Clin. Lab. 2026;72:XXX-XXX

REVIEW ARTICLE

Research Progress on Extracellular Exosome Isolation and Enrichment Techniques

Lin Xu, Yu Tian

Clinical Laboratory, The Affiliated Hospital of Shaoxing University, Shaoxing, China

ABSTRACT

Background: **Exosomes are nanoscale vesicles that encapsulate a broad spectrum of bioactive molecules, including proteins, lipids, mRNA, miRNA, lncRNA, and DNA, and are present in various biological fluids, such as blood, saliva, and urine, where they serve as key mediators in intercellular communication and play critical roles in a wide range of physiological and pathological processes. This review aims to provide a comprehensive overview of current techniques for exosome isolation and enrichment, along with a detailed comparative analysis of their performance characteristics. Methods: Studies concerning exosome isolation and enrichment were systematically reviewed and organized based on methodological approaches, with a particular focus on the principles, procedures, and comparative features of each technique.**

Results: **The principal isolation strategies comprise ultracentrifugation, polymer-based precipitation, filtration, size exclusion chromatography, immunoaffinity capture, and microfluidics-based techniques. Exosome enrichment approaches can be broadly categorized into physical methods (centrifugation, precipitation, filtration, and size exclusion chromatography) and biological methods (immunomagnetic bead–based technique).**

Conclusions: **Exosomes hold considerable potential in the diagnosis, treatment, and prognostic evaluation of clinical diseases. Efficient isolation and enrichment are prerequisites for downstream analyses and applications. Advances in these methodologies are therefore critical for translating exosome research into effective diagnostic and therapeutic strategies.**

(Clin. Lab. 2026;72:xx-xx. DOI: 10.7754/Clin.Lab.2025.250756)

Correspondence:
Yu Tian
Clinical Laboratory
The Affiliated Hospital of Shaoxing University
Shaoxing, 312000
China
Email: tianyushaoxing@outlook.com

Review Article accepted September 4, 2025

KEYWORDS

Exosome, isolation, enrichment, techniques, research progress

INTRODUCTION

Cells release nanoscale particles with a lipid bilayer structure that carry diverse signaling molecules through various mechanisms, thereby transmitting biological information to recipient cells and facilitating intercellular communication. These particles are collectively referred to as extracellular vesicles (EVs) [1]. Exosomes, a subtype of EVs measuring 30 to 200 nm in diameter, are generated within multivesicular bodies (MVBs) in the cytoplasm and are secreted into the extracellular milieu upon fusion of MVBs with the plasma membrane, contributing to communication not only between cells but

also across organs [1,2]. Due to their critical role in signaling, exosomes are involved in a range of physiological and pathological processes, making them key players in disease progression. For example, in pancreatic ductal adenocarcinoma, exosomes derived from cancer cells contain the CD44v6/C1QBP complex, which promotes metastasis by activating the Wnt/β-catenin signaling pathway [3,4]. This illustrates how exosomes can drive disease progression, underscoring their potential as both biomarkers and therapeutic targets.

Exosomes serve vital physiological functions, such as maintaining cellular homeostasis and facilitating the clearance of cellular debris. They achieve this by transporting bioactive molecules, including proteins, lipids, mRNA, miRNA, lncRNA, and DNA [2]. An example of their beneficial role is seen in exosomes derived from adipose mesenchymal stem cells, which transfer mitochondrial components to alveolar macrophages. This transfer enhances mitochondrial integrity and oxidative phosphorylation, ultimately preserving macrophage homeostasis and alleviating pulmonary inflammation [5]. Similarly, in neurological diseases, such as Alzheimer's, exosomes derived from human brain microvascular endothelial cells contain P-glycoprotein, which captures amyloid-β peptides and removes them from the brain, mitigating cognitive impairments [6]. These examples highlight the diverse roles of exosomes in maintaining tissue homeostasis and their potential for treating complex diseases. Notably, exosomes are secreted by all mammalian cells, except mature red blood cells [7]. This widespread secretion capacity makes exosomes versatile and applicable across various medical disciplines. Exosomes derived from stem cells - such as embryonic stem cells, induced pluripotent stem cells, hematopoietic stem cells, mesenchymal stem cells, neural stem cells, and endothelial stem cells - have demonstrated therapeutic potential in treating conditions across multiple specialties, including orthopedics, neurosurgery, plastic surgery, general surgery, cardiothoracic surgery, urology, head and neck surgery, ophthalmology, and obstetrics and gynecology [8]. Their natural ability to serve as nanocarriers also makes them promising candidates for drug delivery systems and therapeutic applications [9]. In addition, exosomes can be utilized as biomarkers for disease diagnosis. For instance, exosomal circ_0005540 has shown promise as a potential marker for atherosclerosis [10]. Currently, exosomes are of significant value in the development of novel diagnostic and therapeutic strategies. The efficient and specific isolation and enrichment of exosomes have become critical prerequisites and major bottlenecks for advancing research and application in this field. Exosomes are characterized by their small size and low abundance, exhibiting considerable heterogeneity in terms of size, composition, function, and origin. Additionally, they exist in complex biological fluids that contain various interfering substances, making their efficient isolation a considerable challenge. Consequently, there has been increasing attention from researchers worldwide on the isolation and enrichment of exosomes in recent years. Xu et al. [11] and Zhang et al. [12] have summarized exosome isolation and enrichment techniques from different perspectives, yet new methods continue to emerge. Therefore, this study aims to provide a comprehensive summary of both traditional and emerging techniques for exosome isolation and enrichment, with the goal of offering a theoretical foundation for methodological advancements in exosome research and its applications.

Significance of the isolation and enrichment

Effective isolation and enrichment of exosomes are pivotal to advancing research and clinical applications in this field. Exosomes, with their small size, low density (1.1 - 1.19 g/mL), and negative surface charge, share similar physical properties with other components in biological fluids, such as cellular debris, soluble proteins, and lipoproteins [13,14]. This overlap in characteristics often leads to challenges in detection, including false positives. For example, during the centrifugation and staining of lymphoblastoid B-cell exosome samples, PKH26 nanoparticles - though not exosomes – frequently form, resembling PKH26-labeled exosomes in terms of size, surface area, and fluorescence intensity. This results in reduced exosome recovery rates and false-positive signals in staining assays [15]. These challenges highlight the critical need for efficient isolation techniques to eliminate impurities, thus ensuring accurate analysis of exosome function and composition. Once isolated, the goal of exosome enrichment is to increase their concentration or purity. This becomes especially important when sample concentrations fall below detection limits or when exosomes fail to generate detectable signals. A notable example of effective enrichment is a strategy developed for plasma exosomes, which demonstrated the ability to enhance detectable protein species and peptides beyond the dynamic range of liquid chromatography mass spectrometry in unfractionated plasma. This advancement provides a more robust tool for plasma proteomics analysis [16]. Furthermore, in cancer diagnostics, exosome-based liquid biopsies can benefit from enriching exosomes with tissue-specific or cancer-specific markers, significantly improving both the sensitivity and specificity of diagnostic tests [17]. In the case of large-volume samples, separate enrichment steps are necessary to ensure the purity of the exosome fraction, further reinforcing the importance of enrichment in maintaining the accuracy and reliability of exosome-based research.

Exosomes, derived from different biological fluids, exhibit distinct membrane compositions depending on their cellular origin. These differences can influence both the purity and yield of isolated exosomes. As a result, high-quality isolation methods, coupled with robust compositional analysis, are essential to fully harness the diagnostic and therapeutic potential of exosomes. The development of such techniques is not only vital for advancing basic biomedical research but also

for translating exosome-based applications into clinical practice.

Isolation techniques

Ultracentrifugation (UC)

In 1987, Johnstone et al. [18] pioneered the isolation of extracellular vesicles (EVs) by culturing sheep reticulocytes *in vitro* and subjecting them to centrifugation at 100,000 x g. This process successfully isolated exosomes, which are EVs with diameters smaller than 50 nm. Ultracentrifugation (UC) has since become a foundational and widely adopted technique for exosome isolation in extracellular vesicle research [13]. UC relies on differences in sedimentation coefficients between exosomes and other components in biological fluids, using high-speed centrifugal force to separate exosomes from contaminants. Among the various UC-based methods, differential ultracentrifugation (DC) is the most commonly used approach [19,20].

The DC process consists of a series of centrifugation steps aimed at sequentially removing larger components and isolating exosomes. Initially, samples are centrifuged at 300 x g and 2,000 x g for 10 minutes each to eliminate dead cells, large apoptotic debris, and larger vesicles. Next, centrifugation at 10,000 X g for 30 minutes removes cellular debris. Finally, a high-speed centrifugation at 100,000 x g for 70 minutes pellets exosomes along with residual impurities. The pellet is then washed in phosphate-buffered saline (PBS) and centrifuged again at 100,000 x g for 70 minutes to purify the exosomes [20,21]. Despite its widespread use, DC is time-intensive and can result in contamination by medium proteins due to the prolonged exposure of exosomes to the culture medium. This typically leads to low yields, often only 5 - 25% of the initial MHC class II concentration in the starting exosome population [22]. Moreover, the high centrifugal forces in UC may disrupt exosome structures, further compromising their purity [23]. As a result, additional enrichment steps are frequently required, increasing the complexity of the extraction process. To address some of these challenges, density gradient ultracentrifugation has been developed as an alternative UC-based method [19]. This technique uses inert centrifugal media, such as sucrose or iodixanol, to create density gradients that enable exosomes to migrate to specific density zones during centrifugation, thereby facilitating their separation from impurities [24]. For example, sucrose gradient centrifugation has been used to isolate ginseng-derived exosomes, which have been shown to inhibit osteoclast differentiation and exhibit anti-osteoporotic effects in a lipopolysaccharide-induced bone resorption mouse model [25]. However, density gradient centrifugation also presents limitations, such as low throughput, long processing times, and the need for skilled personnel to perform the procedure correctly [24]. Although UC-based techniques like DC and density gradient centrifugation are essential for exosome research, they are not without their drawbacks. These challenges highlight the need for continuous optimization and refinement of UC methods to enhance their efficiency, throughput, and reliability, ensuring that exosome isolation remains an accessible and reproducible technique for both basic research and clinical applications.

Polymer-based precipitation (PBP)

Polymer precipitation (PBP) exploits hydrophilic interactions between hydrophilic polymers, such as polyethylene glycol (PEG), and exosomes. These polymers create a hydrophobic microenvironment around the exosomes, reducing their solubility in the surrounding medium. As a result, exosomes aggregate and can be isolated by low-speed centrifugation, which induces sedimentation. PBP offers several advantages over traditional exosome isolation methods. It is cost-effective, significantly faster, with separation speeds approximately six times greater than UC, and yields exosome concentrations that are roughly 2.5 times higher per milliliter compared to UC [7,26,27]. These efficiencies have contributed to the widespread use of PBP-based kits, such as ExoQuick, in clinical applications.

While PBP demonstrates higher exosome recovery than UC, it is somewhat less efficient than ultrafiltration (UF). Nevertheless, one of the key strengths of PBP is its ability to minimize protein contamination, leading to a better particle-to-protein ratio. This enhancement not only improves the purity of the exosome preparation but also helps to preserve the functional integrity of the isolated exosomes, making them more suitable for downstream applications [28,29]. Furthermore, PBP has been optimized for targeted exosome extraction. For example, a modified precipitation method for isolating urinary exosomes incorporates an additional step to remove Tamm-Horsfall protein, which is often co-precipitated with exosomes. This step improves both the efficiency of separation and the yield of exosome particles, making the method particularly well-suited for subsequent RNA analysis [30].

Overall, while PBP may not be as efficient as UF in some cases, its speed, cost-effectiveness, and ability to preserve exosome activity make it an attractive method for isolating exosomes in both research and clinical settings. Its adaptability for targeted extraction further enhances its versatility, offering valuable solutions for various analytical needs.

Filtration methods

Filtration methods provide an efficient approach for purifying fluids and isolating exosomes, offering significant advantages over traditional techniques such as ultracentrifugation (UC) and polymer precipitation (PBP). These methods significantly enhance exosome purity - by up to 3 - 6 times - while also achieving higher protein yields at a reduced cost [29,31]. Common filtration techniques employed in clinical and research settings include ultrafiltration (UF) and tangential flow filtration (TFF).

UF is a simple and reagent-free method that purifies exosomes directly from culture supernatants through the use of filter membranes with specific pore sizes or mo-

lecular weight cutoffs [32]. This allows for the removal of impurities without the need for ultracentrifugation [33]. However, UF typically relies on dead-end filtration (DEF), where the fluid flows in the same direction as the filtration process. As a result, solids quickly accumulate on the membrane surface, forming a filter cake that reduces permeability, eventually leading to membrane clogging and decreased separation efficiency [33, 34]. This inherent limitation of UF can hinder its efficiency, especially for large volumes.

In contrast, TFF overcomes many of these limitations by employing cross-flow filtration principles [35]. Unlike DEF, TFF directs fluid flow at an angle to the membrane surface, significantly slowing the formation of a filter cake. This design not only prolongs the lifespan of the membrane but also improves separation efficiency. Studies have demonstrated that TFF outperforms UC in terms of exosome yield, making it a more suitable method for large-scale applications where both quantity and quality of exosomes are critical [35].

A further advancement in filtration technology is the EXODUS (Ultrafast Isolation System), which specifically addresses the challenge of membrane clogging. EXODUS utilizes negative pressure oscillation and dual-coupled resonators to generate high-frequency reverse vibrations. These vibrations prevent the accumulation of particles on the membrane, ensuring continuous filtration and improving overall separation efficiency by up to 15 times compared to DEF-based methods [36]. Clinically, EXODUS has been successfully applied to isolate and enrich high-purity circulating exosomes from plasma in patients with esophageal squamous cell carcinoma, demonstrating its potential to revolutionize exosome-based liquid biopsies in cancer detection [37].

In summary, filtration methods - such as UF, TFF, and EXODUS - represent significant advancements in exosome isolation. They offer improvements in purity, yield, and efficiency compared to traditional methods, overcoming many of the limitations associated with older techniques. As these methods continue to evolve, they are poised to play a central role in both clinical applications and large-scale research, providing more efficient and reliable tools for exosome-based diagnostics and therapeutics.

Size exclusion chromatography (SEC)

SEC is a well-established technique for separating macromolecules based on their size or hydrodynamic volume [13]. It utilizes a biological fluid as the mobile phase and porous gel filtration polymers - such as Sepharose CL-2B, CL-4B, CL-6B, or Sephacryl S-200 HR, S-300 HR - as the stationary phase [38,39]. SEC is particularly advantageous for exosome isolation due to its ability to separate particles based on size, making it an effective method for purifying exosomes from other cellular components.

In clinical settings, SEC is often combined with other isolation techniques to enhance both efficiency and accuracy. For example, a comparative study demonstrated that when ultracentrifugation (UC) alone was used to isolate bovine serum exosomes, albumin contamination was present in the final preparation. However, when UC was followed by SEC in a sequential approach (UC-SEC or US), albumin contamination was effectively eliminated. This combined method not only reduced false positives but also improved the detection of exosomal markers, highlighting the value of integrating SEC with UC for more reliable exosome isolation [40]. Additionally, SEC has been recommended in combination with filtration for isolating exosomes derived from synovial tissue. This combined approach provides a more effective and robust methodology for osteoarthritis research, where high purity and accurate characterization of exosomes are essential for understanding disease mechanisms and potential therapeutic applications [41].

Overall, the integration of SEC with other isolation methods, such as UC and filtration, enhances the efficiency, purity, and accuracy of exosome isolation. This approach underscores the growing importance of SEC as a versatile tool in both clinical diagnostics and research applications, offering a reliable solution for exosome-based studies across various fields.

Immunoaffinity capture

Exosome membrane trafficking and fusion are regulated by proteins such as CD9, CD63, and CD81 [42]. Immunoaffinity capture takes advantage of the specific binding between antigens/antibodies or ligands/receptors in membrane affinity chromatography. This technique is particularly useful for isolating exosomes with high specificity and purity. For example, CD63, a member of the tetraspanin family, plays a key role in regulating malignancy in various cancers, such as melanoma and breast cancer, and has emerged as a potential cancer biomarker. A CD63-1 aptamer-based magnetic bead system has been developed to efficiently isolate exosomes from the culture media of breast cancer MDA-MB-231 cells and CD63-overexpressing HEK293T cells [43]. This targeted approach enables the isolation of specific exosome subpopulations, offering a high degree of specificity and purity, which is crucial for detailed molecular studies.

Furthermore, immunoaffinity capture has shown superior performance in isolating small RNAs (25 - 200 nt) compared to traditional methods like UC and SEC [40]. This makes it especially useful for applications where the isolation of specific RNA species is required. However, despite its advantages, immunoaffinity capture has some limitations. The high cost of reagents and the stringent storage requirements can make it less feasible for studies that do not require extremely high purity or the isolation of specific exosome subpopulations [33]. Therefore, while immunoaffinity capture offers a highly specialized and effective method for exosome isolation, its use is generally limited to studies where high specificity and purity are essential.

Table 1. Characteristics of isolation methods.

Technology categories	Technical principles	Time - consuming	Operational complexity	Purity	Recovery rate	Cost	Sample suitability	References
UC/DC (gold standard)	isolation based on differences in sedimentation coefficients between exosomes and impurities	> 4 hours	difficult	5% to 25% of the initial EV MHC class II concentration	low	low	large samples	[22,23,52, 53]
Density gradient centri-fugation	uses inert centrifugal media to isolate exosomes by density during centrifugation	> 18 hours	difficult	> UC	low	low	large samples	[54]
PBP	hydrophilic polymers create a hydrophobic microenvironment around exosomes, leading to precipitation by centrifugation	6 times faster than UC	simple	> UC	> UC	low	unlimited	[7,28,29,52, 53]
UF/DEF	exosomes are isolated using filters with specific pore sizes or molecular weight cutoffs to remove impurities	< 4 hours	simple	low	> PBP, > UC	low	large samples	[28,33, 52-54]
TFF	exosomes are concentrated and isolated using cross-flow filtration principles	short	simple	high	> UC	low	large samples	[35,54]
SEC	isolates macromolecules based on size or hydrodynamic volume	< 0.5 hour	simple	> UC	> UC	high	unlimited	[53]
Immuno-affinity capture	uses specific biomolecular binding to isolate exosomes	4 - 20 hours	simple	high	低	high	unlimited	[52,53,55]
Micro-fluidic technology	1) immunoaffinity-based isolation using immobilized biomarkers; 2) isolation using acoustics and dielectrophoresis for label-free isolation	short	simple	high	> UC	low	small samples	[52,53]
ATPS	forms immiscible phases in solution with hydrophilic polymers or a polymer and salt to isolate exosomes	≈ 70 minutes	simple	high	15%	uncertain	uncertain	[48,49]
Clustering and scattering methods	based on size, charge, and chaotic mechanisms for particle isolation	< 20 minutes	simple	3.5 times that of UC	20 times that of UC	uncertain	uncertain	[50]
AF4	isolates exosomes based on size using cross-flow and parabolic flow patterns	> 4 hours	difficult	high	> UC	high	small samples	[54,56]

UC: Ultracentrifugation, DC: Differential ultracentrifugation, EV: Extracellular vesicle, PBP: Polymer-based precipitation, UF: Ultrafiltration, DEF: Dead-end filtration, TFF: Tangential flow filtration, SEC: Size exclusion chromatography, US: Ultracentrifugation and size exclusion chromatography, ATPS: Aqueous two-phase systems, AF4: Asymmetric flow field-flow fractionation.

Table 2. Characteristics of enrichment methods.

Technical name		Technical principles	Advantages	Disadvantages	References
Physical methods (gradual integration of separation and enrichment)	centrifugation (DC)	separates substances with different density differences in biological fluids using different centrifugal forces to achieve dehydration and concentration of exosomes	the most commonly used step for exosome enrichment	time-consuming	[57]
	precipitation	treats biological fluids with 8% PEG to facilitate better precipitation of exosomes, followed by low-speed centrifugation after overnight incubation	1) minimal impact on biological activity, 2) low cost	time-consuming	[57]
	filtration	dead-end ultrafiltration strategy based on a 300 kDa molecular weight cutoff membrane	preserves exosome integrity	membrane clogging	[61]
	SEC	separates macromolecules based on molecular size or hydrodynamic volume	1) short time consumption, 2) preserves the activity of separated exosomes	high cost	[63]
Biological methods	immunomagnetic bead technology	novel enrichment technology based on biological specific recognition	efficient enrichment, precise localization	lack of large-scale research currently	[63]

DC: Differential ultracentrifugation, SEC: Size exclusion chromatography.

Microfluidics-based techniques

Microfluidic technology has demonstrated remarkable integration capabilities, consolidating exosome separation, detection, and analysis functions onto a compact microchip platform [44]. Currently, microfluidic-based separation techniques are divided into two main categories. The first relies on immunoaffinity principles, where specific biomarkers are immobilized within microchannels or microstructures to isolate target exosomes [45]. Similar to traditional immunoaffinity capture, this approach offers high sensitivity and specificity [45], making it highly effective for precise exosome isolation. In clinical practice, microfluidic chips based on immunoaffinity mechanisms employ 300 nm magnetic nanoparticles conjugated with anti-CD63 antibodies to prepare immunomagnetic beads, enabling the specific capture of exosomes derived from gastric cancer cells. These chips achieve an exosome recovery rate exceeding 80%, a purity greater than 83%, and detection rates of 70% or higher for stage I and II gastric cancer [46]. The second category combines microfluidics with acoustics or dielectrophoresis to enable label-free separation of exosomes based on their electrical and physical properties [45]. For example, an acoustofluidic platform uses separation modules that can differentiate submicron particles, allowing for the distinction between larger EV subpopulations and exosomes [47]. This technique not only eliminates the need for labels but also provides an efficient, non-invasive method for exosome isolation.

Additionally, microfluidic technology offers a low detection limit, making it particularly advantageous for small sample volumes. Precisely fabricated micro/nanochannels allow for the accurate manipulation of minute fluid volumes, ranging from microliters to nanoliters. This precision enables highly efficient isolation of exosomes, even from small samples, which reduces reagent and sample consumption, promotes cost-effective bioanalysis, and enhances the efficiency of liquid biopsies [13,45]. These features position microfluidic platforms as a promising tool in both clinical diagnostics and biomedical research, offering a more streamlined and efficient alternative to traditional exosome isolation techniques.

Other methods

In addition to the aforementioned methods, several other techniques have been developed for exosome separation. One such method is the aqueous two-phase system, which commonly uses two hydrophilic polymers (e.g., polyethylene glycol and dextran) or a polymer combined with a salt (e.g., polyethylene glycol with phosphate or sulfate) to create immiscible phases in an aqueous solution, facilitating the separation of exosomes. The polyethylene glycol/dextran system, the most widely used aqueous two-phase system, allows for efficient exosome recovery in a short time, with a recovery rate of approximately 15% [48,49].

Another notable approach includes clustering and scattering techniques, which are based on the size, charge, and chaotic mechanisms of exosomes. These methods have demonstrated impressive results, achieving the highest exosome recovery rate - approximately 20 times greater than that of UC - and a purity ratio that is 3.5 times higher than UC [50].

Additionally, asymmetric flow field-flow fractionation (AF4) technology has emerged as an effective method for separating extracellular nanoparticles. This technique significantly reduces the separation time compared to UC, offering a faster alternative for isolating exosomes from cell culture media. Research suggests

that AF4 could play a pivotal role in advancing high-quality molecular diagnostic and therapeutic approaches based on exosomes and their cargo [51].

The characteristics of these various separation techniques are summarized in Table 1, highlighting their unique advantages and applications in exosome isolation. Each method offers distinct benefits depending on the specific requirements of the research or clinical application, providing versatile tools for advancing exosome-based diagnostics and therapeutics.

Enrichment techniques

Physical methods

Centrifugation

The core principle of enrichment lies in "concentration", aiming to enhance both the concentration and purity of target exosomes. In recent years, to improve the efficiency of exosome extraction, separation and enrichment have become increasingly integrated. Among the various enrichment methods, DC serves not only as a foundational technique for exosome isolation but also as the most commonly employed method for exosome enrichment [57]. DC utilizes varying centrifugal forces to separate substances based on their density differences in biological fluids, effectively concentrating and dehydrating exosomes. However, despite its widespread use, both the separation and enrichment processes via DC are hindered by challenges such as complex operation and prolonged processing times. As a result, researchers are continuously working to optimize DC-based enrichment methods.

One notable advancement in enhancing the efficiency of DC involves the use of DNA nanostructures as mass tags to increase centrifugal force, thereby enabling rapid and specific enrichment of cancer-related exosomes [58]. Beyond improving the centrifugal efficiency of DC, DNA-related technologies are also emerging as viable alternatives for exosome enrichment.

DNAzymes, catalytically active DNA sequences, have demonstrated exceptional performance and sensitivity in real-time detection of exosomes from various sources [24,59]. For exosome enrichment, DNAzyme probes hybridize with complementary sequences of substrate probes, triggering the self-assembly of exosomes into micrometer-scale clusters. This method bypasses the complexity of ultracentrifugation (UC) and facilitates rapid enrichment using conventional centrifugation at 10,000 x g [60].

These innovations in DNA-based technologies offer promising alternatives to traditional methods, improving both the efficiency and simplicity of exosome enrichment while maintaining high specificity.

Precipitation

ExtraPEG, a polymer-based precipitation (PBP) method, involves treating biological fluids with 8% polyethylene glycol (PEG) to enhance the precipitation of exosomes. Following overnight incubation, low-speed centrifugation is used to achieve both enrichment and purification. This approach offers a rapid and cost-effective solution for enriching exosomes from large volumes of culture media [57].

One of the key advantages of ExtraPEG is its minimal impact on the biological activity of exosomes. Confocal microscopy has confirmed that exosome bioactivity remains intact after the precipitation process, ensuring the preserved functional integrity of the isolated exosomes. These findings underscore the suitability of ExtraPEG for the enrichment of a wide range of exosome types, making it a versatile tool for exosome-based research and applications [57].

By providing an efficient, low-cost method for exosome isolation without compromising bioactivity, ExtraPEG represents a promising alternative to more complex and time-consuming techniques, facilitating large-scale exosome isolation for diagnostic, therapeutic, and research purposes.

Filtration

Fast and Efficient Exosome Enrichment using Filter-Aided Extracellular Vesicle Enrichment (FAEVEr) is a dead-end ultrafiltration strategy utilizing a 300 kDa molecular weight cutoff membrane. This membrane is designed to effectively retain exosomes while allowing smaller molecular weight contaminants, such as proteins, to pass through. By minimizing non-specific protein adsorption on the membrane, FAEVEr helps preserve the integrity of the exosomes and enhances their purity [61].

One of the main advantages of this approach is its efficiency in isolating exosomes, as it prevents the accumulation of unwanted proteins that could potentially interfere with subsequent analyses. The result is a higher purity and more intact exosome population, making FAEVEr an effective and reliable method for exosome enrichment. This strategy not only simplifies the isolation process but also improves the overall quality of the exosomes, supporting their use in a variety of research and clinical applications.

SEC

In the extraction of bovine milk exosomes, a method that combines initial isolation of EVs through UC followed by exosome enrichment using SEC has been reported. This UC-SEC combination offers a significant improvement in exosome yield compared to conventional enrichment methods, such as density gradient centrifugation [62].

By utilizing UC for initial EV isolation and then applying SEC for further enrichment, this approach effectively enhances both the purity and quantity of exosomes, ensuring more reliable and higher yields. The combination of these two techniques offers a more efficient and streamlined process, making it a promising option for exosome extraction in various research and clinical applications.

Biological methods

In addition to traditional physical methods that "concentrate" exosomes for purification, novel enrichment technologies based on biological specific recognition are advancing rapidly. One such method is immunomagnetic

bead technology, which combines immunological specificity with magnetic separation to achieve exosome enrichment. This technique involves conjugating antibodies to magnetic materials, allowing for targeted isolation of exosomes.

For example, a Strep-tag II-based immunomagnetic bead separation system has been developed, where capture antibodies are modified onto magnetic nanoparticles through the specific and reversible recognition between Strep-Tactin and Strep-tag II. This system enables efficient enrichment and precise localization of exosomes, offering several advantages over conventional methods. It simplifies the process compared to UC, accelerates processing time, and enhances sensitivity, making it a highly effective tool for exosome isolation and analysis [63].

The integration of immunomagnetic bead technology represents a promising advancement in exosome enrichment, providing a more efficient, rapid, and precise alternative for applications in research, diagnostics, and therapeutics. The characteristics of the various enrichment methods are summarized in Table 2.

Summary and outlook

The present review has provided a comprehensive overview of current techniques for exosome isolation and enrichment. The strength of this review lies in its thorough and comparative analysis of both traditional and emerging techniques for exosome isolation and enrichment. By synthesizing a wide range of methods, this review offers a comprehensive overview of the technological advances in the field, addressing key challenges such as exosome purity, yield, and cost-efficiency. Furthermore, it emphasizes the significance of methodological innovations that have enhanced the efficiency and specificity of exosome isolation, thus providing a valuable resource for researchers and clinicians alike.

In recent years, the field of exosome research has made significant strides, progressing from a basic understanding of exosomes to exploring their potential applications in human health. The use of exosomes in medicine and biology continues to expand, with an increasing number of studies focused on improving exosome isolation efficiency. The ultimate goal is to achieve exosome separation and enrichment methods that are simple, cost-effective, and capable of yielding high purity. To achieve this, there has been a growing trend toward integrating, automating, and miniaturizing techniques for isolating exosomes from small-volume samples. For larger-volume samples, two key areas need further advancement: first, enhancing exosome recovery rates by optimizing existing separation systems, and second, developing independent enrichment methods that are easier to operate, more sensitive, and capable of establishing efficient exosome extraction platforms. Despite these advancements, our current understanding of exosomes, particularly at the microscale level, remains incomplete, which limits the widespread application of sophisticated biotechnologies such as immunoaffinity and immunomagnetic bead methods. As such, future efforts should focus on two main objectives: deepening our understanding of the complex nature of exosomes and developing separation and enrichment techniques that are tailored to the heterogeneity of exosomes derived from different sources.

These advancements will be pivotal in unlocking the full potential of exosomes in medicine and biology. They will provide critical technical support for the future of precision medicine and biological research, enabling more efficient and targeted applications of exosomes for diagnostic, therapeutic, and research purposes.

Declaration of Interest:

Both authors declare no competing interests.

References:

1. Rezaie J, Feghhi M, Etemadi T. A review on exosomes application in clinical trials: perspective, questions, and challenges. Cell Commun Signal 2022;20(1):145. (PMID: 36123730)
2. Krylova SV, Feng D. The Machinery of Exosomes: Biogenesis, Release, and Uptake. Int J Mol Sci 2023;24(2):1337. (PMID: 36674857)
3. Zhang H, Xing J, Dai Z, Wang D, Tang D. Exosomes: the key of sophisticated cell-cell communication and targeted metastasis in pancreatic cancer. Cell Commun Signal 2022;20(1):9. (PMID: 35033111)
4. Xie Z, Gao Y, Ho C, et al. Exosome-delivered CD44v6/C1QBP complex drives pancreatic cancer liver metastasis by promoting fibrotic liver microenvironment. Gut 2022;71(3):568-79. (PMID: 33827783)
5. Xia L, Zhang C, Lv N, et al. AdMSC-derived exosomes alleviate acute lung injury via transferring mitochondrial component to improve homeostasis of alveolar macrophages. Theranostics 2022; 12(6):2928-47. (PMID: 35401830)
6. Pan J, He R, Huo Q, Shi Y, Zhao L. Brain Microvascular Endothelial Cell Derived Exosomes Potently Ameliorate Cognitive Dysfunction by Enhancing the Clearance of Abeta Through Up-Regulation of P-gp in Mouse Model of AD. Neurochem Res 2020;45(9):2161-72. (PMID: 32583212)
7. Coughlan C, Bruce KD, Burgy O, et al. Exosome Isolation by Ultracentrifugation and Precipitation and Techniques for Downstream Analyses. Curr Protoc Cell Biol 2020;88(1):e110. (PMID: 32633898)
8. Tan F, Li X, Wang Z, Li J, Shahzad K, Zheng J. Clinical applications of stem cell-derived exosomes. Signal Transduct Target Ther 2024;9(1):17. (PMID: 38212307)
9. Mondal J, Pillarisetti S, Junnuthula V, et al. Hybrid exosomes, exosome-like nanovesicles and engineered exosomes for therapeutic applications. J Control Release 2023;353:1127-49. (PMID: 36528193)
10. Wang C, Li Z, Liu Y, Yuan L. Exosomes in atherosclerosis: performers, bystanders, biomarkers, and therapeutic targets. Theranostics 2021;11(8):3996-4010. (PMID: 33664877)

11. Xu WM, Li A, Chen JJ, Sun EJ. Research Development on Exosome Separation Technology. J Membr Biol 2023;256(1):25-34. (PMID: 36040494)

12. Zhang Y, Bi J, Huang J, Tang Y, Du S, Li P. Exosome: A Review of Its Classification, Isolation Techniques, Storage, Diagnostic and Targeted Therapy Applications. Int J Nanomedicine 2020;15: 6917-34. (PMID: 33061359)

13. Al-Madhagi H. The Landscape of Exosomes Biogenesis to Clinical Applications. Int J Nanomedicine 2024;19:3657-75. (PMID: 38681093)

14. Wang HY, Xie PJ, Qiao XQ, Zhang LY. [Typical strategy and research progress of efficient isolation methods of exosomes based on affinity interaction]. Se Pu 2025;43(5):413-23. (PMID: 40331606)

15. Puzar Dominkus P, Stenovec M, Sitar S, et al. PKH26 labeling of extracellular vesicles: Characterization and cellular internalization of contaminating PKH26 nanoparticles. Biochim Biophys Acta Biomembr 2018;1860(6):1350-61. (PMID: 29551275)

16. Wu CC, Tsantilas KA, Park J, et al. Enrichment of extracellular vesicles using Mag-Net for the analysis of the plasma proteome. Nat Commun 2025;16(1):5447. (PMID: 40595564)

17. Yu W, Hurley J, Roberts D, et al. Exosome-based liquid biopsies in cancer: opportunities and challenges. Ann Oncol 2021;32(4): 466-77. (PMID: 33548389)

18. Johnstone RM, Adam M, Hammond JR, Orr L, Turbide C. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). J Biol Chem 1987;262(19):9412-20. (PMID: 3597417)

19. Hou GS, Yuan HM, Liang Z, Zhang LH, Zhang YK. [Exosome separation and enrichment technologies and their applications in disease diagnosis and treatment]. Se Pu 2025;43(5):434-45. (PMID: 40331608)

20. Momen-Heravi F, Balaj L, Alian S, et al. Current methods for the isolation of extracellular vesicles. Biol Chem 2013;394(10):1253-62. (PMID: 23770532)

21. Thery C, Amigorena S, Raposo G, Clayton A. Isolation and characterization of exosomes from cell culture supernatants and biological fluids. Curr Protoc Cell Biol 2006;Chapter 3:Unit 3.22. (PMID: 18228490)

22. Lamparski HG, Metha-Damani A, Yao JY, et al. Production and characterization of clinical grade exosomes derived from dendritic cells. J Immunol Methods 2002;270(2):211-26. (PMID: 12379326)

23. Xing YH, Ren XS, Li DH, Liu L. [Exosome separation and analysis based on microfluidics technology and its clinical applications]. Se Pu 2025;43(5):455-71. (PMID: 40331610)

24. Zhang H, Tang Y, Zhou Y, Wang Y, Si H, Li L, Tang B. DNAzyme-RCA-based colorimetric and lateral flow dipstick assays for the point-of-care testing of exosomal m5C-miRNA-21. Chem Sci 2024;15(24):9345-52. (PMID: 38903234)

25. Seo K, Yoo JH, Kim J, et al. Ginseng-derived exosome-like nanovesicles extracted by sucrose gradient ultracentrifugation to inhibit osteoclast differentiation. Nanoscale 2023;15(12):5798-808. (PMID: 36857681)

26. Garcia-Romero N, Madurga R, Rackov G, et al. Polyethylene glycol improves current methods for circulating extracellular vesicle-derived DNA isolation. J Transl Med 2019;17(1):75. (PMID: 30871557)

27. Rahmatinejad F, Kharat Z, Jalili H, Renani MK, Mobasheri H. Comparison of morphology, protein concentration, and size distribution of bone marrow and Wharton's jelly-derived mesenchymal stem cells exosomes isolated by ultracentrifugation and polymer-based precipitation techniques. Tissue Cell 2024;88:102427. (PMID: 38833940)

28. Niu Z, Pang RTK, Liu W, Li Q, Cheng R, Yeung WSB. Polymer-based precipitation preserves biological activities of extracellular vesicles from an endometrial cell line. PLoS One 2017;12(10): e0186534. (PMID: 29023592)

29. Gao M, Cai J, Zitkovsky HS, Chen B, Guo L. Comparison of Yield, Purity, and Functional Properties of Large-Volume Exosome Isolation Using Ultrafiltration and Polymer-Based Precipitation. Plast Reconstr Surg 2022;149(3):638-49. (PMID: 35196679)

30. Kanchi Ravi R, Khosroheidari M, DiStefano JK. A modified precipitation method to isolate urinary exosomes. J Vis Exp 2015(95):51158. (PMID: 25651044)

31. Zhao X, Liu X, Chen T, et al. Fully Integrated Centrifugal Microfluidics for Rapid Exosome Isolation, Glycan Analysis, and Point-of-Care Diagnosis. ACS Nano 2025 Mar 11;19(9):8948-65. (PMID: 40014808)

32. Ikezaki T, Kuroda Y, Kawai T, et al. Exosomes secreted from human-derived adipose stem cells prevent progression of osteonecrosis of the femoral head. J Orthop Surg Res 2024;19(1):793. (PMID: 39587645)

33. Chen J, Li P, Zhang T, et al. Review on Strategies and Technologies for Exosome Isolation and Purification. Front Bioeng Biotechnol 2021;9:811971. (PMID: 35071216)

34. Busatto S, Vilanilam G, Ticer T, et al. Tangential Flow Filtration for Highly Efficient Concentration of Extracellular Vesicles from Large Volumes of Fluid. Cells 2018;7(12):273. (PMID: 30558352)

35. Visan KS, Lobb RJ, Ham S, et al. Comparative analysis of tangential flow filtration and ultracentrifugation, both combined with subsequent size exclusion chromatography, for the isolation of small extracellular vesicles. J Extracell Vesicles 2022;11(9): e12266. (PMID: 36124834)

36. Chen Y, Zhu Q, Cheng L, et al. Exosome detection via the ultrafast-isolation system: EXODUS. Nat Methods 2021;18(2):212-8. (PMID: 33432243)

37. Zhang C, Guo Z, Jing Z. Prediction of Response to Chemoradiotherapy by Dynamic Changes of Circulating Exosome Levels in Patients with Esophageal Squamous Cell Carcinoma. Int J Nanomedicine 2024;19:1351-62. (PMID: 38352821)

38. Sidhom K, Obi PO, Saleem A. A Review of Exosomal Isolation Methods: Is Size Exclusion Chromatography the Best Option? Int J Mol Sci 2020;21(18):6466. (PMID: 32899828)

39. Monguio-Tortajada M, Galvez-Monton C, Bayes-Genis A, Roura S, Borras FE. Extracellular vesicle isolation methods: rising impact of size-exclusion chromatography. Cell Mol Life Sci 2019; 76(12):2369-82. (PMID: 30891621)

40. Bok EY, Seo SY, Lee HG, et al. Exosomes isolation from bovine serum: qualitative and quantitative comparison between ultracentrifugation, combination ultracentrifugation and size exclusion chromatography, and exoEasy methods. J Anim Sci Technol 2024;66(5):1021-33. (PMID: 39398302)

41. Chen P, Ruan A, Zhou J, et al. Extraction and identification of synovial tissue-derived exosomes by different separation techniques. J Orthop Surg Res 2020;15(1):97. (PMID: 32151262)

42. Ruivo CF, Adem B, Silva M, Melo SA. The Biology of Cancer Exosomes: Insights and New Perspectives. Cancer Res 2017; 77(23):6480-8. (PMID: 29162616)

43. Song Z, Mao J, Barrero RA, Wang P, Zhang F, Wang T. Development of a CD63 Aptamer for Efficient Cancer Immunochemistry and Immunoaffinity-Based Exosome Isolation. Molecules 2020;25(23):5585. (PMID: 33261145)

44. Contreras-Naranjo JC, Wu HJ, Ugaz VM. Microfluidics for exosome isolation and analysis: enabling liquid biopsy for personalized medicine. Lab Chip 2017;17(21):3558-77. (PMID: 28832692)

45. Wang J, Ma P, Kim DH, Liu BF, Demirci U. Towards Microfluidic-Based Exosome Isolation and Detection for Tumor Therapy. Nano Today 2021;37:101066. (PMID: 33777166)

46. Yu Z, Lin S, Xia F, et al. ExoSD chips for high-purity immunomagnetic separation and high-sensitivity detection of gastric cancer cell-derived exosomes. Biosens Bioelectron 2021;194: 113594. (PMID: 34474280)

47. Wu M, Ouyang Y, Wang Z, et al. Isolation of exosomes from whole blood by integrating acoustics and microfluidics. Proc Natl Acad Sci U S A 2017;114(40):10584-9. (PMID: 28923936)

48. Shin H, Han C, Labuz JM, et al. High-yield isolation of extracellular vesicles using aqueous two-phase system. Sci Rep 2015;5: 13103. (PMID: 26271727)

49. Su B, Jeyhani M, Thillainadesan G, et al. Next Generation Aqueous Two-Phase System for Gentle, Effective, and Timely Extracellular Vesicle Isolation and Transcriptomic Analysis. J Extracell Vesicles 2025;14(3):e70058. (PMID: 40108918)

50. Kim J, Lee H, Park K, Shin S. Rapid and Efficient Isolation of Exosomes by Clustering and Scattering. J Clin Med 2020;9(3): 650. (PMID: 32121214)

51. Zhang H, Lyden D. Asymmetric-flow field-flow fractionation technology for exomere and small extracellular vesicle separation and characterization. Nat Protoc 2019;14(4):1027-53. (PMID: 30833697)

52. Wang W, Sun H, Duan H, et al. Isolation and usage of exosomes in central nervous system diseases. CNS Neurosci Ther 2024; 30(3):e14677. (PMID: 38497529)

53. Yang D, Zhang W, Zhang H, et al. Progress, opportunity, and perspective on exosome isolation - efforts for efficient exosome-based theranostics. Theranostics 2020;10(8):3684-707. (PMID: 32206116)

54. Jia Y, Yu L, Ma T, et al. Small extracellular vesicles isolation and separation: Current techniques, pending questions and clinical applications. Theranostics 2022;12(15):6548-75. (PMID: 36185597)

55. Bharti R, Kumar M, Devi V, Rao A, Aggarwal A, Gupta T. Efficient isolation and characterization of Serum-Derived Exosomes: evaluating ultracentrifugation and Total Exosome Isolation Reagent based precipitation. Ultrastruct Pathol 2025;49(4):340-53. (PMID: 40413775)

56. Hu L, Zheng X, Zhou M, et al. Optimized AF4 combined with density cushion ultracentrifugation enables profiling of high-purity human blood extracellular vesicles. J Extracell Vesicles 2024; 13(7):e12470. (PMID: 39001700)

57. Rider MA, Hurwitz SN, Meckes DG Jr. ExtraPEG: A Polyethylene Glycol-Based Method for Enrichment of Extracellular Vesicles. Sci Rep 2016;6:23978. (PMID: 27068479)

58. Lu Z, Shi Y, Ma Y, et al. Fast and specific enrichment and quantification of cancer-related exosomes by DNA-nanoweight-assisted centrifugation. Anal Chem 2022;94(26):9466-71. (PMID: 35731982)

59. Hu W, Su H, Zeng X, Duan X, Li Y, Li L. Exo-III Enzyme and DNAzyme-Assisted Dual Signal Recycles for Sensitive Analysis of Exosomes by Using Personal Glucose Meter. Appl Biochem Biotechnol 2023;195(2):861-70. (PMID: 36219333)

60. Yu X, Chen X, Sun Z, et al. Ultracentrifugation-Free Enrichment and Quantification of Small Extracellular Vesicles. Anal Chem 2022;94(29):10337-45. (PMID: 35792105)

61. Pauwels J, Van de Steene T, Van de Velde J, et al. Filter-Aided Extracellular Vesicle Enrichment (FAEVEr) for Proteomics. Mol Cell Proteomics 2025;24(2):100907. (PMID: 39842778)

62. Vaswani K, Koh YQ, Almughlliq FB, Peiris HN, Mitchell MD. A method for the isolation and enrichment of purified bovine milk exosomes. Reprod Biol 2017;17(4):341-8. (PMID: 29030127)

63. Guo X, Hu F, Zhao S, Yong Z, Zhang Z, Peng N. Immunomagnetic Separation Method Integrated with the Strep-Tag II System for Rapid Enrichment and Mild Release of Exosomes. Anal Chem 2023;95(7):3569-76. (PMID: 36661256)