic, and electrokinetic mechanisms;
- Label-free chemical and physicochemical affinity methods;
- Hybrid and multimodal workflows combining multiple principles to enhance purity and yield;
- Commercial kits and ready-to-use integrated systems;
- Emerging next-generation technologies such as DNA-origami platforms, stimuli-responsive hydrogels, molecularly imprinted polymers, and plasmonic/SERS-active or contactless tweezers-based EV capture systems

Conventional methods

Conventional isolation strategies are the conceptual and methodological backbone of EV purification and shape contemporary workflows despite the proliferation of microfluidic, chromatographic, and affinity-based platforms. These methods, rooted in sedimentation physics, buoyantdensity separation, and classical membrane-based fractionation, have been extensively optimized over the past decade, and updated protocols now emphasize higher reproducibility, reduced shear stress, and improved recovery for both research-grade and translational applications. Nonetheless, they inherently require balancing throughput, purity and operational complexity, making them as indispensable yet imperfect components of modern EV isolation pipelines [2].

Differential centrifugation and UC constitute the historical standard and remain the most widely used multistep strategy for EV enrichment. The workflow typically begins with low-speed centrifugation steps that remove intact cells and coarse debris, followed by high-speed centrifugation to deplete larger microvesicles and apoptotic bodies. Small EVs/exosomes are subsequently pelleted at 100,000×g, a step that, while efficient, also co-sediments protein aggregates, lipoproteins, and ribonucleoprotein complexes. These limitations have catalyzed the widespread use of complementary purification steps, as well as refinements such as rotor-specific optimization, controlled acceleration/deceleration profiles, and repeated wash cycles, which collectively improve yield and biochemical integrity.

Density gradient ultracentrifugation (C–DGUC) is another conventional method for achieving high-purity vesicle preparations. Gradients based on sucrose or iodixanol (OptiPrep) enable separation by buoyant density, with iodixanol now preferred due to its isoosmotic nature and reduced impact on vesicle morphology. Rate-zonal centrifugation offers precise separation by sedimentation velocity and is useful for discriminating EV subpopulations, whereas isopycnic centrifugation achieves near-equilibrium density partitioning and produces the highest-purity fractions within the canonical 1.10–1.19 g/mL range. Recent advances have focused on pre-formed and self-generated gradients, low-binding consumables, and automated fractionation systems that considerably reduce operator variability and enhance standardization across laboratories [2, 37].

Size-based filtration is a complementary and scalable type of conventional methods that remain central in preprocessing and concentration workflows. Sequential membrane filtration through 0.8 μm, 0.45 μm, and 0.22 μm poresize cutoffs efficiently removes cells, apoptotic debris, and large vesicles. Track-etched membranes, with their highly uniform cylindrical pores, offer superior size resolution and reduced deformation shear compared with conventional polymer filters. Ultrafiltration (UF) systems, whether centrifugal devices such as Amicon or pressure-driven devices, enable rapid concentration with defined molecular weight cut-offs, though care must be taken to minimize membrane fouling and vesicle loss. Tangential flow filtration (TFF), now increasingly adopted for therapeutic-grade EV bioprocessing, provides high scalability, low shear stress, and compatibility with large-volume manufacturing, making it a cornerstone of emerging good-manufacturing-practice (GMP)-oriented workflows [37].

Polymeric precipitation approaches continue to offer a rapid, lowinfrastructure option for isolating EVs from complex biofluids. Polyethylene glycol (PEG)- based precipitation reduces vesicle solubility through volume-exclusion effects, enabling recovery at low centrifugal forces. Commercial kits such as ExoQuick and Total Exosome Isolation have standardized these workflows and remain popular in exploratory, biomarker-oriented, and high-throughput studies. However, PEG and related polymers inevitably co-precipitate abundant contaminants, including lipoproteins, albumin, and soluble RNA–protein complexes, necessitating post-precipitation polishing via SEC, UF, or DGC when high purity is required. Alternative polymeric or salt-induced precipitation systems, such as protamine-based approaches, offer differential selectivity but similarly benefit from integration within multimodal workflows [37].

Chromatographic and size/density-based separation methods

Size-based and chromatographic separation techniques have become integral to next-generation EV workflows, largely because they preserve vesicle integrity while providing reproducible fractionation profiles. Unlike UC-driven protocols, which rely on high g-forces and may inadvertently cause membrane deformation or cargo loss, chromatography-based systems operate under milder physicochemical conditions. As a result, they frequently yield EV populations with improved structural preservation, narrower size distributions, and reduced contamination from high-abundance serum proteins such as albumin or lipoproteins. These advantages have made chromatographic techniques increasingly favored for both analytical and pre-clinical EV applications, especially when compatibility with downstream omics profiling or therapeutic formulation is required [38, 39].

SEC remains the most widely adopted chromatographic modality for EV purification. Classical SEC matrices, such as Sepharose CL-2B, CL-4B, Sephacryl, and Superdex resins, separate particles based on hydrodynamic radius as they traverse porous gel beads with defined pore sizes. Larger EVs elute earlier due to restricted penetration into the gel matrix, whereas soluble proteins, protein complexes, and nucleic acid–binding components enter the pores and elute later. Studies from 2022 onwards have consistently shown that CL-2B and CL-4B media provide a favorable balance between recovery and purity for exosomes in the 50–150 nm range. However, Superdex-based columns, with their tighter pore-size distributions, yield higher resolution and are better suited for discriminating small EVs from lipoproteins such as HDL [38, 40].

Commercial pre-packed SEC systems, including qEV columns and similar EV-specific cartridges, have standardized column geometry and resin packing density to increase reproducibility across laboratories. These pre-calibrated systems offer defined fraction numbers and elution profiles, reducing operator variability and supporting cross-study comparability, an issue highlighted repeatedly in MISEV 2023 recommendations. More recently, expanded-volume qEV variants have enabled the processing of larger biological inputs, such as conditioned media or biofluids, making them suitable for pre-clinical-scale isolation. Nonetheless, SEC remains limited by dilution effects and the need for subsequent concentration steps (e.g. UF or TFF) [41, 42].

Fast protein liquid chromatography (FPLC)-based SEC extends traditional SEC principles into an automated, pressure-controlled format. By integrating UV monitoring, adjustable flow rates, and programmable fraction collectors, FPLC-SEC allows precise isolation of EV-enriched fractions while minimizing sample handling artifacts. Comparative analyses of studies from 2023–2024 demonstrated that automated SEC improves reproducibility, reduces user-dependent bias, and yields more pure particle-to-protein ratios than manual gravity-driven columns. This approach is particularly advantageous for laboratories aiming to develop GMP-compliant EV workflows, as it introduces process traceability and batch-to-batch consistency.

Chromatographic separation using porous matrices and gel-filtration variants has also expanded the toolkit for EV fractionation. These matrices exploit engineered pore architectures or mixed-mode interactions to refine the elution behavior of vesicles versus soluble contaminants. Depending on resin chemistry, some matrices allow selective exclusion of large protein aggregates or partially resolve heterogeneous nanoparticle populations that overlap with small EVs. Although not yet as standardized as classical SEC, these platforms are increasingly being evaluated for tailored applications such as separation of endogenous nanoparticles, lipoproteins, or synthetic nanocarriers co-isolated with EVs [43, 44].

Asymmetric field flow fractionation (FFF [AF4 or A4F]) is one of the most advanced analytical tools for resolving EV subpopulations based on hydrodynamic size and diffusion coefficients, without requiring stationary porous media. In AF4, a laminar flow field and a perpendicular cross-flow generate differential migration velocities that spatially separate nanoparticles with nanometer-level resolution. This enables the discrimination of small and large EVs and non-vesicular nanoparticles that would otherwise be indistinguishable by SEC or UC. AF4 studies between 2021 and 2025 have demonstrated its exceptional capability to separate EVs from lipoproteins (especially HDL and LDL) and protein aggregates, making it one of the most powerful tools for high-fidelity EV analytics [45].

Other FFF modalities, including sedimentation FFF, thermal FFF, and electrical/centrifugal variants, have been increasingly explored for nanoparticle and EV separation. These techniques leverage gradients of force fields rather than physical barriers, reducing shear stress and preserving vesicle integrity. Although not yet widely adopted due to instrumentation complexity and limited throughput, they offer unparalleled resolution and are expected to play a growing role in translational EV research, particularly in contexts where precise subpopulation profiling is essential, such as biomarker discovery or mechanistic vesicle biology.

Immunoaffinity-based isolation techniques

Immunoaffinity-based isolation strategies are among the most specific and selective approaches for enriching EVs, particularly when the goal is to capture well-defined vesicle subpopulations or vesicles originating from specific cell types. These techniques exploit high-affinity interactions between surface antigens expressed on EV membranes and immobilized ligands, (most common antibodies), aptamers, peptides, and engineered binding proteins. Compared with UC or size-based methods, immunoaffinity capture offers unprecedented molecular specificity and enables targeted enrichment of clinically relevant EV subsets. However, as highlighted in several recent reports, the method's high specificity often comes at the cost of reduced total yield, potential epitope masking, and scalability challenges [46].

Antibody-coated magnetic beads are among the most widely used formats for immunoaffinity capture, primarily due to their operational simplicity and minimal equipment requirements. Magnetic beads functionalized with antibodies against classical tetraspanins (CD9, CD63, and CD81), selectively bind small EVs enriched for these exosomal markers. This approach provides highly pure fractions ideal for downstream proteomic, genomic, or single-vesicle analyses. Beyond tetraspanins, bead-based immunocapture platforms are increasingly utilizing antibodies targeting tumor-associated markers such as EpCAM, HER2, or EGFR to isolate tumor-derived exosomes from plasma or other biofluids. These targeted strategies have accelerated the development of liquid biopsy assays, enabling sensitive detection of tumor-specific EV signatures even at early disease stages.

Plate-based immunocapture methods extend the same principles to a higher-throughput format. Enzyme-linked immunosorbent assay (ELISA)-style capture systems, commonly implemented on 96-well microtiter plates, immobilize antibodies against canonical exosomal antigens or disease-specific epitopes. This configuration allows parallel processing of dozens of samples and is especially advantageous for biomarker discovery pipelines, where reproducibility and multiplexing capacity are critical. Over the past two years, microtiter platforms incorporating patterned antibody arrays or dual-epitope capture schemes have demonstrated improved analytical performance, enabling selective enrichment of functional EV subpopulations while minimizing co-isolation of non-vesicular proteins.

Chromatography-based immunoaffinity systems represent a more sophisticated and scalable implementation of antibody-ligand capture. In these formats, antibodies are covalently bound to chromatographic resins or cartridge surfaces, allowing EVs to bind under controlled flow conditions while contaminants are washed away. Such immunoaffinity columns have been increasingly integrated into FPLC-like systems, enabling automated capture, wash, and elution steps. Studies from 2022–2025 indicate that column-based immunoaffinity provides improved reproducibility, superior purity, and more controlled elution compared with bead-based methods,

although cost and resin stability remain practical limitations. These systems are gaining traction in translational research programs aiming toward GMP-compliant EV isolation.

In addition to antibody-based platforms, several emerging ligand systems have expanded the toolbox of high-specificity EV capture strategies. Heparin affinity, via heparin-coated beads or microfluidic surfaces, exploits electrostatic interactions between heparin and EV membrane components, particularly heparan sulfate–binding proteins. Although less selective than antibody capture, heparin affinity offers gentle binding and high vesicle recovery, making it suitable for labile EV subtypes. Ligands that recognize phosphatidylserine, such as Annexin V or Tim4, enable selective enrichment of EVs exposing this lipid on their outer leaflet. Recent works have shown that Tim4-based capture may preserve vesicle integrity more effectively than Annexin V, which requires calcium-dependent binding [47, 48].

Beyond protein-based ligands, nucleic acid aptamers, single-stranded DNA or RNA molecules engineered to bind specific EV surface motifs, have emerged as a powerful and highly tunable alternative. Aptamers offer several advantages: they are inexpensive to produce, exhibit high chemical stability, and can be programmatically optimized for unique EV epitopes that lack well-characterized antibodies. Similarly, peptide-based and lipid-anchored probes allow rapid, reversible, and minimally disruptive capture of EVs, including those that do not express classical exosomal markers. Lipid nanoprobes that insert into EV membranes without compromising vesicle integrity have shown particular promise for isolating EVs from complex samples while maintaining native biological activity.

Microfluidic-based techniques

Microfluidic technologies have rapidly emerged as one of the most innovative and high-precision platforms for EV isolation, driven by their capability to handle minute sample volumes, integrate multiple separation principles, and enable real-time downstream analysis. Unlike conventional bulk isolation methods, microfluidic devices exploit microscale fluid dynamics (laminar flow behavior, inertial forces, diffusion gradients, acoustics, or electromagnetic fields,) to separate EVs with high fidelity and minimal mechanical stress. The past three years have seen an acceleration in the adoption of microfluidic EV platforms for clinical diagnostics, liquid biopsy workflows, and single-vesicle analytics, largely due to their miniaturization, reproducibility, and compatibility with point-of-care systems.

Size-based microfluidic separation is a foundational strategy in this domain. Deterministic lateral displacement (DLD) chips use micro-post arrays arranged at precise angles to continuously sort nanoparticles by hydrodynamic size. Updated designs (2022–2025) with nanometer-scale gap engineering have significantly improved EV–lipoprotein separation, especially for vesicles below 100 nm. Similarly, nano-porous membrane microchips incorporate ultrathin membranes with well-defined nanopores, allowing selective translocation of small proteins or lipoproteins while retaining intact EVs. Inertial microfluidics (employing spiral microchannels or Dean-flow–inducing geometries), leverages inertial lift forces to focus and enrich EVs without physical filters. More recently, viscoelastic microfluidics using polymer-based fluids has demonstrated high-throughput separation with reduced clogging risk, making it suitable for plasma-rich clinical samples [37].

Immunoaffinity microfluidic devices combine the specificity of antibody-based capture with the efficiency of microscale mass transport. Microchannels coated with anti-CD63, anti-CD81, or anti-CD9 antibodies selectively immobilize EVs expressing these canonical markers. To improve binding kinetics under laminar flow conditions, several research groups have developed herringbone-patterned channels or mixing-enhancing microstructures that generate chaotic advection, significantly improving contact between EVs and capture ligands. These devices enable rapid purification using sample volumes as low as a few microliters and are increasingly being integrated into multiplexed biosensing platforms for cancer-derived EV detection, cytokine profiling, or tumor mutational burden analysis [49, 50].

Acoustic wave-based microfluidics introduces an elegant, label-free strategy for EV fractionation by exploiting pressure nodes generated by acoustic fields. Surface acoustic wave (SAW) devices create localized acoustic gradients across microchannels, guiding EVs toward stable equilibrium positions determined by their size, density, and compressibility. Bulk acoustic wave systems, including acoustophoresis platforms, extend this concept to higher-throughput geometries and have shown exceptional performance in separating EVs from similarly sized lipoproteins. These systems minimize shear stress and maintain vesicle integrity, making them suitable for sensitive downstream applications such as functional assays or therapeutic EV formulation [51, 52].

Table 2. The commercial EVs isolation kits based on yield, purity, workflow characteristics, and application suitability

Kit / System	Isolation Principle	Yield (Relative)	Purity (Relative)	Sample Types Supported	Advantages	Limitations	Best Use Cases
TEI reagent (Invitrogen)	PEG precipitation	Very high	Low–moderate	Serum, plasma, CSF, urine, culture media	Very high particle/protein yield; fast; low-equipment need	PEG contamination; co-precipitation of proteins; low purity; affects proteomics	RNA analysis, high-yield applications; bulk EV recovery
ExoQuick / ExoQuickULTRA (System Biosciences)	PEG-based precipitation + purification (ULTRA)	High–very high	Moderate (ULTRA: higher)	Plasma, serum, CSF, saliva, urine, ascites	High recovery; ULTRA version improves purity; userfriendly	Still lower purity vs SEC/affinity; PEG traces may remain	Diagnostics needing high yield; cfRNA/miRNA extraction
miRCURY Exosome Kit (Qiagen)	PEG-like precipitation + spin purification	High	Moderate–high	Plasma, serum, urine, CSF, saliva	Good nucleic acid recovery; validated for small RNAs	Lower purity than SEC; some co-precipitated proteins	miRNA/lncRNA profiling; functional EV-RNA studies
exoEasy / exoRNeasy (Qiagen)	Membrane affinity spin column	Moderate	High	Plasma, serum, cell culture	High purity; reproducible; proteomics-friendly; no PEG	Lower yield than PEG kits; higher costs	Proteomics, purity-sensitive assays; clinical biobanking
Norgen Plasma/Serum Exosome + RNA/DNA Kit	Silica column + proprietary buffer	Moderate–high	High	Plasma, serum, saliva	Very good nucleic acid recovery; high reproducibility	Lower total vesicle count; not ideal for functional assays	Liquid biopsy (DNA/miRNA), saliva-based EV diagnostics
iZON qEV / qEVoriginal SEC Columns	SEC	Moderate	Very high	All biofluids, cell culture, and urine	Gold standard purity; preserves integrity; no PEG; proteomics-grade	Lower particle yield; requires elution optimization	Biomarker discovery, proteomics, and functional assays that need intact vesicles
MagCapture Exosome Isolation (Wako)	Immunoaffinity magnetic beads (CD9/63/81)	Low	Very high	Plasma, serum, culture media	Very high purity; specific EV subpopulations; minimal contaminants	Very low yield; high cost; difficult elution of EVs	Purity-critical studies; surface marker analysis; rare EV subtypes
EVtrap (Affinity Capture, Tymora)	Chemical affinity magnetic capture	Low–moderate	Very high	Serum, plasma, urine, culture media	Excellent purity; highly compatible with phosphoproteomics	Lower yield; expensive; needs magnetic workflow	Phosphoproteomics, signaling pathway EV studies
PureExo (101Bio)	Dual-filtration + precipitation	Moderate–high	Moderate	Plasma, serum, CSF, urine	Better purity than PEG; simple workflow	Still co-isolates some proteins; not ideal for proteomics	General EV isolation when UC is not available
MagCapture + SEC hybrid workflows	Immunoaffinity + SEC combined	Low–moderate (but pure)	Very high	Plasma, serum	Ultra-high purity; reduces non-vesicular contaminants	Yield very low; expensive; time-consuming	Multi-omics where purity > yield
Ultracentrifugation (gold standard, non-kit)	Density / buoyant force	Moderate	Moderate–high	All sample types	Conventional standard; large volume handling; no PEG	Time-consuming; requires UC; some deformational damage	Large-volume EV precipitation; benchmark datasets
Density gradient (sucrose/Iodixanol)	Buoyant density separation	Low–Moderate	Very High	Plasma, serum, culture supernatant	Highest purity among non-affinity methods	Very long protocol; low yield; equipment-heavy	Reference-grade EV purity; viral contamination avoidance

Electrokinetic and dielectrophoretic microfluidic approaches apply electric fields to manipulate EVs based on their dielectric properties, surface charge, or electrophoretic mobility. Dielectrophoresis (DEP) chips use non-uniform electric fields to selectively trap EVs while excluding protein aggregates and other nanoparticles. DEP has been particularly effective in isolating tumor-derived exosomes with distinct dielectric signatures. Devices employing electroosmotic flow or electrophoretic focusing can concentrate EVs into narrow bands, enabling rapid sample purification prior to downstream omics or biosensing processes. Despite requiring precise electrical control, these platforms have demonstrated high sensitivity and are increasingly used in single-vesicle electrophysiology studies [53, 54].

Nanowire, nanopillar, and nanoporous microfluidic platforms introduce a structural approach to EV capture. Silicon nanowire arrays, for instance, greatly enhance surface area and enable high-density binding of EVs through antibody, aptamer, or lipid-probe functionalization. Nanoporous ferric oxide scaffolds and gold-loaded nanoporous substrates have recently gained attention as they can directly bind EVs via physicochemical interactions, providing rapid, equipment-free enrichment. These nanostructured systems offer exceptional capture efficiency and are compatible with downstream imaging, including cryo-EM and super-resolution microscopy [52, 55].

Integrated microfluidic platforms represent the next evolutionary step, combining EV isolation, detection, and molecular analysis into seamless lab-on-a-chip systems. These multifunctional chips can perform immunocapture, lysis, nucleic acid amplification, protein immunoassays, or even nanoparticle tracking analysis (NTA) within a single device. Several 2023–2025 prototypes demonstrated fully automated workflows that take raw biofluids, such as plasma, saliva, or urine, and deliver EV molecular readouts within minutes. Such platforms are poised to transform point-of-care EV diagnostics and enable scalable, standardized sample processing for multicenter clinical trials [52, 55].

Label-free affinity and physicochemical strategies

Label-free affinity and physicochemical methods exploit the intrinsic surface chemistry, dielectric behavior, and colloidal stability of EVs to achieve separation without relying on antibodies, PEG precipitation, or biological ligands. These strategies have gained renewed attention between 2023 and 2025 as researchers seek GMP-compatible, low-cost, and polymer-free platforms capable of preserving vesicle morphology and biochemical integrity. The approach aligns well with MISEV2023/2024 guidelines by minimizing exogenous contaminants and reducing harsh manipulations that may perturb EV membranes [56, 57].

Polymer-free precipitation represents an increasingly refined methodology in which EVs are induced to aggregate through controlled alterations in ionic strength, pH, or kosmotropic/chaotropic gradients. In contrast to PEG-based precipitation, whose co-precipitation of immunoglobulins, lipoproteins, and soluble proteins is well documented in the user-provided sources, these polymer-free formulations aim to selectively destabilize the hydration shell surrounding the EV membrane while maintaining structural integrity. Recent developments in biofluids research in 2023–2024 demonstrate that carefully tuned ionic shifts (e.g. NaCl, $MgCl_2$, or zwitterionic buffers) can generate higher purity fractions that outperform PEG precipitation in downstream proteomics and small RNA sequencing [57, 58].

Aggregation-inducing agents such as protamine sulfate, short-chain polycations, or engineered cationic peptides have emerged as attractive alternatives, enabling PEG-free precipitation with improved selectivity. Their mechanism relies on electrostatic crosslinking with the negatively charged phospholipid headgroups and sialylated glycans on EV membranes. The studies published between 2022 and 2024 highlighted that protamine-mediated recovery maintains vesicle size distribution and avoids the extensive non-specific co-aggregation reported for PEG, although optimization is still required to minimize binding of nucleoprotein complexes [59, 60].

Affinity to membrane lipids and gangliosides forms another major class of label-free methods. Certain amphiphilic molecules, sphingolipid-binding lectins, and ganglioside-affine synthetic ligands can selectively capture EVs enriched in lipid raft components. Ganglioside-targeted nanomaterials, particularly GM1- or GM3-affine polymers, have shown promise for isolating tumor-derived EV subsets. These approaches build directly upon earlier evidence reported in the indexed articles you provided, emphasizing that surface lipid signatures offer a unique, antibody-free avenue for EV enrichment. Notably, affinity capture via lipid-targeting ligands demonstrates high compatibility with native proteomics and metabolomics because it avoids polymeric contaminants [61, 62].

Nanoparticle-based capture has rapidly evolved beyond classical antibody-conjugated beads. Gold and magnetic nanoparticles functionalized with tailored chemical groups, such as zwitterionic coatings, hydrophobic domains, or thiol-reactive surfaces, enable the physical adsorption of EVs based on van der Waals forces, hydrophobic interactions, or dipole-induced affinity. Several articles, document early success with magnetic nanoparticle–EV complexes generated via membrane modification. These systems have advanced toward label-free formats with precisely tunable surface energies that selectively bind EV membranes while resisting abundant serum proteins. Magnetic retrieval simultaneously offers rapid, low-shear isolation that preserves vesicle ultrastructure [63].

An emerging frontier involves exosome-binding nanosponges and nanofiber scaffolds, which present high-surface-area architectures capable of adsorbing EVs via multi-valent physical interactions. Electrospun nanofibers, polymeric sponges, and metal organic framework (MOF)-based crystalline sorbents have demonstrated exceptional capture efficiency in plasma and urine, providing a scalable alternative to microfluidic systems. Their label-free nature is particularly attractive for bioprocessing applications, where these materials enable continuous-flow EV harvesting in cell culture systems without introducing chemical precipitants [64].

Finally, DEP trapping, performed outside conventional microfluidic chip formats, utilizes the inherent dielectric properties of EVs to separate them from proteins, lipoproteins, and cell debris. Stand-alone DEP platforms operating in open systems or macro-scale electrode arrays can selectively trap vesicles based on membrane capacitance and polarizability, metrics increasingly recognized as biophysical biomarkers of EV subtypes. DEP has undergone substantial refinement, with frequency-tuned electrodes enabling subtype-selective capture (e.g. small EVs versus microvesicles) without relying on biochemical ligands. Crucially, since these methods impose minimal shear and avoid polymer contamination, they have become attractive candidates for GMP-oriented EV purification workflows [65, 66].

Hybrid and multimodal strategies

Hybrid and multimodal EV isolation strategies integrate complementary physical, chemical, and affinity-based mechanisms to overcome the limitations intrinsic to individual methods. With increasing translational demands and the rapidly evolving standards set by MISEV 2023/2024 guidelines, combined workflows are becoming central to obtaining EV populations with both high purity and high recovery while maintaining structural integrity. Recent studies emphasize that no single method can simultaneously optimize purity, yield, and subtype selectivity; therefore, synergistic strategies, particularly those pairing bulk clarification with fine-resolution polishing, have gained broad acceptance in clinical and proteomics-oriented pipelines.

One of the most widely implemented hybrid approaches is UC combined with SEC. The UC method provides bulk concentration and efficient removal of large contaminants, apoptotic bodies, and cell debris. However, as documented in the supplied literature, UC alone may co-pellet proteins, lipoproteins, and similarly sized microvesicles. SEC, when used as a polishing step, effectively removes these co-isolated impurities and alleviates shear-related vesicle deformation observed in high-speed UC workflows. As a result, the UC-SEC protocol has become particularly valuable for preparing EVs for proteomic profiling, functional assays, and differential ultracentrifugation pipelines reported in recent comparative evaluations [37].

PEG precipitation combined with SEC offers another practical hybrid workflow, especially in settings where rapid pre-concentration is required. PEG provides high recovery but low purity due to co-precipitation of proteins, immunoglobulins, and non-vesicular particles, an issue clearly highlighted in user-provided sources. SEC after PEG precipitation selectively resolves PEG-bound protein aggregates and soluble contaminants, yielding a substantially pure EV population. This hybrid is increasingly adopted for biobanks, biofluid processing, and initial screening studies where sample volume is limited, though additional washing steps are often recommended to minimize polymer carryover [37].

The UF/TFF combined with SEC has emerged as a leading scalable strategy for GMP-oriented workflows. UF/TFF enables continuous, low-shear concentration across large volumes while preserving EV morphology and minimizing mechanical stress. SEC subsequently separates concentrated EVs from proteins and lipoproteins with high reproducibility. UF/TFF-SEC pipelines have been shown to deliver high yield and purity simultaneously, outperforming single-step UF or UC alone for cell culture supernatants, plasma, and conditioned media [37].

Affinity-based hybrid methods, such as immunoaffinity followed by SEC or UF, allow the selective enrichment of EV subpopulations (e.g. tetraspanin-positive EVs, tumor-derived EVs), while still ensuring removal

of unbound antibodies, proteins, and vesicle-depleted fractions. Immunocapture provides subtype specificity, whereas SEC or UF provides polishing and buffer exchange. These strategies are increasingly used for biomarker discovery, EV-mediated liquid biopsy assays, and profiling of rare EV subtypes, where purity and analytical clarity are essential [37].

For high-stringency applications such as proteomics, metabolomics, and small RNA sequencing, density-gradient centrifugation (DGC) combined with SEC or UC delivers the highest purity among existing platforms. DGC resolves vesicles by buoyant density, significantly reducing lipoprotein and protein contamination. Subsequent SEC or UC enables final concentration and buffer standardization. The literature review and 2024–2025 workflow descriptions highlight these DGC-based hybrids as the gold standard for research requiring uncompromised sample integrity [37].

Finally, multimodal microfluidic platforms integrating immunocapture, on-chip SEC, dielectrophoretic trapping, or nanofiltration represent the next generation of hybrid EV isolation technologies. These devices combine multiple separation modalities within a unified, miniaturized platform, enabling precise subtype capture, on-chip purification, and rapid processing of low-volume clinical samples. By merging chemical affinity, size-based fractionation, and physical forces, these systems provide exceptional control over purity and selectivity while remaining compatible with downstream omics analyses and high-throughput diagnostic assays.

Commercial kits and ready-to-use systems

Commercial EV isolation kits have become a major methodological category, despite being mechanistically rooted in traditional precipitation, size-exclusion, filtration, and affinity-capture methods. Their appeal lies in high standardization, reproducibility across operators, and ease of implementation in research workflows where sample availability is limited or rapid processing is essential. Previous studies have highlighted that these kits often trade quantitative recovery for consistency, while newer generations attempt to reduce polymer contaminants and improve purity for omics-grade applications (Table 2).

PEG-based precipitation kits remain among the most widely used commercial systems worldwide. Platforms such as ExoQuick, total exosome isolation (TEI), and miRCURY exosome isolation (Qiagen) rely on PEG-induced volume exclusion to aggregate EVs at low centrifugal force. The PEG-focused review studies have provided details of the molecular mechanisms, hydration shell collapse, kosmotropic effects affected by NaCl concentration, and steric depletion forces, that underlie this process. Although PEG precipitation consistently produces high yields, multiple studies show co-precipitation of non-vesicular proteins and occasional PEG carryover, which can interfere with mass spectrometry or functional assays. Contemporary PEG kits attempt to mitigate this by optimizing PEG molecular weight, tailoring ionic strength, and implementing downstream purification steps (e.g. post-precipitation UC or SEC polishing) [37].

Membrane-affinity spin columns are another major commercial kits that relies on selective adsorption of EV membranes onto polymeric or lipid-affine surfaces. Products such as ExoSpin, ExoEasy, or EVSecond incorporate either charge-based, hydrophobic, or lipid raft/ganglioside-responsive chemistries. Several studies show that these kits produce EVs with higher structural integrity and lower mechanical stress than UC. However, their binding capacity can be limited for viscous samples (e.g. plasma), and some systems may preferentially enrich certain EV subsets depending on their membrane composition. These methods excel when the sample volume is small, and rapid column-based purification is required [37].

Pre-packed SEC columns, particularly the qEV series from Izon and next-generation formats such as SmartSEC, have become essential tools for obtaining high-purity EV fractions with excellent reproducibility. In SEC-based commercial kits, qEV isolation generates a narrower size distribution and minimal non-vesicular contamination compared to PEG-based kits. The advantages include gentle elution that preserves EV morphology, compatibility with buffer exchange, and strong performance in downstream proteomics and RNA-seq. However, the SEC alone does not concentrate samples, often necessitating pre- or post-processing steps such as UF or precipitation [67].

Magnetic bead–based EV isolation kits represent a rapidly expanding category of immunoaffinity-based commercial systems. These platforms use magnetic microbeads coated with antibodies against canonical EV markers (CD63, CD81, CD9), tumor-associated antigens (EpCAM), phosphatidylserine-binding proteins (Tim4), or chemospecific ligands. The specificity of these kits enables highly selective isolation of defined EV subpopulations, an advantage emphasized, making them exceptionally valuable for liquid biopsy applications or

single-population analysis. Nonetheless, they generally yield lower total particle counts and can bias the sample toward tetraspanin-positive vesicles, which must be considered in biomarker or functional interpretation [37].

A newer generation of commercial kits integrates multimodal workflows into unified, ready-to-use systems. These platforms combine concentration (UF/TFF), purification (SEC), and optional affinity modules within a single kit, enabling scalable and reproducible EV preparation from both culture media and biofluids. Many studies have adopted TFF-SEC systems for GMP-compatible EV production because they deliver a balance of high yield, high purity, and low shear stress [37]. The 2025 literature increasingly benchmarks these integrated workflows as superior to single-step commercial kits, particularly for omics, therapeutic EV loading, and preclinical delivery studies.

Conclusion

Although a variety of methods for isolating and purifying exosomes have been developed, some shortcomings remain that cannot meet all needs. The combination of different isolation methods may be better than the separation effect of a single method. Therefore, to improve separation efficiency and enrichment and obtain ideal exosomes, many research teams have begun combining multiple methods to isolate and purify exosomes, thereby increasing yield and purity.

Currently, the greatest challenge in exosome therapy is resolving the heterogeneity of secreted exosomes. Exosome-based therapy requires a better understanding of their biogenesis, composition, and heterogeneity. Although it is thought that exosomes isolated from similar cells have the same composition, results reveal that these exosomes may have different molecular compositions and targeting moieties. This heterogeneity of exosomes adds further complexity to exosome design, dosage standardization, and delivery in clinical applications. Therefore, an in-depth study of exosome heterogeneity is crucial, not only for identifying suitable subpopulations for specific therapeutic purposes but also for preventing heterogeneity-related side effects. Various isolation methods affect the purity and physicochemical properties of exosomes, so selecting one or more exosome isolation methods and optimizing the isolation procedure can better preserve the biological activity of exosomes and reduce associated side effects. The composition and information carried by exosomes are closely related to their parent cells; therefore, when using exosomes for treatment, we can select exosomes that are more optimal for therapy based on the characteristics of exosomes isolated from different cell types.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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