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Review

Polyethylene glycol (PEG)-based precipitation for exosome enrichment: A review on recent developments, current challenges, and future perspectives

Reham M. Marzouk[1], Mohamed A. Gab-Allah[1,2], and Jeongkwon Kim[1,★]

[1]*Department of Chemistry, Chungnam National University, Daejeon, Republic of Korea*
[2]*Organic Analysis Laboratory, National Institute of Standards, Tersa St., Al-Haram, P. O. Box; 136, Giza 12211, Egypt*
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Abstract: It is becoming increasingly evident that nearly all living cells can generate and secrete various categories of membrane-enclosed structures known as extracellular vesicles (EVs). Exosomes represent one of the major subclasses of EVs and play crucial roles in many biological processes, such as intercellular communication, tissue homeostasis, and the transfer of genetic material. Therefore, the development of efficient, high-throughput, and cost-effective methods for isolating high-yield exosomes remains an active area of scientific investigation. However, current isolation techniques face challenges related to purity, yield, scalability, and cost-effectiveness. Among various enrichment strategies employed for exosomes, polyethylene glycol (PEG)-based precipitation has gained significant acceptance from researchers due to its simplicity, affordability, cost-effectiveness, and capacity for processing small and limited quantities of samples. PEG precipitation demonstrates superior performance in exosome yield compared to commercial kits and other commonly used isolation techniques. This review offers a comprehensive overview of various exosome enrichment techniques, focusing on the underlying mechanism, method, advantages, and limitations of PEG precipitation. The recent applications of PEG precipitation in exosome isolation from various biological samples are revisited. Furthermore, efforts aimed at enhancing the efficiency of this method, emerging trends, and future perspectives are reviewed. This review also offers a summary of the biogenesis, composition, and function of exosomes to support a deeper understanding of the applications of PEG precipitation methods in exosome research.

Key words: exosomes, extracellular vesicles, polyethylene glycol, precipitation, isolation

1. Introduction

Exosomes are a distinct subclass of EVs, typically ranging from 30 to 200 nm in diameter, and play a vital role in many biological processes and disease pathogenesis.[1,2] These vesicles can be found in different biological fluids, including blood, saliva, urine, bile, breast milk, tears, sweat, and others.[3-8] They are secreted

★ Corresponding author
Phone : +82-(0)42-821-5477
E-mail : jkkim48105@cnu.ac.kr

by almost all cell types and have been identified as a novel mechanism of intercellular communication.[1,9] By facilitating molecular exchange between cells, exosomes contribute to numerous physiological and pathological processes, such as immune regulation, tissue repair, and tumor progression.[10,11] Exosomes contain a lipid bilayer membrane that encapsulates various active biomolecules, including proteins, DNA, RNA, lipids, and metabolites, which serve as molecular cargos reflecting the profile of their cells of origin.[12,13] In recent years, there has been a growing interest in investigating these cargos, primarily due to their potential as a biomarker source for liquid biopsy. This is particularly important because the isolation of these cargos from exosomes rather than whole body fluids greatly reduces sample complexity, enhancing detection sensitivity and specificity.[12,14] Unlike solid biopsies, which are often inaccessible or limited in size, analyzing exosomal molecular cargo in biofluids offers a key advantage since these samples can be collected through rapid, minimally invasive procedures that are suitable for clinical applications.[15] Moreover, exosome content more accurately reflects intratumor heterogeneity and serves as a valuable source of diagnostic and prognostic biomarkers, complementing patient data obtained through conventional methods.[15,16]

To fully exploit the potential of exosomes in biomedical research and clinical practice, robust and efficient isolation techniques are essential. Despite significant advancements in the exosome field over the past decade, a clear consensus on the most effective methods for their isolation and characterization has yet to be established. Ultracentrifugation (UC) is widely regarded as the gold standard for exosome isolation and remains the most commonly used method, implemented in numerous exosome studies.[12,17] This technique, often combined with sucrose density gradients, enables the purification of high-quality exosomes.[18,19] However, it requires expensive and specialized equipment, prolonged processing times (up to 8 – 10 h), and multiple high-speed centrifugation steps, thus limiting its practicality.[12,20,21] Additionally, concerns have been raised about its efficiency, as the high shear forces involved may cause potential damage to exosomes.[22, 23] Alternative techniques can address these limitations by isolating exosomes based on specific surface markers, as seen in immunoaffinity-based methods,[24,25] or by size-based separation approaches, including ultrafiltration, dialysis, microfluidics, and size exclusion chromatography.[26-32] These methods enhance exosome purity and preserve membrane integrity; however, they often result in lower yield and require costly instrumentation, which may limit their widespread application.[26] These constraints have driven the search for alternative methods that can provide high efficiency, reproducibility, and cost-effectiveness.

PEG-based precipitation has gained widespread recognition among researchers due to its simplicity, affordability, applicability to small sample volumes, cost-effectiveness, and scalability, making it an attractive choice for clinical research and large-scale applications.[15,33] PEG is a synthetic polymer consisting of repeated units of ethylene oxide and can offer beneficial properties such as non-irritability, non-toxicity, and high scalability.[33] Originally developed for early virus enrichment,[34] the PEG precipitation method has been used to isolate and purify exosomes from various biological fluids, further demonstrating its versatility.[7,9,33-36] Unlike ultracentrifugation and other specific techniques, PEG-based methods do not require specialized equipment, making them an attractive option for both basic research and clinical applications. Despite its advantages, concerns regarding the co-precipitation of contaminants and potential effects on downstream analyses remain due to its non-specific mechanism, thus highlighting the need for further optimization and refinement. Multiple-cycle PEG-based enrichment and hybrid/combined approaches that integrate PEG precipitation with other exosome enrichment techniques are being explored to enhance the purity and specificity of exosome isolation.[9,37-40] This review provides a comprehensive overview of exosome enrichment strategies, with a particular emphasis on the PEG-based precipitation technique. It also discusses the biological principles underlying PEG precipitation, its method, and related positive aspects. Furthermore, recent applications and challenges

of PEG-based exosome enrichment are summarized. Finally, the review addresses emerging trends and future directions in the field, offering valuable insights into the advancing frontier of exosome research.

Despite significant progress in exosome research, several critical knowledge gaps remain that limit the effective application of exosome enrichment methods in both research and clinical practice. First, there is no universally accepted standard for exosome isolation, and commonly used techniques such as ultracentrifugation, immunoaffinity capture, and size-based separation each present distinct trade-offs in terms of purity, yield, scalability, and cost-effectiveness. This lack of consensus complicates method selection and hinders direct comparison across studies, which in turn affects reproducibility and translational potential. Furthermore, many isolation methods, including PEG-based precipitation, are challenged by the co-precipitation of contaminants such as proteins and lipoproteins, which can interfere with downstream analyses and functional studies. The scalability and accessibility of conventional methods like ultracentrifugation are also limited by the need for specialized equipment and time-consuming protocols, making them impractical for high-throughput or clinical applications. Additionally, the effects of different isolation methods on exosome integrity, molecular cargo, and biological function are not fully understood, raising concerns about the reliability of exosome-based biomarkers and therapeutics. This review directly addresses these knowledge gaps by providing a comprehensive comparison of exosome enrichment techniques, with a particular focus on the mechanisms, advantages, and limitations of PEG-based precipitation. It highlights recent advances and hybrid strategies aimed at improving the purity and specificity of PEG-based methods, and contextualizes method selection for diverse downstream applications. By explicitly linking these gaps to the review's aims, the coverage clarifies how the analysis advances the field and supports the development of more robust, reproducible, and clinically relevant exosome isolation protocols.

2. The Biological Origin of Exosomes

Exosomes originate as intraluminal vesicles (ILVs) inside multivesicular bodies (MVBs), which are essential components of the endosomal system that regulate the sorting and transport of cellular vesicles.[41] Their release occurs when MVBs fuse with the plasma membrane, as illustrated in *Fig.* 1. The process begins with the inward budding of the plasma membrane via endocytosis, forming a cup-shaped pocket containing extracellular proteins and membrane components.[10] This leads to the formation of early endosomes, which can merge with other endosomes originating from organelles like mitochondria, the endoplasmic reticulum, and the trans-Golgi network. Early endosomes mature into late endosomes or MVBs, where a second inward budding of the endosomal membrane generates ILVs.[42,43] Exo- somes, typically 70 – 150 nm in size, are distinct from microvesicles, which range from 100 to 1000 nm.[44] Exosome formation is largely regulated by the ESCRT mechanism, comprising ESCRT-0, I, II, and III, along with associated proteins like ALIX, TSG101, VPS4, and VTA1, which function sequentially.[10,45,46] ESCRT-0 recruits ubiquitinated cargo to the endosomal membrane, while ESCRT-I and II drive membrane budding and cargo sorting. ESCRT-III, disassembled by Vps4 ATPase, enables vesicle detachment from the cytoplasmic membrane. Exosome release is mediated by Rab-GTPases (e.g., RAB2B, 5A, 7, 9A, 11, 27, 35) and possibly SNARE proteins (VAMP7, YKT6). ALIX also contributes to membrane budding, vesicle scission, and cargo selection by interacting with syndecan and tetraspanins (CD9, CD81, CD63).[46,47]

3. Biological Composition and Functions of Exosomes

Exosomes serve as carriers of biologically active molecules, thereby reflecting the molecular composition of their parent cells.[48,49] The structure and composition of exosomes are shown in *Fig.* 1. They are enriched with proteins that are involved in membrane transport,

vesicle fusion, cell adhesion, biogenesis of multivesicular bodies (MVBs), and exosome release. Key exosomal markers, such as tetraspanins (CD9, CD63, and CD81), ALIX, and TSG101, help distinguish them from other extracellular vesicles and facilitate their isolation and detection.[42,50,51] In addition to proteins, exosomes contain a variety of lipids, including cholesterol, sphingolipids, and phospholipids, which contribute to their stability, biogenesis, and cellular uptake.[43,47] Lipids are essential for preserving exosome structure, facilitating their biogenesis, and regulating homeostasis in recipient cells.[47] Exosomes also carry genetic material, including mRNA, miRNA, and noncoding RNAs, which play crucial roles in cell signaling, gene regulation, and disease progression.[52] These RNAs can be transferred to recipient cells, where they influence cellular functions such as proliferation, migration, and immune response.[53,54] The ability of exosomes to transport bioactive molecules across cells and tissues highlights their significance in intercellular communication and their potential as biomarkers for disease diagnosis and therapeutic applications.[13]

Exosomes play a crucial role in intercellular communication by transporting proteins, lipids, and nucleic acids between cells.[19,55,56] This exchange influences various biological processes, including gene regulation, immune responses, and disease progression. The molecular content of exosomes reflects the physiological or pathological state of their parent cells. For instance, exosomes from virus-infected cells may contain viral RNA, while those from cancer cells carry distinct miRNA profiles that contribute to tumor growth and metastasis.[57] In the immune system, exosomes can modulate immune responses by either activating or suppressing immune cells. Dendritic cell-derived exosomes enhance immune activity by

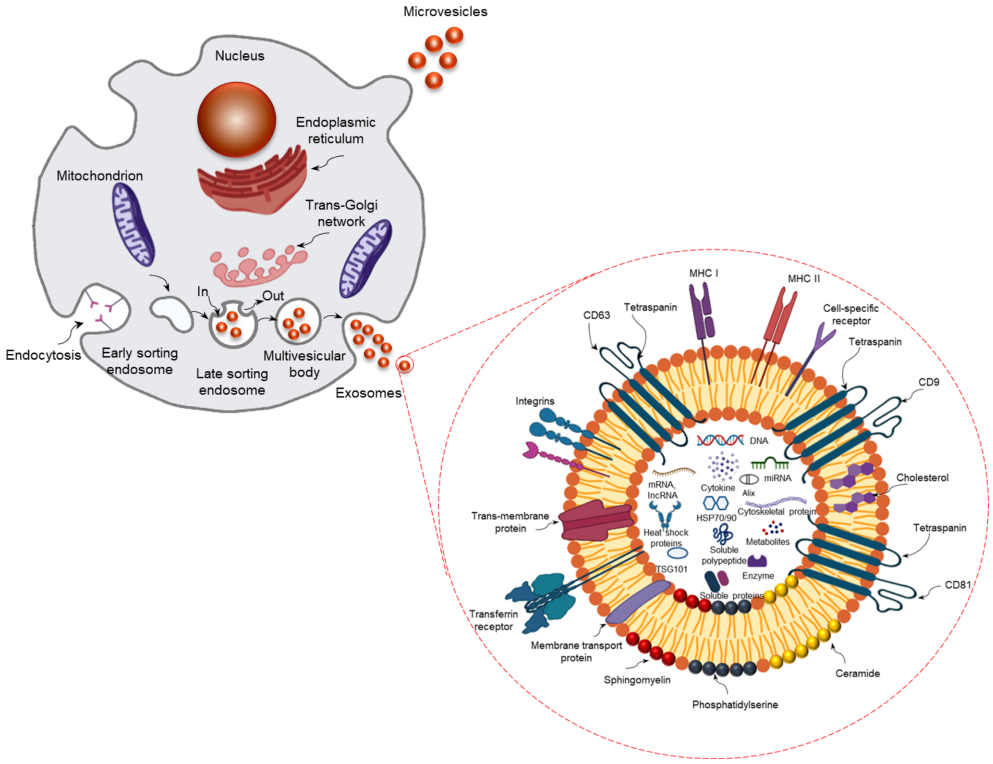

Fig. 1. Schematic representation of exosome biological origin, structural composition, and molecular content. Exosomes (30 – 150 nm) are generated within multivesicular bodies and released into the extracellular environment through fusion with the plasma membrane. In contrast, larger extracellular vesicles such as microvesicles and apoptotic bodies (up to 1 μm) originate from direct membrane budding. Exosomes are enriched with diverse biomolecules, including proteins, nucleic acids, lipids, and metabolites. Created using BioRender.com.

stimulating T cells, whereas cancer cell-derived exosomes may aid in immune evasion.[58] Additionally, exosomes facilitate tumor progression by transporting oncogenic factors, promoting angiogenesis, and mediating drug resistance.[43,59] They influence the tumor microenvironment, supporting cancer cell survival and dissemination. Beyond cancer, exosomes are implicated in neurodegenerative diseases by spreading misfolded proteins and inflammatory signals.[60,61] They also contribute to tissue repair, as mesenchymal stem cell-derived exosomes promote regeneration by delivering growth factors and cytokines.[55,56] Their regenerative potential has been explored in treatments for cardiovascular, neurological, and wound-healing applications.[62,63] Furthermore, exosomal RNAs play a significant role in regulating gene expression in recipient cells, affecting processes such as cell cycle progression and histone modification. Their unique RNA cargo makes them promising biomarkers for disease diagnosis and prognosis.[64] Furthermore, exosomes participate in metabolic regulation by transferring metabolic enzymes and substrates, influencing cellular energy balance and disease development.[65] Their lipid composition also affects membrane fluidity and signaling pathways, further highlighting their diverse biological significance.[66]

4. Overview of Exosome Enrichment Methods

With the growing research on exosomes, their potential applications are increasingly being explored. Hence, reliable isolation and enrichment of exosomes are essential for evaluating their biological functions and possible applications. However, the heterogeneity of exosomes in size, content, function, and origin presents challenges for their isolation. Furthermore, most existing isolation methods struggle to fully separate exosomes from lipoproteins with similar biophysical properties and EVs from non-endosomal pathways, leading to low exosomal purity.[11] Therefore, developing efficient exosome enrichment methods for accurate downstream analysis remains a key challenge, as it directly influences the reliability of biomarker discovery, functional studies, and therapeutic applications. For different purposes and applications, various enrichment methods have been established to isolate and purify exosomes from biological samples, such as blood, plasma, breast milk, saliva, and cells.[3,4,7,9,36] These methods include ultracentri-

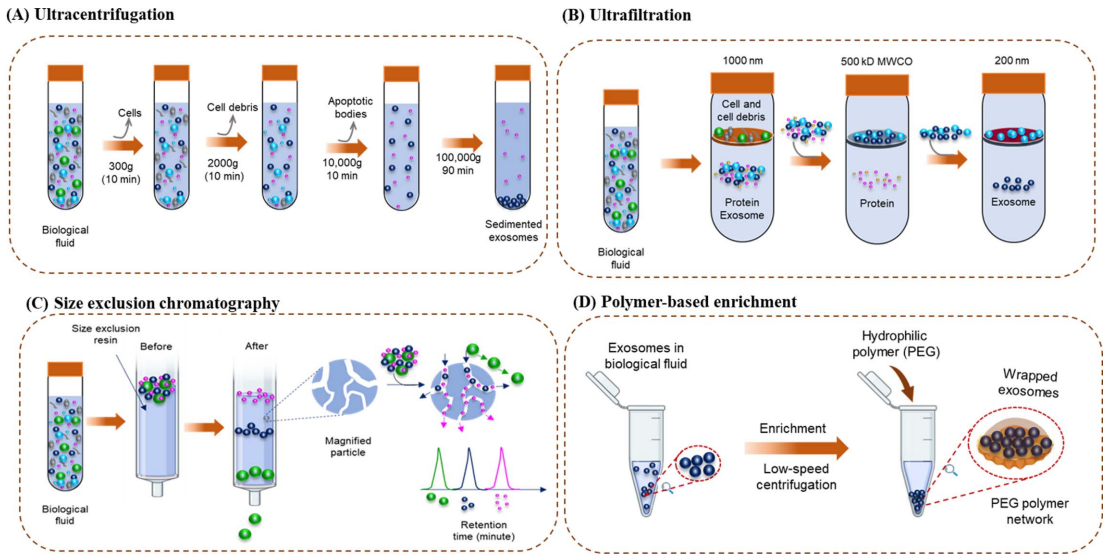

Fig. 2. Common techniques used for exosome enrichment and purification, including (A) ultracentrifugation, (B) ultrafiltration, (C) size exclusion chromatography, and (D) polymer-based precipitation. Among these, ultracentrifugation remains the most widely studied method for exosome isolation.

Table 1. Principles, advantages, and limitations of commonly available exosome isolation methods.

Method	Isolation principle	Advantages	Limitations
Ultracentrifugation	High-speed centrifugation separates and pellets exosomes based on size and density. Multiple spins at increasing speeds isolate exosomes, while other smaller particles are eliminated in the supernatant.	– The gold standard for exosome isolation (most widely used). – No chemical contamination. – Suitable for large sample volumes. – Ease of operation.	– Time-consuming and labor-intensive. – Requires expensive equipment. – Requires large sample volumes. – Potential loss of exosomes due to pelleting. – Co-precipitation of contaminants (e.g., protein aggregates, lipoproteins). – May lead to exosomal rupture.
Ultrafiltration	Membrane filters with specific molecular weight cut-offs are employed to separate exosomes from other components based on size.	– Faster and simpler than ultracentrifugation – No need for specialized equipment – Suitable for high-throughput processing – Can be very well combined with other methods.	– Risk of membrane clogging. – Loss of smaller exosomes. – Shear stress may damage exosomes. – Large starting sample volume. – Protein contamination.
Size exclusion chromatography	Separation and enrichment of exosomes using a porous gel matrix (stationary phase) based on their size. Exosomes elute rapidly due to their size, while smaller molecules are retained within the stationary phase.	– Preserves exosome integrity. – Minimal contamination from proteins/lipoproteins. – Ease-of-use.	– Potentially significant contamination with non-EV particles (e.g., lipoproteins). – Limited sample capacity. – Low exosome yield. – No specificity. – Specialized equipment requirement.
Immunoaffinity methods	Antibodies targeting exosome surface protein markers (e.g., CD9, CD63, CD81) are employed to capture exosomes selectively.	– High specificity. – Very pure exosome isolations. – Suitable for biomarker discovery. – Minimal contamination.	– Expensive (antibody costs) – Low yield. – Specialized equipment requirement. – May miss subpopulations of exosomes (those without specific surface markers).
Microfluidics	Exosomes are captured and isolated based on their unique properties (size-based filtration, density gradient separation, acoustic or electrophoretic sorting, and immunoaffinity capture).	– High efficiency and speed. – Minimal sample volume. – Exosome isolation can be combined with characterization tools. – Exosome isolation can be performed on small or limited sample quantities.	– Low throughput. – Potential loss of exosome subtypes. – Requires a specific level of expertise. – Not suitable for preparative purposes (e.g., therapeutic applications).
PEG-based precipitation	PEG wraps abundant free exosomes and induces their aggregation, followed by low-speed centrifugation (1500 × g) for precipitation.	– Simple, cost-effective, and scalable. – Preserves exosome integrity – Suitable for small or limited sample quantities. – High exosome yield. – Many kits are commercially available. – Possibility for clinical application.	– Co-precipitation of contaminants in the final exosome pellet. – Additional purification may be required.

fugation, ultrafiltration, size exclusion chromatography, immunoaffinity methods, microfluidics, and PEG precipitation. *Fig.* 2 illustrates schematic representations of the four widely utilized methods for exosome enrichment. Each isolation method has unique strengths and limitations that significantly influence the characteristics of the final isolated products.[12] However, the choice of a technique should align with specific research goals and sample characteristics. *Table* 1 summarizes common exosome enrichment methods, including their advantages and limitations. *Table* 2 presents a comparative analysis of isolation techniques, highlighting typical yields, purity metrics, and protein-to-vesicle ratios. These data emphasize

Table 2. Summary of typical yields, purity measures, and protein-to-vesicle ratios for major exosome isolation methods

Method	Typical Yield (particles/mL)	Purity (protein-to-vesicle ratio)*	Key Features/Notes
Ultracentrifugation	10^{10}–10^{11}	1 – 10 μg protein/10^9 vesicles	Gold standard; high purity but time-consuming; may cause vesicle damage and lower yield compared to precipitation methods.
Size Exclusion Chromatography (SEC)	10^9–10^{10}	0.5 – 2 μg protein/10^9 vesicles	High purity; gentle on vesicles; lower yield; scalable for clinical use.
Immunoaffinity Capture	10^8–10^9	<1 μg protein/10^9 vesicles	Highest specificity and purity; very low yield; expensive and limited to known markers.
PEG-based Precipitation	10^{11} – 10^{12}	10 – 50 μg protein/10^9 vesicles	Highest yield; simple and scalable; lower purity due to co-precipitation of contaminants.
Commercial Precipitation Kits	10^{11} – 10^{12}	10 – 60 μg protein/10^9 vesicles	Similar to PEG; convenient but costly; variable purity and yield.

*Protein-to-vesicle ratio is a commonly used indicator of exosome preparation purity. Lower ratios indicate higher purity, as less protein contamination is present per vesicle.

the quantitative aspects discussed in the review and support the overall evaluation of each method.

4.1. Ultracentrifugation

Ultracentrifugation is widely considered the gold standard and the most commonly employed method for exosome isolation.[17] This method separates vesicles based on size and density through sequential centrifugation steps: low-speed centrifugation (300 × g to 2000 × g) removes cells and debris, moderate-speed centrifugation (10,000 × g) excludes larger vesicles, and high-speed ultracentrifugation (>100,000 × g) pellets the exosomes, which are resuspended in a buffer for downstream applications.[19,67] *Fig.* 2(A) illustrates the ultracentrifugation method used for exosome enrichment from biological samples. This technique is particularly suitable for processing large sample volumes and is relatively straightforward to perform with appropriate equipment.[68] However, this method has some limitations, including being time-consuming and labor-intensive, making it less practical for high-throughput applications. Additionally, ultracentrifugation requires expensive instrumentation, which may not be accessible to all laboratories. Another drawback is the potential co-precipitation of contaminants such as protein aggregates and lipoproteins, which can compromise sample purity.[69,70] Furthermore, the high centrifugal forces applied during the process may lead to exosomal rupture, potentially affecting the integrity and functionality of the isolated vesicles. Techniques like density gradient ultracentrifugation are often used for additional refinement.[26] Lower-speed centrifugation at 40,000 × g has also been explored for its simplicity, reduced costs, and the availability of suitable centrifuges.[67]

4.2. Ultrafiltration

Ultrafiltration uses membranes with specific molecular weight cut-offs to isolate exosomes based on size exclusion. Sequential filtration removes large particles (1000 nm filter), free proteins (500 kDa cut-off), and smaller vesicles (<200 nm filter), effectively enriching exosomes.[65] *Fig.* 2(B) presents a schematic representation of the sequential ultrafiltration approach used for exosome enrichment and isolation from biological fluids. Key benefits include its rapid processing of large sample volumes and scalability, particularly in clinical applications requiring quick isolation.[71] However, issues such as membrane clogging, vesicle adhesion, exosome damage due to shear stress, and co-isolation of similarly sized particles may reduce yield and purity.[26,72] Combining ultrafiltration with methods like SEC, precipitation-based enrichment, or ultracentrifugation often improves the specificity of exosome enrichment.[71,73-76]

4.3. Size exclusion chromatography

Size exclusion chromatography (SEC) is a widely

employed technique for isolating and purifying exosomes due to its ability to separate particles based on their size, offering a gentle and non-denaturing method.[31,77] SEC uses a column packed with porous materials that allow smaller molecules to enter the pores, while larger particles, such as exosomes, elute faster because they are excluded from the pores.[78] The process of SCE is shown in *Fig.* 2(C). One of the major advantages of SEC is its ability to separate exosomes with minimal contamination from protein aggregates, lipoproteins, and other extracellular vesicles, improving the purity of the isolated exosomes. Unlike other methods, SEC does not rely on harsh chemical treatments or centrifugation, making it suitable for sensitive downstream analyses. However, SEC does require careful optimization of buffer conditions to maintain exosome integrity, and the process can be time-consuming when large-scale isolation is needed.[16] To enhance its efficiency and reduce the limitations associated with co-isolating non-exosomal particles, SEC can be combined with other techniques like polymer-based precipitation or ultrafiltration.[31,71,73,74] This combination allows for better purification and more effective separation of exosomes from unwanted contaminants, improving the quality of the isolated exosome preparations for further analysis.[71,73-76]

4.4. Immunoaffinity methods

Immunoaffinity methods utilize antibodies that target specific exosomal surface proteins (e.g., CD9, CD63) for selective isolation.[24,25] Magnetic beads or nanostructured supports coated with antibodies allow the efficient capture of exosomes from complex samples.[79,80] Immunoaffinity-based exosome isolation offers high specificity and purity by selectively capturing exosomes expressing specific surface markers. This makes it particularly suitable for biomarker discovery, as it minimizes contamination from non-exosomal components. However, the method has limitations, including high costs due to antibody expenses, low yield, and the potential exclusion of exosome subpopulations lacking the targeted surface markers. Preprocessing with ultracentrifugation or SEC can mitigate some limitations by reducing non-specific contaminants.[81,82]

4.5. Microfluidics

Microfluidics is an emerging and highly innovative approach for exosome isolation, utilizing the manipulation of small fluid volumes in microchannels. This method allows for precise control over the isolation process, often integrating multiple steps such as separation, enrichment, and detection into a single device.[83] Microfluidic platforms utilize various mechanisms, including immunoaffinity capture, size-based filtration, and acoustic or electrophoretic sorting, to isolate exosomes with high specificity and efficiency. These systems are particularly advantageous for their ability to process small sample volumes rapidly, making them ideal for applications requiring high sensitivity, such as clinical diagnostics and personalized medicine.[83] For example, devices like the ExoChip, which incorporates CD63 antibodies, are commonly employed for their high specificity and efficiency in capturing exosomes.[84] Additionally, the integration of microfluidic devices with downstream analytical tools enhances their utility in comprehensive exosomal analysis. Despite their potential, challenges such as device fabrication costs and limited throughput remain areas for further development.[16,85]

4.6. PEG-based precipitation: mechanism, method, and positive aspects

PEG-based precipitation is an emerging and widely used method for exosome isolation. This precipitation method is based on the fact that water-excluding polymers such as PEG can induce phase separation by wrapping abundant free exosomes, reducing their solubility, and thereby promoting their aggregation.[36,76] PEG is a synthetic polymer consisting of repeating units of ethylene oxide, and its structure is commonly expressed as $H\text{-}(O\text{-}CH_2\text{-}CH_2)_n\text{-}OH$. PEG offers beneficial properties such as non-irritability, non-toxicity, high scalability, and good biodegradability, making it (along with its derivatives) widely used in pharmacokinetics and drug delivery, with approval from the U.S. FDA.[86] It plays a crucial role in enhancing therapeutic efficacy by improving pharma- cokinetic

properties and enabling the formulation of long-circulating nanoparticle-based drugs.[86] These applications further validate PEG's biocompatibility, non-toxicity, and safety profile. As a hydrophilic polymer, PEG creates a dehydrating effect, leading to exosome clustering, which can then be efficiently harvested through a single low-speed centrifugation step.[87] The exact mechanism underlying PEG-induced precipitation remains incompletely understood. However, two theories have been proposed to explain this process: the excluded volume theory and the attractive depletion force theory.[88] The excluded volume model suggests that PEG reduces the hydration and solubility of biomolecules, leading to their aggregation and precipitation. Meanwhile, the attractive depletion force theory attributes precipitation to the osmotic pressure exerted by PEG, which induces molecular attraction and facilitates aggregation.[89]

Originally developed for virus isolation,[34,90] PEGs of varying molecular weights and concentrations have been widely adopted for exosome enrichment due to their similar biophysical properties.[36,44,76,87] This precipitation method has also been extensively applied to precipitate nucleic acids and other biomolecules.[91] In detail, biological samples can be diluted with phosphate-buffered saline and undergo initial centrifugation at 1,000-2,000 × g for 30 min at 4 °C to remove cellular debris and dead cells.[33] Following this, the supernatant is filtered through a 0.22 μm membrane to further remove non-exosomal components. The resulting filtrate is then mixed with an aqueous PEG solution with an average molecular weight of 10 kDa and incubated for a specified time, allowing sufficient interaction between PEG and exosomes. Typically, PEG concentrations ranging from 5 % to 15 % (w/v) are used based on the biological sample and the desired efficiency.[92] This incubation promotes exosome aggregation by enhancing their self-assembly and increasing their mass, facilitating efficient recovery at relatively low centrifugal forces. The final centrifugation step performed at 1,500 – 16,000 × g for 10 – 60 min at 4 °C can isolate exosome-rich precipitates and form the exosome pellet.[33] The addition of sodium chloride (NaCl; 1 M) during PEG dissolution serves several important purposes.[33] NaCl influences exosome stability, enhancing their separation from smaller molecules and unwanted proteins during incubation. It also modulates the solution's ionic strength, facilitating the enrichment and purification of exosomes. Moreover, NaCl plays a crucial role in regulating the solubility and interactions of proteins and other biomolecules, promoting the efficient separation of exosomes from non-exosomal components.[33] Notably, an optimal NaCl concentration can weaken electrostatic interactions among proteins,[93,94] aiding in the dissociation of impurities from the exosome surface and thereby improving exosome purity.

Notably, the PEG-based precipitation technique is highly regarded for its simplicity, rapid processing, scalability for large sample sizes, and cost-effectiveness, making it particularly advantageous as an accessible alternative for high-throughput studies at laboratories with limited resources.[87] PEG-based methods require minimal specialized equipment, making them more accessible for clinical research. In contrast to ultracentrifugation and ultrafiltration, which may distort vesicle shape, the PEG precipitation method preserves the morphological and functional integrity of exosomes, thus preserving their structural and functional properties for downstream proteomic and functional studies.[36,95] PEG could also be an effective option for processing small and limited sample volumes, such as serum and plasma, rather than large-scale preparations, and often results in a higher exosome yield than differential ultracentrifugation.[7,96,97] Moreover, PEG-precipitated exosomes have shown particular suitability for downstream nucleic acid analysis, especially RNA and DNA profiling.[98-100] Notably, studies assessing DNA quantification from exosomes isolated via PEG precipitation revealed superior total DNA recovery, underscoring the potential of PEG-based methods for DNA-related downstream applications, particularly in the context of malignant solid neoplasm.[15] PEG was also found to enhance the bio-functional properties of exosomes. For example, Warren *et al.* demonstrated that PEG coating significantly improved the mucin permeability and stability of milk-derived exosomes, even under the

harsh conditions of acidic gastric environments.[8]

However, due to its non-specific mechanism, PEG precipitation can co-isolate various macromolecules, including lipoproteins and protein complexes, which may compromise exosomal purity. As shown in *Table* 2, this method typically results in high yield but low purity compared to other exosome isolation techniques. Consequently, additional purification steps, such as size-exclusion chromatography or density gradient centrifugation, may be required to enhance sample specificity.[11,12,33] Despite this limitation, this highly hydrophilic polymer remains effective in clinical research settings, and its advantages make it a valuable tool for rapid and efficient exosome extraction, especially from small sample volumes which is fundamental in the context of translational and clinical research.[101]

5. Recent Applications of PEG Precipitation in Exosome Isolation

5.1. Serum

Human serum is one of the most commonly used biological fluids for exosome research due to its clinical relevance. PEG-based precipitation has been widely employed for isolating serum-derived exosomes. For instance, Andreu *et al.*[99] evaluated PEG precipitation (50 % PEG 6000 in 375 mM NaCl) for enriching EVs from frozen human serum for miRNA analysis. They compared PEG with ultracentrifugation and commercial kits, assessing purity and yield using nanoparticle tracking analysis, Western blot, and cytometry. PEG effectively enriched EV-associated miRNAs (e.g., miR-126, miR-30c, miR-143) while showing minimal recovery of non-EV miRNAs. The study demonstrated PEG's efficiency, cost-effectiveness, and compatibility with long-term frozen samples (up to 8 years), making it a viable alternative for EV-based biomarker studies. In another research, the potential of serum-derived EVs for lung cancer diagnosis by profiling EV-derived proteins was investigated.[102] EVs were isolated from serum by PEG-based precipitation and immunoaffinity separation. First, serum (600 μL) was centrifuged at 12,000 × g for 30 min at 4 °C to remove debris. Then, 25% PEG 2000 was added to achieve a final percentage of 12 % PEG, and the mixture was incubated on ice for 1 h. The process was repeated twice, and the pellet was resuspended in 200 μL PBS. Proteomic analysis, including 2-D gel electrophoresis and MALDI-TOF MS, identified 55 upregulated protein spots. Seven proteins (CD5L, CLEC3B, ITIH4, SERFINF1, SAA4, SERFINC1, and C20ORF3) were highly expressed in cancer patient-derived EVs. García-Romero *et al.*[15] compared the efficiency of common EV isolation methods in human serum, including ultracentrifugation, PEG precipitation (10 % (w/v) of PEG 8000), and two commercial kits. The incubation was performed overnight at 4 °C, followed by centrifugation at 16,100 × g for 1 h. Digital PCR was used for the first time to detect specific gDNA sequences, offering diagnostic and monitoring potential for various diseases. The results highlighted PEG precipitation as the most cost-effective and feasible EV isolation method.

Functionalizing nanoparticles with PEG, known as PEGylation, enhances stability, biocompatibility, and ability to selectively interact with biological targets. PEGylation reduces nanoparticle aggregation, minimizes nonspecific interactions, and provides functional groups for conjugating biomolecules, making it a valuable strategy for exosome isolation and biomedical applications.[103] More recently, Guru *et al.*[104] explored a novel and rapid method for isolating small exosomes (≤50 nm) from human serum using a bench-top centrifuge. This approach was enhanced by functionalizing citrate-capped gold nanoparticles (CGNPs) with PEG to form PEGylated GNPs (PGNPs). The PEG was activated using EDC/SNHS chemistry to conjugate antibodies targeting exosomal surface proteins. The antibody-conjugated PGNPs were then incubated with serum, forming PGNP-exosome complexes. These complexes were efficiently separated through low-speed centrifugation (7000 × g), a significant improvement over standard ultracentrifugation (100,000 × g), enabling exosome isolation in less than 2 h. In another interesting work, Chang *et al.*[105] presented a novel method for isolating exosomes using PEG-coated Fe_3O_4 magnetic nanoparticles (MNPs). The

nanoparticles, synthesized via chemical co-precipitation, were approximately 20 nm in diameter, with larger agglomerates reaching several hundred nanometers. The PEG-coated Fe_3O_4 MNPs effectively reduced the protein concentration in fetal bovine serum (FBS) to 39.89 % of the original without affecting the exosome concentration, as evidenced by particle size distribution and gel electrophoresis. The method removed most serum proteins, including albumins and immunoglobulins, ensuring purified exosomes for further analysis. This technique offers a simple, effective approach for protein removal in serum during exosome isolation.

5.2. Plasma

Plasma, as a widely accessible biological fluid, has become a key source for the isolation of exosomes due to its abundance of extracellular vesicles.[7] In a recent study, Tangwattanachuleeporn's group compared the efficacy of different PEG concentrations with two commercial exosome isolation kits, ExoQuick and Total Exosome Isolation, in human plasma.[7] They found that PEG concentrations of 10 – 20 % yielded exosomes with similar size and concentration to ExoQuick, outperforming TEI. Notably, 10 % PEG enhanced the detection of miR-122 and miR-16 expression compared to both ExoQuick and TEI. In proteomics, PEG at 10 % also showed better protein identification, although serum contamination remained a challenge. These results suggest that 10 % PEG is an optimal and cost-effective choice for clinical exosome isolation.

Dextran Blue (DEXB), a high-molecular-weight polysaccharide (2000 kDa), has been utilized to enhance the aggregation of EVs, allowing for more efficient precipitation of membrane-coated particles while minimizing the co-isolation of membrane-free microparticles, large biopolymers, and supramolecular complexes. Konoshenko *et al.*[98] developed a simple and cost-effective aggregation–precipitation method for isolating EVs from plasma using dextran blue for aggregation and PEG for precipitation. 500 μL of human plasma was mixed with NaCl, PBS, Tris-HCl, DEXB, and 25 % PEG 20000 in PBS. The mixture was incubated for 30 min at 4 °C, followed by centrifugation at 17,000 × g for 20 min. The supernatant was discarded, and the pellet was resuspended in 500 μL PBS, frozen in liquid nitrogen, and stored at -80 °C for miRNA isolation or EV analysis. This method demonstrated comparable efficacy to ultracentrifugation in terms of EV isolation, miRNA recovery, and absence of polymerase inhibitors, as confirmed by transmission electron microscope (TEM), dynamic light scattering (DLS), and miRNA analyses. The approach proved to be fast, requires no complex equipment, and is adaptable to various biofluids, making it suitable for both research and clinical applications. Gámez-Valero *et al.*[106] compared plasma EV isolation methods, including SEC, 50 % PEG6000, and PRotein Organic Solvent PRecipitation (PROSPR), assessing their impact on EV purity, composition, and biological effects. SEC effectively removed abundant plasma proteins, as confirmed by low protein content and Cryo-EM, while PEG and PROSPR retained contaminants. Only SEC preserved key EV markers (CD9, CD63, CD81, LGALS3BP, CD5L), suggesting that precipitating agents may alter EV integrity. Additionally, EVs isolated via PEG and PROSPR reduced cell viability in vitro. These findings highlight the importance of selecting an appropriate isolation method based on downstream applications.

5.3. Urine

Urine is a markedly favorable biological specimen due to its non-invasive nature, and urinary EVs (UEVs) serve as a promising source of biomarkers for the non-invasive detection of various diseases. Sight *et al.*[107] highlighted the potential of UEVs as biomarkers for diagnosing and monitoring various diseases. The study optimized a PEG-based UEV isolation method following MISEV guidelines, ensuring efficiency, reliability, and suitability for diverse downstream applications. The purified urine (20 mL) was mixed with 24 % w/v PEG Mn6000 in 1 M NaCl (1:1 ratio), achieving a final PEG concentration of 12 % w/v. The UEVs were finally pelleted by centrifugation at 10,000 × g for 60 min and resuspended

in PBS for storage at -80 °C. This cost-effective and accessible approach is well-suited for clinical labs and longitudinal studies, supporting the use of UEVs as a robust biomarker source. In another interesting study, Lv *et al*.[108] evaluated a cost-effective and efficient PEG-based method for isolating urinary exosomes to diagnose renal fibrosis (RF). A 30 mL urine sample was centrifuged at 3,000 × g for 10 min to remove debris. The supernatant was mixed with 12.5 mL of 24 % PEG 6000, incubated overnight at 4 °C, and centrifuged at 4,000 × g for 1 h. The pellet was resuspended in PBS and divided for exosome identification and RNA isolation. Exosomal miR-29c and miR-21 were significantly dysregulated in RF patients, with diagnostic potential demonstrated by ROC analysis (AUC: 0.8333 and 0.7639, respectively, $P < 0.05$). miR-29c levels showed a strong negative correlation with estimated glomerular filtration rate (eGFR) and interstitial fibrosis. This PEG-based approach proved to be a simple, low-cost alternative for urinary exosome isolation, supporting miRNA-based RF diagnostics. Furthermore, Konoshenko *et al*.[98] developed a simple and cost-effective aggregation–precipitation method for isolating EVs from urine using dextran blue for aggregation followed by precipitation with PEG. Human urine (5 mL) was mixed with NaCl, $NaHCO_3$, DEXB, and 25 % PEG 20000 in PBS, followed by incubation at 4 °C for 30 min. After centrifugation at 17,000 × g for 20 min, the supernatant was discarded, and the pellet was resuspended in 500 μL PBS, frozen in liquid nitrogen, and stored at -80 °C for RNA extraction or EV analysis. Compared to ultracentrifugation, this method demonstrated comparable efficiency in isolating urinary EVs, as confirmed by TEM, dynamic light scattering, and miRNA analysis. The approach effectively preserved miRNA integrity and was free of polymerase inhibitors, making it suitable for clinical and research applications.

5.4. Saliva

Saliva is a non-invasive, easily accessible, and simple-to-collect biological fluid. It offers higher patient compliance than other fluids and holds the potential as a source of biomarkers for oral and systemic diseases.[109] Li *et al*.[110] compared two methods for isolating salivary EVs (ultracentrifugation and PEG-based precipitation) using proteomics. Both methods yielded EVs ranging from 40 to 210 nm, with PEG-separated EVs exhibiting a broader size distribution and irregular aggregation, while UC-separated EVs were more monodispersed and teacup-shaped. UC-separated EVs showed higher expression of EV-specific markers. Proteomic analysis identified 1217 salivary exosomal proteins, with 361 differential proteins, demonstrating that UC isolated a broader range of EV-related proteins. These findings suggest that UC is a more efficient method for isolating salivary EVs. Rider *et al*.[87] developed the ExtraPEG method (8 % PEG+wash), a cost-effective and efficient approach for isolating EVs, including exosomes, using PEG-based precipitation. ExtraPEG enabled rapid EV enrichment from large saliva volumes with low-speed centrifugation, followed by a single ultracentrifugation step. The method yields EVs with sufficient RNA and protein quality for proteomics and sequencing, making it suitable for biomarker discovery. Moreover, confocal microscopy confirmed that vesicle integrity and biological activity were preserved. In another research, Deregibus's group introduced a charge-based precipitation method for isolating EVs from saliva, using protamine (P) in combination with PEG.[111] For EV isolation, samples were mixed with a protamine/polyethylene glycol (PEG 35,000) solution at a 1:4 ratio and incubated overnight at 4 °C. After centrifugation at 1,500 × g for 30 min at 22 °C, the pellet was resuspended in buffer for further analysis. To remove lipoproteins, samples were passed through Sephadex G-100 spin columns, and EVs were collected from the void volume. This technique leverages the negative charge of EVs to facilitate precipitation without the need for ultracentrifugation. Compared to ultracentrifugation, the P/PEG method demonstrated higher EV recovery, as confirmed by NanoSight analysis, while maintaining similar EV size and exosomal marker expression (CD63, CD9, CD81). RNA yield was comparable, and the isolated EVs retained biological activity,

promoting wound closure and cell proliferation. This simple, cost-effective approach enables efficient EV isolation from small saliva samples, supporting its application in biomarker research.

5.5 Cell culture and cell tissue

Cell culture is commonly used to isolate exosomes because it provides a controlled environment to study and harvest exosomes from specific cell types, enabling consistent and reproducible results for research and diagnostic applications.[36,44,112] Weng *et al.*[36] demonstrated a PEG-based approach for efficiently and cost-effectively isolating exosomes from HeLa cell culture supernatant. The exosome preparation was carried out in two steps. The conditioned medium was mixed with the PEG stock to a final concentration of 10 % PEG 1000, incubated at 4 °C for 30 min, and centrifuged at 3,000 × g for 10 min. The exosome pellet was collected for further analysis. In the second step, the exosome pellet was resuspended in PBS, and PEG was added again (10 % final concentration). After another 30-min incubation at 4 °C, the sample was centrifuged at 3,000 × g for 10 min, and the pellet was finally harvested for downstream biological analysis. High-resolution electron microscopy confirmed the size and morphology of PEG-precipitated exosomes, while proteomic analysis identified 6,299 protein groups encoded by 5,120 genes, including 97 % of the Top 100 exosome markers in the ExoCarta database. Additionally, the study revealed a higher ratio of neo-cleavage sites in exosomal proteins, suggesting a potential role for exosomes in protein degradation and transport. These findings highlight the efficacy of PEG-based exosome isolation for biomarker discovery and biological studies. In a recent study, Ludwig *et al.*[44] developed an optimized PEG-based precipitation method for scalable and reproducible enrichment of EVs from cell culture supernatants. Compared to traditional methods like UC and SEC, this approach offers a more efficient alternative while maintaining EV functionality. Additional washing and reprecipitation steps helped reduce PEG co-precipitated contaminants such as bovine serum albumin (BSA), though some non-EV molecules remained. Despite this, PEG-enriched EVs retained therapeutic efficacy in an ischemic stroke model, indicating that co-purified components did not impair their biological activity. While not ideal for the molecular profiling of pure EVs, the PEG method provides a practical and scalable solution for enriching functional EVs from cell-derived tissues.

On the other hand, prostate cancer (PCa) is the most common solid malignant disease worldwide.[10] Yi *et al.*[112] demonstrated that PEG precipitation is an efficient and cost-effective method for isolating exosomes from prostate cancer cell lines (LnCap, PC3, and DU-145), yielding purity comparable to commercial kits. A PEG solution (16 g PEG, 5.844 g NaCl in 100 mL water) was prepared and mixed with prostate cancer cell cultures. After sequential centrifugation at 500 g and 2000 g to remove debris, cells were resuspended, incubated overnight with PEG at 4 °C, and centrifuged at 16,000 g for 1 h. The pellet was washed and resuspended in PBS for further analysis. Liquid chromatography-mass spectrometry has become one of the most sensitive analytical methods to provide information on the molecular mass and structural features of molecules.[113-127] By using this technique, they analyzed the lipid composition of these exosomes and found that LnCap-derived exosomes were enriched in phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol compared to whole cells. Interestingly, lysophosphatidylcholine (LPC), a toxic metabolite absent in LnCap cells, was detected in their exosomes, suggesting a role in cancer progression. This study underscores the effectiveness of PEG precipitation for exosome isolation from cell-derived tissues and its potential in biomarker discovery for prostate cancer screening.[112]

5.6. Mesenchymal stem cells

Exosomes have been identified as cell-cell communication agents, and those derived from mesenchymal stem cells (MSCs) exhibit therapeutic effects similar to those of the cells of origin. MSCs are multipotent cells primarily known for their role in immune regulation through paracrine effects, aiding in tissue repair and cancer therapy. In a recent study, Jia *et*

al.[96] demonstrated that PEG precipitation is a more efficient method than UC for isolating EVs from amniotic fluid-derived mesenchymal stem cells (AF-MSCs). Large vesicles were eliminated by centrifugation at 10,000 × g for 45 min at 4 °C, and the resulting supernatant was utilized for EV isolation. Afterwards, an equal volume of PEG 20000 solution (16 g PEG in 100 mL 1 M NaCl) was added to 125 mL of cell culture medium and incubated at 4 °C for 12 h. The mixture was then centrifuged at 10,000 × g for 20 min at 4 °C to collect the EV pellet, which was resuspended in 500 μL of precooled PBS. While both methods produced EVs with similar morphology, size, and marker expression, PEG precipitation yielded a higher number of EV particles, protein, and RNA. In a THP-1 infection model, MSC-derived EVs suppressed LPS-induced cytokine secretion, with UC-EVs more effective at inhibiting TNF-α, while PEG-EVs more effectively reduced IL-10 levels. Börger *et al.*[128] developed a PEG-based precipitation method for efficient extraction of MSC-EVs from large volumes of conditioned medium. This scalable protocol allows the preparation of MSC-EVs without the need for specialized equipment. The PEG-based method has been successfully applied to treat a human graft-versus-host disease patient and several animal models, demonstrating its therapeutic potential. While the method has some limitations, it provides a practical approach for initial evaluations of EV-based therapies.

6. Limitations of PEG Precipitation Methods

As mentioned earlier, PEG precipitation is a widely used approach for exosome isolation due to its attractive features, including but not limited to simplicity, cost-effectiveness, and scalability. Despite these advantages, the method presents challenges and limitations that can impact exosome isolation's purity, reproducibility, and overall efficiency.[11, 129] One major drawback is the possible co-precipitation of other contaminants, resulting in low-quality exosome isolation. Since PEG is a non-ionic polymer that easily alters the dielectric constant of water and induces phase separation primarily based on solubility rather than specific exosome properties, it lacks selectivity, leading to impure preparations.[95] Besides exosomes, other components, such as some cell fragments, lipoproteins, immunoglobulins, viral particles, immune complexes, and other contaminants, are present in the final exosome pellets precipitated by PEG.[26,130] These contaminants can interfere with downstream applications such as proteomic, lipidomic, and RNA-based analyses, necessitating additional purification steps. Therefore, it could be argued that many studies may have overestimated their EV yield when using precipitation techniques, as the assessment was primarily based on total protein content rather than on EV-specific markers.[76] Unlike ultracentrifugation or immunoaffinity-based methods, which provide greater selectivity, PEG-based approaches often require complementary techniques to effectively distinguish exosomes from other vesicle populations.[9,39] Another limitation of PEG precipitation is the potential retention of residual PEG molecules in the final exosome preparation.[26,129] PEG is a polymer with high viscosity and strong water-binding properties, making it difficult to remove completely through conventional washing steps.[86] The presence of residual PEG can interfere with various biochemical assays, affect nanoparticle tracking analysis (NTA) measurements, and compromise mass spectrometry-based proteomic studies. These challenges necessitate both pre- and post-isolation steps. Pre-isolation focuses on eliminating subcellular particles like lipoproteins, while post-isolation involves removing polymeric residues using a Sephadex G-25 column.[26]

The efficiency of PEG precipitation is also influenced by various factors, including PEG molecular weight, concentration, sample composition, incubation time, and incubation temperature.[36,87,95] These parameters must be carefully optimized, as deviations can significantly affect exosome yield and quality. For instance, while high PEG concentrations enhance precipitation efficiency, they may also increase the co-precipitation of unwanted macromolecules.[131,132] Additionally, prolonged incubation times can lead to exosome aggregation, altering their size distribution

and potentially affecting their biological activity.[33] Such variability poses challenges in achieving consistent and reproducible results, particularly in comparative studies or large-scale clinical applications. To address these limitations, optimization strategies such as combining PEG-based isolation with other techniques of exosomal enrichment, refining PEG formulations, and establishing standardized protocols are necessary.

7. Efforts for Enhancing PEG Precipitation Methods

The limitations of PEG-based exosome precipitation can be mitigated by integrating it with other exosome isolation techniques and optimizing the process through multiple PEG precipitation cycles to enhance its performance. Although exosomes isolated through PEG precipitation may contain impurities, their purity improves significantly when combined with complementary methods. For instance, coupling PEG precipitation with UC or SEC has proven to be an effective strategy for minimizing contaminants, such as bovine serum albumin (BSA), lipoproteins, and immunoglobulins that are often found in the final pellet.[9,39] While UC alone efficiently separates exosomes, it may also introduce unwanted impurities, a challenge that can be addressed by the stabilizing effect of PEG, which promotes more stable exosome aggregates.[12] This combination reduces background contaminants, thereby enhancing the purity and quality of the isolated exosomes, which is advantageous for functional, proteomic, and molecular downstream analyses. Ludwig *et al*.[9] optimized an integrated strategy combining PEG precipitation with ultracentrifugation UC to enhance EV enrichment from cell culture supernatants. This scalable and reproducible protocol addresses the limitations of conventional EV isolation methods by improving yield while maintaining functional activity. Washing the PEG-precipitated pellet and performing UC re-precipitation significantly reduced non-EV contaminants, which was validated by the calculated ratio of particles per mg protein. Although a minor percentage of non-EV molecules may still co-precipitate, PEG-enriched EVs demonstrated similar therapeutic efficacy to parent cells in an ischemic stroke model indicating that these co-purified molecules did not compromise the functional properties of the isolated EV samples. In another research, Martínez-Greene *et al*.[39] developed an integrated workflow combining PEG precipitation with SEC to enhance EV isolation, addressing challenges in EV purity and heterogeneity. By separating EV subtypes based on CD9, CD63, and CD81 content and elution time, they identified distinct early- and late-eluting EV fractions with unique proteomic profiles. Proteomic analysis revealed 286 exclusive proteins in early fractions and 148 proteins with differential concentrations. This method improved EV purity and allowed for subtype characterization, contributing to functional EV studies and standardizing EV isolation protocols.[39]

On the other hand, Park *et al.*[40] emphasized that a single-cycle PEG precipitation may not be sufficient for efficient exosome isolation due to the potential co-precipitation of non-exosomal proteins. To address this, they developed a multiple-cycle PEG precipitation strategy, which significantly enhanced the purity of EV fractions from clinical specimens while minimizing sample volume requirements. The purity was assessed using multiple reaction monitoring (MRM) of key proteins (A2M, THBS1, LGALS3BP, and ALB) and validated by shotgun proteomics, which revealed that 89 % of identified proteins were EV-related, as confirmed by the EVpedia database. This optimized, scalable method offers higher EV yield, better purity, and improved efficiency compared to UC, making it more suitable for proteomics research and clinical applications.

8. Precipitation-based Commercial Kits

To date, various companies have developed a range of exosome isolation kits utilizing precipitation-based methods to facilitate the extraction of exosomes from small biofluid volumes, such as ExoQuick™ precipitation solution (System Biosciences), Total Exosome isolation kit (TEI, Invitrogen), ExoGAG (Nasasbiotech),

miRCURY (Exiqon), Exoprep (HansaBioMed), miRCURY exosome kits (QIAGEN), Exosome precipitation solutions (Immunostep), and PureExo Exosome isolation kit (101Bio).[7,22,94,95] These kits typically contain pre-formulated polymeric reagents designed for selective vesicle precipitation while minimizing contaminants and significantly simplifying the workflow by eliminating the need for tedious reagent preparation and optimizing PEG concentration, molecular weight, and ionic strength. The kits also offer standardized protocols that enhance reproducibility, preserve exosome integrity, and ensure high-quality yields.[22] However, commercial kits provide limited flexibility in experimental design, and the inherent variations between batches, brands, and models contribute to exosome heterogeneity, potentially affecting experimental consistency and result reliability.[7,15] Despite significant advancements by biotechnology companies and the widespread availability of exosome isolation kits, their high cost remains a major limitation, thereby restricting their broader applications. Furthermore, studies have shown that exosomes isolated using PEG-based kits resulted in reduced cell viability when applied to tumor cells in functional assays, compared to EVs isolated via differential ultracentrifugation.[133,134] This suggests that certain chemical component (s) present in the precipitation reagent, which may co-precipitate with the exosomes, could exert cytotoxic effects on cells, potentially confounding the results of functional analyses. While commercial kits provide convenience, studies suggest that optimizing the conventional PEG precipitation protocols, such as adjusting PEG molecular weight and concentration or incorporating additional purification steps, can enhance exosome enrichment efficiency while maintaining greater cost-effectiveness than commercially available alternatives.[7,15,36,87,99,112,135] For instance, the efficiency of common exosome isolation methods, including ultracentrifugation, PEG precipitation (10 % (w/v) of PEG 8000), and two commercial kits (ExoQuick® and PureExo®) were evaluated, and the obtained results highlighted PEG precipitation as the most cost-effective and feasible isolation method.[15] In this study, exosomes isolated through PEG precipitation demonstrated the highest total DNA recovery, highlighting their potential value for downstream DNA analysis, particularly in studies related to malignant solid tumors.[15] A similar trend was also observed in Alvarez's study, where PEG-based precipitation also demonstrated superior performance, yielding higher quantities of exosomes along with enhanced recovery of microRNA and mRNA.[37] This method proved to be particularly advantageous for RNA profiling and downstream proteomic analyses, especially in settings where ultracentrifugation is unavailable or when processing large sample volumes.

9. Future Perspectives and Considerations

Despite the advancements in optimizing PEG precipitation methods, several key areas remain for improvement to enhance the overall quality and efficiency of exosome isolation. Future advancements in exosome isolation techniques should aim to enhance the specificity, reproducibility, and efficiency of PEG-based methods by exploring novel and cheaper polymer formulations that might minimize the unwanted co-isolation of contaminants. By overcoming these challenges, PEG precipitation can be further refined to provide a reliable and scalable approach for exosome research and therapeutic applications. One promising avenue could be the incorporation of engineered nanoparticles or affinity ligands that target exosome-specific surface markers, further enhancing purity and specificity in the precipitation process. Additionally, the development of new, more affordable commercial kits that incorporate optimized protocols could broaden access to exosome research, making these technologies more widely accessible. The combination of automated and high-throughput systems with improved isolation methods could lead to faster, more reliable results in clinical and research applications.

Another important area for future work is the standardization of protocols across research labs and clinical facilities. While PEG precipitation offers significant advantages in terms of cost-effectiveness

and scalability, the variability in experimental conditions, including PEG concentration, incubation time, and temperature, makes it difficult to compare results across studies. A unified protocol that ensures consistency and reproducibility across different laboratories could significantly enhance the reliability of exosome-related findings.

10. Conclusion

In the past few years, the secretion of membrane-enclosed vesicles, such as exosomes, by various cell types has been identified as a novel mechanism of intercellular communication. Moreover, exosomes have become valuable tools for understanding disease progression and hold great potential for use in diagnostic applications, thanks to their ability to preserve molecular markers from released cells. Therefore, achieving high-yield exosome isolation has become a key focus for researchers in recent years. However, researchers face challenges such as labor-intensive processes, low exosome yields, high equipment costs, large sample volume requirements, and lengthy procedures with current exosome isolation techniques. PEG, recognized by the FDA for its biosafety, is an excellent precipitation agent for exosome isolation due to its versatile colloidal properties. PEG precipitation often offers a cost-effective, efficient, and scalable method for exosome isolation, making it a widely used approach in various research and clinical applications. Its simplicity, minimal equipment requirements, and compatibility with small sample volumes contribute to its popularity. Notably, PEG precipitation is comparable to or even better than standard or commercial methods and outperforms other techniques in terms of exosome yield. Moreover, studies have shown that exosomes coated with PEG are effectively shielded from mechanical damage and exhibit enhanced compatibility for subsequent analyses involving DNA, RNA, and proteins. Therefore, using PEG for exosome preparation is considered one of the most effective methods for isolation.

Despite these attributes, some challenges in the PEG precipitation method should be addressed. The non-selective nature of PEG poses limitations, as it leads to the co-precipitation of unwanted contaminants, including lipoproteins, immunoglobulins, and cellular debris. These contaminants not only reduce the purity of isolated exosomes but also hinder downstream analyses, such as proteomics, lipidomics, and RNA-based profiling. However, combining PEG precipitation with complementary exosome isolation techniques or using multiple PEG precipitation cycles has shown promising results in enhancing exosome purity. These strategies effectively reduce contaminant levels, improve exosome yield, and ensure the functional integrity of the isolated vesicles. On the other hand, optimizing PEG parameters, such as molecular weight, concentration, and incubation time, is critical to balancing efficient exosome precipitation with minimizing the co-precipitation of non-exosomal proteins and other contaminants. Commercial PEG-based exosome isolation kits have simplified the process, offering standardized protocols for researchers; however, their high cost limited their broader applications.

In conclusion, the PEG precipitation method remains an essential tool in exosome research. By refining the method through optimization, integration with other isolation techniques, and addressing the challenges of purity and contamination, PEG precipitation can continue to be an efficient method for exosome isolation. This review article provides a comprehensive overview of the latest advancements in exosome isolation using PEG, offering a deeper insight into PEG-based methods. It also highlights the potential of PEG precipitation as a highly promising approach for advancing exosome isolation techniques in both basic research and clinical applications.

Conflict of Interest

We declare that we have no conflict of interest.

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References

1. M. Onozato, Y. Tanaka, M. Arita, T. Sakamoto, H. Ichiba, K. Sadamoto, M. Kondo, and T. Fukushima, *Practical Laboratory Medicine*, **12**, e00099 (2018). https://doi.org/10.1016/j.plabm.2018.e00099
2. R. M. Johnstone, M. Adam, J. R. Hammond, L. Orr, and C. Turbide, *Journal of Biological Chemistry*, **262**(19), 9412-9420 (1987). https://doi.org/10.1016/S0021-9258(18)48095-7
3. F. Gao, F. Jiao, C. Xia, Y. Zhao, W. Ying, Y. Xie, X. Guan, M. Tao, Y. Zhang, W. Qin, and X. Qian, *Chemical Science*, **10**(6), 1579-1588 (2019). https://doi.org/10.1039/C8SC04197K
4. S. A. Melo, L. B. Luecke, C. Kahlert, A. F. Fernandez, S. T. Gammon, J. Kaye, V. S. LeBleu, E. A. Mittendorf, J. Weitz, N. Rahbari, C. Reissfelder, C. Pilarsky, M. F. Fraga, D. Piwnica-Worms, and R. Kalluri, *Nature*, **523**(7559), 177-182 (2015). https://doi.org/10.1038/nature14581
5. H. Miyake, C. Lee, S. Chusilp, M. Bhalla, B. Li, M. Pitino, S. Seo, D. L. O'Connor, and A. Pierro, *Pediatric Surgery International*, **36**(2), 155-163 (2020). https://doi.org/10.1007/s00383-019-04599-7
6. H.-Q. Nguyen, D. Lee, Y. Kim, M. Paek, M. Kim, K.-S. Jang, J. Oh, Y.-S. Lee, J. E. Yeon, D. M. Lubman, and J. Kim, *Analytical Chemistry*, **91**(20), 13297-13305 (2019). https://doi.org/10.1021/acs.analchem.9b04198
7. M. Tangwattanachuleeporn, P. Muanwien, Y. Teethaisong, and P. Somparn, *Medicina*, **58**(11), 1600 (2022). https://doi.org/10.3390/medicina58111600
8. M. R. Warren, C. Zhang, A. Vedadghavami, K. Bokvist, P. K. Dhal, and A. G. Bajpayee, *Biomaterials Science*, **9**(12), 4260-4277 (2021). https://doi.org/10.1039/D0BM01497D
9. A.-K. Ludwig, K. De Miroschedji, T. R. Doeppner, V. Börger, J. Ruesing, V. Rebmann, S. Durst, S. Jansen, M. Bremer, E. Behrmann, B. B. Singer, H. Jastrow, J. D. Kuhlmann, F. El Magraoui, H. E. Meyer, D. M. Hermann, B. Opalka, S. Raunser, M. Epple, P. A. Horn, and B. Giebel, *Journal of Extracellular Vesicles*, **7**(1), 1528109 (2018). https://doi.org/10.1080/20013078.2018.1528109
10. H. Shao, H. Im, C. M. Castro, X. Breakefield, R. Weissleder, and H. Lee, *Chemical Reviews*, **118**(4), 1917-1950 (2018). https://doi.org/10.1021/acs.chemrev. 7b00534
11. Y. Zhang, J. Bi, J. Huang, Y. Tang, S. Du, and P. Li, *Int. J. Nanomedicine,* **15**, 6917-6934 (2020). https://doi.org/10.2147/ijn.S264498
12. I. Jalaludin, D. M. Lubman, and J. Kim, *Mass Spectrometry Reviews*, **42**(2), e21749 (2023). https://doi.org/10.1002/mas.21749
13. R. Kalluri and V. S. LeBleu, *Science*, **367**(6478), eaau6977 (2020). https://doi.org/10.1126/science.aau6977
14. A. L. S. Revenfeld, R. Bæk, M. H. Nielsen, A. Stensballe, K. Varming, and M. Jørgensen, *Clinical Therapeutics*, **36**(6), 830-846 (2014). https://doi.org/10.1016/j.clinthera.2014.05.008
15. N. García-Romero, R. Madurga, G. Rackov, I. Palacín-Aliana, R. Núñez-Torres, A. Asensi-Puig, J. Carrión-Navarro, S. Esteban-Rubio, H. Peinado, A. González-Neira, V. González-Rumayor, C. Belda-Iniesta, and A. Ayuso-Sacido, *Journal of Translational Medicine*, **17**(1), 75 (2019). https://doi.org/10.1186/s12967-019-1825-3
16. N. Garcia-Romero, S. Esteban-Rubio, G. Rackov, J. Carrión-Navarro, C. Belda-Iniesta, and A. Ayuso-Sacido, *Molecular Aspects of Medicine*, **60**, 27-37 (2018). https://doi.org/10.1016/j.mam.2017.11.009
17. J. J. Lai, Z. L. Chau, S. Y. Chen, J. J. Hill, K. V. Korpany, N. W. Liang, L. H. Lin, Y. H. Lin, J. K. Liu, and Y. C. Liu, *Advanced Science*, **9**(15), 2103222 (2022). https://doi.org/10.1002/advs.202103222
18. S. Gupta, S. Rawat, V. Arora, S. K. Kottarath, A. K. Dinda, P. K. Vaishnav, B. Nayak, and S. Mohanty, *Stem Cell Research & Therapy*, **9**, 1-11 (2018). https://doi.org/10.1186/s13287-018-0923-0
19. I. Jalaludin, D. M. Lubman, and J. Kim, *Mass Spectrometry Letters*, **12**(3), 93-105 (2021). https://doi.org/10.5478/MSL.2021.12.3.93
20. C. Théry, S. Amigorena, G. Raposo, and A. Clayton, *Current Protocols in Cell Biology*, **30**(1), 3.22. 21-23.22. 29 (2006). https://doi.org/10.1002/0471143030.cb0322s30

21. D. D. Taylor and S. Shah, *Methods*, **87**, 3-10 (2015). https://doi.org/10.1016/j.ymeth.2015.02.019
22. M. Ding, C. Wang, X. Lu, C. Zhang, Z. Zhou, X. Chen, C.-Y. Zhang, K. Zen, and C. Zhang, *Analytical and Bioanalytical Chemistry*, **410**, 3805-3814 (2018). https://doi.org/10.1007/s00216-018-1052-4
23. K. W. Witwer, E. I. Buzás, L. T. Bemis, A. Bora, C. Lässer, J. Lötvall, E. N. Nolte-'t Hoen, M. G. Piper, S. Sivaraman, and J. Skog, *Journal of Extracellular Vesicles*, **2**(1), 20360 (2013). https://doi.org/10.3402/jev.v2i0.20360
24. D. W. Greening, R. Xu, H. Ji, B. J. Tauro, and R. J. Simpson, A Protocol for Exosome Isolation and Characterization: Evaluation of Ultracentrifugation, Density-Gradient Separation, and Immunoaffinity Capture Methods. In 'Proteomic Profiling: Methods and Protocols', Posch, A. Ed.; Springer New York, 2015.
25. G. Yousif, S. Qadri, A. Parray, N. Akhthar, A. Shuaib, and Y. Haik, *NeuroMolecular Medicine*, **24**(3), 339-351 (2022). https://doi.org/10.1007/s12017-021-08696-6
26. P. Li, M. Kaslan, S. H. Lee, J. Yao, and Z. Gao, *Theranostics*, **7**(3), 789 (2017). https://doi.org/10.7150/thno.18133
27. M. Macías, V. Rebmann, B. Mateos, N. Varo, J. L. Perez-Gracia, E. Alegre, and Á. González, *Clinical Chemistry and Laboratory Medicine (CCLM)*, **57**(10), 1539-1545 (2019). https://doi.org/10.1515/cclm-2018-1297
28. I. Helwa, J. Cai, M. D. Drewry, A. Zimmerman, M. B. Dinkins, M. L. Khaled, M. Seremwe, W. M. Dismuke, E. Bieberich, and W. D. Stamer, *PloS one*, **12**(1), e0170628 (2017). https://doi.org/10.1371/journal.pone.0170628
29. O. El Ouahabi, H. Salim, R. Pero-Gascon, and F. Benavente, *Journal of Chromatography A*, **1635**, 461752 (2021). https://doi.org/10.1016/j.chroma.2020.461752
30. W. Nakai, T. Yoshida, D. Diez, Y. Miyatake, T. Nishibu, N. Imawaka, K. Naruse, Y. Sadamura, and R. Hanayama, *Scientific Reports*, **6**(1), 1-11 (2016). https://doi.org/10.1038/srep33935
31. S. Park, I. Jalaludin, H. Hwang, M. Ko, M. Adelipour, M. Hwan, N. Cho, K. K. Kim, D. M. Lubman, and J. Kim, *Journal of Chromatography B*, **1228**, 123828 (2023). https://doi.org/10.1016/j.jchromb.2023.123828
32. M. L. Heinemann, M. Ilmer, L. P. Silva, D. H. Hawke, A. Recio, M. A. Vorontsova, E. Alt, and J. Vykoukal, *Journal of Chromatography A*, **1371**, 125-135 (2014). https://doi.org/10.1016/j.chroma.2014.10.026
33. Q. Huang, J. Wang, H. Ning, W. Liu, and X. Han, *Molecular and Cellular Biochemistry* (2024). https://doi.org/10.1007/s11010-024-05191-x
34. C. Gardiner, D. D. Vizio, S. Sahoo, C. Théry, K. W. Witwer, M. Wauben, and A. F. Hill, *Journal of Extracellular Vesicles*, **5**(1), 32945 (2016). https://doi.org/10.3402/jev.v5.32945
35. A. Gámez-Valero, M. Monguió-Tortajada, L. Carreras-Planella, M. Franquesa, K. Beyer, and F. E. Borràs, *Sci. Rep.*, **6**, 33641 (2016). https://doi.org/10.1038/srep33641
36. Y. Weng, Z. Sui, Y. Shan, Y. Hu, Y. Chen, L. Zhang, and Y. Zhang, *Analyst*, **141**(15), 4640-4646 (2016). https://doi.org/10.1039/C6AN00892E
37. M. L. Alvarez, M. Khosroheidari, R. Kanchi Ravi, and J. K. DiStefano, *Kidney International*, **82**(9), 1024-1032 (2012). https://doi.org/10.1038/ki.2012.256
38. J. J. Lai, Z. L. Chau, S.-Y. Chen, J. J. Hill, K. V. Korpany, N.-W. Liang, L.-H. Lin, Y.-H. Lin, J. K. Liu, Y.-C. Liu, R. Lunde, and W.-T. Shen, *Advanced Science*, **9**(15), 2103222 (2022). https://doi.org/10.1002/advs.202103222
39. J. A. Martínez-Greene, K. Hernández-Ortega, R. Quiroz-Baez, O. Resendis-Antonio, I. Pichardo-Casas, D. A. Sinclair, B. Budnik, A. Hidalgo-Miranda, E. Uribe-Querol, M. d. P. Ramos-Godínez, and E. Martínez-Martínez, *Journal of Extracellular Vesicles*, **10**(6), e12087 (2021). https://doi.org/10.1002/jev2.12087
40. J. Park, E.-B. Go, J. S. Oh, J. K. Lee, and S.-Y. Lee, *International Journal of Molecular Sciences*, **22**(9), 4311 (2021). https://doi.org/10.3390/ijms22094311
41. E. E. Burkova, S. E. Sedykh, and G. A. Nevinsky, *International Journal of Molecular Sciences*, **22**(4), 2158 (2021). https://doi.org/10.3390/ijms22042158
42. M. Frydrychowicz, A. Kolecka-Bednarczyk, M. Madejczyk, S. Yasar, and G. Dworacki, *Scandinavian Journal of Immunology*, **81**(1), 2-10 (2015). https://doi.org/10.1111/sji.12247
43. L. Moeinzadeh, I. Razeghian-Jahromi, Z. Zarei-Behjani, Z. Bagheri, and M. Razmkhah, *Stem Cells International*, **2022**(1), 8392509 (2022). https://doi.org/10.1155/2022/8392509
44. A.-K. Ludwig, K. De Miroschedji, T. R. Doeppner, V. Börger, J. Ruesing, V. Rebmann, S. Durst, S. Jansen,

M. Bremer, and E. Behrmann, *Journal of Extracellular Vesicles*, **7**(1), 1528109 (2018). https://doi.org/10.1080/20013078.2018.1528109

45. B. Yue, H. Yang, J. Wang, W. Ru, J. Wu, Y. Huang, X. Lan, C. Lei, and H. Chen, *Cell Proliferation*, **53**(7), e12857 (2020). https://doi.org/10.1111/cpr.12857
46. S. V. Krylova and D. Feng, *International Journal of Molecular Sciences*, **24**(2), 1337 (2023). https://doi.org/10.3390/ijms24021337
47. C. Théry, L. Zitvogel, and S. Amigorena, *Nature Reviews Immunology*, **2**(8), 569-579 (2002). https://doi.org/10.1038/nri855
48. M. Xu, J. Ji, D. Jin, Y. Wu, T. Wu, R. Lin, S. Zhu, F. Jiang, Y. Ji, B. Bao, M. Li, W. Xu, and M. Xiao, *Genes & Diseases*, **10**(5), 1894-1907 (2023). https://doi.org/10.1016/j.gendis.2022.03.021
49. M. Adelipour, D. M. Lubman, and J. Kim, *Expert Opinion on Biological Therapy*, **23**(6), 491-507 (2023). https://doi.org/10.1080/14712598.2023.2211203
50. L. M. Doyle and M. Z. Wang, *Cells*, **8**(7), 727 (2019). https://doi.org/10.3390/cells8070727
51. A. E. Vitha, A. W. Kollefrath, C.-Y. C. Huang, and F. Garcia-Godoy, *Stem Cells and Development*, **28**(2), 141-150 (2018). https://doi.org/10.1089/scd.2018.0205
52. J. Zhang, S. Li, L. Li, M. Li, C. Guo, J. Yao, and S. Mi, *Genomics Proteomics Bioinformatics*, **13**(1), 17-24 (2015). https://doi.org/10.1016/j.gpb.2015.02.001
53. J. G. van den Boorn, J. Daßler, C. Coch, M. Schlee, and G. Hartmann, *Advanced Drug Delivery Reviews*, **65**(3), 331-335 (2013). https://doi.org/10.1016/j.addr.2012.06.011
54. Y. Xie, W. Dang, S. Zhang, W. Yue, L. Yang, X. Zhai, Q. Yan, and J. Lu, *Molecular Cancer*, **18**(1), 37 (2019). https://doi.org/10.1186/s12943-019-0984-4
55. G. Lou, Z. Chen, M. Zheng, and Y. Liu, *Experimental & Molecular Medicine*, **49**(6), e346-e346 (2017). https://doi.org/10.1038/emm.2017.63
56. B. Yu, X. Zhang, and X. Li, *International Journal of Molecular Sciences*, **15**(3), 4142-4157 (2014). http://doi.org/10.3390/ijms15034142
57. B. J. Crenshaw, L. Gu, B. Sims, and Q. L. Matthews, *Open Virol. J.*, **12**, 134-148 (2018). https://doi.org/10.2174/1874357901812010134
58. C. Théry, M. Ostrowski, and E. Segura, *Nat. Rev. Immunol.*, **9**(8), 581-593 (2009). https://doi.org/10.1038/nri2567
59. H. Aheget, L. Mazini, F. Martin, B. Belqat, J. A. Marchal, and K. Benabdellah, *Cancers*, **13**(1), 84 (2021). https://doi.org/10.3390/cancers13010084
60. B. M. Coleman and A. F. Hill, *Seminars in Cell & Developmental Biology*, **40**, 89-96 (2015). https://doi.org/10.1016/j.semcdb.2015.02.007
61. A. M. Janas, K. Sapoń, T. Janas, M. H. B. Stowell, and T. Janas, *Biochimica et Biophysica Acta (BBA) - Biomembranes*, **1858**(6), 1139-1151 (2016). https://doi.org/10.1016/j.bbamem.2016.02.011
62. S. Gurunathan, M. H. Kang, and J. H. Kim, *Int. J. Nanomedicine*, **16**, 1281-1312 (2021). https://doi.org/10.2147/ijn.S291956
63. Y. Zhang, Y. Liu, H. Liu, and W. H. Tang, *Cell & Bioscience*, **9**(1), 19 (2019). https://doi.org/10.1186/s13578-019-0282-2
64. S. W. Ferguson and J. Nguyen, *Journal of Controlled Release*, **228**, 179-190 (2016). https://doi.org/10.1016/j.jconrel.2016.02.037
65. D. Yang, W. Zhang, H. Zhang, F. Zhang, L. Chen, L. Ma, L. M. Larcher, S. Chen, N. Liu, and Q. Zhao, *Theranostics*, **10**(8), 3684 (2020). https://doi.org/10.7150/thno.41580
66. M. Record, K. Carayon, M. Poirot, and S. Silvente-Poirot, *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids*, **1841**(1), 108-120 (2014). https://doi.org/10.1016/j.bbalip.2013.10.004
67. J. Kim, Z. Tan, and D. M. Lubman, *Electrophoresis*, **36**(17), 2017-2026 (2015). https://doi.org/10.1002/elps.201500131
68. L. M. Doyle and M. Z. Wang, *Cells*, **8**(7), 727 (2019).
69. R. Vago, G. Radano, D. Zocco, and N. Zarovni, *Scientific Reports*, **12**(1), 17663 (2022). https://doi.org/10.1038/s41598-022-22577-3
70. L. Wang, X. Yu, J. Zhou, and C. Su, *Biological Procedures Online*, **25**(1), 28 (2023). https://doi.org/10.1186/s12575-023-00220-3
71. S. L. Shu, C. L. Allen, S. Benjamin-Davalos, M. Koroleva, D. MacFarland, H. Minderman, and M. S. Ernstoff. A Rapid Exosome Isolation Using Ultrafiltration and Size Exclusion Chromatography (REIUS) Method for Exosome Isolation from Melanoma Cell Lines. In 'Melanoma: Methods and Protocols', Hargadon, K. M. Ed.; Springer US, 2021.
72. G. Vergauwen, B. Dhondt, J. Van Deun, E. De Smedt,

G. Berx, E. Timmerman, K. Gevaert, I. Miinalainen, V. Cocquyt, G. Braems, R. Van den Broecke, H. Denys, O. De Wever, and A. Hendrix, *Scientific Reports*, **7**(1), 2704 (2017). https://doi.org/10.1038/s41598-017-02599-y

73. J. Z. Nordin, Y. Lee, P. Vader, I. Mäger, H. J. Johansson, W. Heusermann, O. P. B. Wiklander, M. Hällbrink, Y. Seow, J. J. Bultema, J. Gilthorpe, T. Davies, P. J. Fairchild, S. Gabrielsson, N. C. Meisner-Kober, J. Lehtiö, C. I. E. Smith, M. J. A. Wood, and S. E. L. Andaloussi, *Nanomedicine: Nanotechnology, Biology and Medicine*, **11**(4), 879-883 (2015). https://doi.org/10.1016/j.nano.2015.01.003
74. B. J. Benedikter, F. G. Bouwman, T. Vajen, A. C. A. Heinzmann, G. Grauls, E. C. Mariman, E. F. M. Wouters, P. H. Savelkoul, C. Lopez-Iglesias, R. R. Koenen, G. G. U. Rohde, and F. R. M. Stassen, *Scientific Reports*, **7**(1), 15297 (2017). https://doi.org/10.1038/s41598-017-15717-7
75. M. Gao, J. Cai, H. S. Zitkovsky, B. Chen, and L. Guo, *Plastic and Reconstructive Surgery*, **149**(3), (2022). https://doi.org/10.1097/PRS.0000000000008830
76. J. Stam, S. Bartel, R. Bischoff, and J. C. Wolters, *Journal of Chromatography B*, **1169**, 122604 (2021). https://doi.org/10.1016/j.jchromb.2021.122604
77. J. Li, Y. Zhang, P.-Y. Dong, G.-M. Yang, and S. Gurunathan, *Biomedicine & Pharmacotherapy*, **165**, 115087 (2023). https://doi.org/10.1016/j.biopha.2023.115087
78. Y. Zhang, J. Bi, J. Huang, Y. Tang, S. Du, and P. Li, *International Journal of Nanomedicine*, **15**, 6917-6934 (2020). 10.2147/IJN.S264498
79. N. Koliha, Y. Wiencek, U. Heider, C. Jüngst, N. Kladt, S. Krauthäuser, I. C. D. Johnston, A. Bosio, A. Schauss, and S. Wild, *Journal of Extracellular Vesicles*, **5**(1), 29975 (2016). https://doi.org/10.3402/jev.v5.29975
80. K. Boriachek, M. K. Masud, C. Palma, H.-P. Phan, Y. Yamauchi, M. S. A. Hossain, N.-T. Nguyen, C. Salomon, and M. J. A. Shiddiky, *Analytical Chemistry*, **91**(6), 3827-3834 (2019). https://doi.org/10.1021/acs.analchem.8b03619
81. S. Sharma, M. LeClaire, and J. K. Gimzewski, *Nanotechnology*, **29**(13), 132001 (2018). https://doi.org/10.1088/1361-6528/aaab06
82. E. Song, C. Zhang, B. Israelow, A. Lu-Culligan, A. V. Prado, S. Skriabine, P. Lu, O.-E. Weizman, F. Liu, Y. Dai, K. Szigeti-Buck, Y. Yasumoto, G. Wang, C. Castaldi, J. Heltke, E. Ng, J. Wheeler, M. M. Alfajaro, E. Levavasseur, B. Fontes, N. G. Ravindra, D. Van Dijk, S. Mane, M. Gunel, A. Ring, S. A. J. Kazmi, K. Zhang, C. B. Wilen, T. L. Horvath, I. Plu, S. Haik, J.-L. Thomas, A. Louvi, S. F. Farhadian, A. Huttner, D. Seilhean, N. Renier, K. Bilguvar, and A. Iwasaki, *Journal of Experimental Medicine*, **218**(3), e20202135 (2021). https://doi.org/10.1084/jem.20202135
83. S. Gholizadeh, M. Shehata Draz, M. Zarghooni, A. Sanati-Nezhad, S. Ghavami, H. Shafiee, and M. Akbari, *Biosensors and Bioelectronics*, **91**, 588-605 (2017). https://doi.org/10.1016/j.bios.2016.12.062
84. F. W. Chen, C. Li, and Y. A. Ioannou, *PloS one*, **5**(11), e15054 (2010). https://doi.org/10.1371/journal.pone.0015054
85. F. S. Iliescu, D. Vrtačnik, P. Neuzil, and C. Iliescu, *Micromachines*, **10**(6), 392 (2019). https://doi.org/10.3390/mi10060392
86. A. A. D'souza and R. Shegokar, *Expert Opinion on Drug Delivery*, **13**(9), 1257-1275 (2016). https://doi.org/10.1080/17425247.2016.1182485
87. M. A. Rider, S. N. Hurwitz, and D. G. Meckes, *Scientific Reports*, **6**(1), 1-14 (2016). https://doi.org/10.1038/srep23978
88. L. J. Lohmann and J. Strube, *Processes*, **8**(1), 58 (2020). https://doi.org/10.3390/pr8010058
89. D. H. Atha, and K. C. Ingham, *Journal of Biological Chemistry*, **256**(23), 12108-12117 (1981). https://doi.org/10.1016/S0021-9258(18)43240-1
90. D. K. Oh, C. K. Hyun, J. H. Kim, and Y. H. Park, *Biotechnology and Bioengineering*, **32**(4), 569-573 (1988). https://doi.org/10.1002/bit.260320421
91. Y. Zhou, R. P. McNamara, and D. P. Dittmer, *Viruses*, **12**(9), 917 (2020). https://doi.org/10.3390/v12090917
92. J. Rech, A. Getinger-Panek, S. Gałka, and I. Bednarek, *Applied Sciences*, **12**(23), 12259 (2022). https://doi.org/10.3390/app122312259
93. A. Meng, B. Luan, W. Zhang, Y. Zheng, B. Guo, and B. Zhang, *International Journal of Biological Macromolecules*, **273**, 132911 (2024). https://doi.org/10.1016/j.ijbiomac.2024.132911
94. J. Yu, D. Huang, H. Liu, and H. Cai, *Biotechnology Journal*, **19**(9), e202400374 (2024). https://doi.org/

10.1002/biot.202400374

95. E. Yakubovich, A. Polischouk, and V. Evtushenko, *Biochemistry (Moscow), Supplement Series A: Membrane and Cell Biology*, **16**(2), 115-126 (2022). https://doi.org/10.1134/S1990747822030096
96. L. Jia, B. Li, C. Fang, X. Liang, Y. Xie, X. Sun, W. Wang, L. Zheng, and D. Wang, *Stem Cells Int.*, **2022**, 3577015 (2022). https://doi.org/10.1155/2022/3577015
97. E. Serrano-Pertierra, M. Oliveira-Rodríguez, M. Rivas, P. Oliva, J. Villafani, A. Navarro, M. C. Blanco-López, and E. Cernuda-Morollón, *Bioengineering*, **6**(1), 8 (2019). https://doi.org/10.3390/bioengineering6010008
98. M. Y. Konoshenko, E. A. Lekchnov, O. E. Bryzgunova, E. Kiseleva, I. A. Pyshnaya, and P. P. Laktionov, *Diagnostics*, **11**(3), 384 (2021). https://doi.org/10.3390/diagnostics11030384
99. Z. Andreu, E. Rivas, A. Sanguino-Pascual, A. Lamana, M. Marazuela, I. González-Alvaro, F. Sánchez-Madrid, H. de la Fuente, and M. Yáñez-Mó, *Journal of Extracellular Vesicles*, **5**(1), 31655 (2016). https://doi.org/10.3402/jev.v5.31655
100. R. Szatanek, J. Baran, M. Siedlar, and M. Baj-Krzyworzeka, *International Journal of Molecular Medicine*, **36**(1), 11-17 (2015). https://doi.org/10.3892/ijmm.2015.2194
101. T. Baranyai, K. Herczeg, Z. Onódi, I. Voszka, K. Módos, N. Marton, G. Nagy, I. Mäger, M. J. Wood, S. El Andaloussi, Z. Pálinkás, V. Kumar, P. Nagy, A. Kittel, E. I. Buzás, P. Ferdinandy, and Z. Giricz, *Plos One*, **10**(12), e0145686(2015). https://doi.org/10.1371/journal.pone.0145686
102. E.-S. Choi, H. A. Faruque, J.-H. Kim, K. J. Kim, J. E. Choi, B. A. Kim, B. Kim, Y. J. Kim, M. H. Woo, J. Y. Park, K. Hur, M.-Y. Lee, D. S. Kim, S. Y. Lee, and E. Kim, *Diagnostics*, **11**(4), 620 (2021). https://doi.org/10.3390/diagnostics11040620
103. N. Dilsiz, *Translational Oncology*, **50**, 102121 (2024). https://doi.org/10.1016/j.tranon.2024.102121
104. K. T. Pammi Guru, J. S. Sreeja, D. Dharmapal, S. Sengupta, and P. K. Basu, *Nanomaterials*, **12**(10), 1660 (2022). https://doi.org/10.3390/nano12101660
105. M. Chang, Y.-J. Chang, P. Y. Chao, and Q. Yu, *PloS one*, **13**(6), e0199438 (2018). https://doi.org/10.1371/journal.pone.0199438
106. A. Gámez-Valero, M. Monguió-Tortajada, L. Carreras-Planella, M. Franquesa, K. Beyer, and F. E. Borràs, *Scientific Reports*, **6**, 33641 (2016). https://doi.org/10.1038/srep33641
107. A. D. Singh, S. Patnam, A. Manocha, L. Bashyam, A. K. Rengan, and M. V. Sasidhar, *MethodsX*, **11**, 102310 (2023). https://doi.org/10.1016/j.mex.2023.102310
108. C.-Y. Lv, W.-J. Ding, Y.-L. Wang, Z.-Y. Zhao, J.-H. Li, Y. Chen, and J. Lv, *International Urology and Nephrology*, **50**(5), 973-982 (2018). https://doi.org/10.1007/s11255-017-1779-4
109. N. van der Lubbe, P. M. Jansen, M. Salih, R. A. Fenton, A. H. van den Meiracker, A. H. Danser, R. Zietse, and E. J. Hoorn, *Hypertension*, **60**(3), 741-748 (2012). https://doi.org/10.1161/hypertensionaha.112.198135
110. M. Li, D. Lou, J. Chen, K. Shi, Y. Wang, Q. Zhu, F. Liu, and Y. Zhang, *Analytical and Bioanalytical Chemistry*, **413**(2), 365-375 (2021). https://doi.org/10.1007/s00216-020-03004-w
111. M. C. Deregibus, F. Figliolini, S. D'Antico, P. M. Manzini, C. Pasquino, M. De Lena, C. Tetta, M. F. Brizzi, and G. Camussi, *Int. J. Mol. Med.*, **38**(5), 1359-1366 (2016). https://doi.org/10.3892/ijmm.2016.2759
112. X. Yi, Y. Li, X. Hu, F. Wang, and T. Liu, *J. Cancer*, **12**(10), 2893-2902 (2021). https://doi.org/10.7150/jca.48906
113. R. V. Bhupatiraju, P. Peddi, V. S. Tangeti, and B. S. Rao, *Analytical Science and Technology*, **37**(5), 280-294 (2024). 10.5806/ast.2024.37.5.280
114. J. Noh and S. W. Myung, *Analytical Science and Technology*, **37**(3), 131-142 (2024). 10.5806/ast.2024.37.3.131
115. M. A. Gab-Allah, K. Choi, and B. Kim, *Food Chemistry*, **344**, 128698 (2021). https://doi.org/10.1016/j.foodchem.2020.128698
116. M. A. Gab-Allah, K. Choi, and B. Kim, *Food Control*, **121**, 107557 (2021). https://doi.org/10.1016/j.foodcont.2020.107557
117. M. A. Gab-Allah, K. Choi, and B. Kim, *Analytical and Bioanalytical Chemistry*, **414**(5), 1867-1879 (2022). https://doi.org/10.1007/s00216-021-03817-3
118. M. A. Gab-Allah, K. Choi, and B. Kim, *Toxins*, **5**(2), 85 (2023). https://doi.org/10.3390/toxins15020085
119. M. A. Gab-Allah, Y. Getachew Lijalem, H. Yu, S. Lee, S.-Y. Baek, J. Han, K. Choi, and B. Kim, *Food Chemistry*,

404, 134542 (2023). https://doi.org/10.1016/j.foodchem.2022.134542

120. M. A. Gab-Allah and J. Kim, *Mass Spectrometry Letters*, **15**(1), 1-25 (2024). https://doi.org/10.5478/MSL.2024.15.1.1
121. M. A. Gab-Allah, I. F. Tahoun, R. N. Yamani, E. A. Rend, and A. B. Shehata, *Food Control*, **137**, 108974 (2022). https://doi.org/10.1016/j.foodcont.2022.108974
122. M. A. Gab-Allah, K. G. Mekete, K. Choi, and B. Kim, *Journal of Food Composition and Analysis*, **99**, 103851 (2021). https://doi.org/10.1016/j.jfca.2021.103851
123. Y. G. Lijalem, M. A. Gab-Allah, K. Choi, and B. Kim, *Food Chemistry*, **384**, 132483 (2022). https://doi.org/10.1016/j.foodchem.2022.132483
124. M. A. Gab-Allah, I. F. Tahoun, R. N. Yamani, E. A. Rend, and A. B. Shehata, *Journal of Food Composition and Analysis*, **107**, 104395 (2022). https://doi.org/10.1016/j.jfca.2022.104395
125. Y. G. Lijalem, M. A. Gab-Allah, H. Yu, K. Choi, and B. Kim, *Analytical and Bioanalytical Chemistry*, **416**(13), 3173-3183 (2024). https://doi.org/10.1007/s00216-024-05265-1
126. Y. G. Lijalem, M. A. Gab-Allah, H. Yu, K. Choi, and B. Kim, *Journal of Food Composition and Analysis*, **126**, 105896 (2024). https://doi.org/10.1016/j.jfca.2023.105896
127. I. F. Tahoun, M. A. Gab-Allah, R. N. Yamani, and A. B. Shehata, *Microchemical Journal*, **169**, 106599 (2021). https://doi.org/10.1016/j.microc.2021.106599
128. V. Börger, S. Staubach, R. Dittrich, O. Stambouli, and B. Giebel, *Curr. Protoc. Stem. Cell Biol.*, **55**(1), e128 (2020). https://doi.org/10.1002/cpsc.128
129. K. Sidhom, P. O. Obi, and A. Saleem, *International Journal of Molecular Sciences*, **21**(18), 6466 (2020). https://doi.org/10.3390/ijms21186466
130. M. Y. Konoshenko, E. A. Lekchnov, A. V. Vlassov, and P. Laktionov, *Biomed. Res. Int.*, **2018**, 8545347 (2018). https://doi.org/10.1155/2018/8545347
131. V. B. E, D. Ramesh, M. C. Shaju, A. Kumar, S. Pandey, R. Nayak, V. Alka, S. Munjal, A. Salimi, K. S. R. Pai, and S. M. Bakkannavar, *Oncol. Res.*, **32**(1), 73-94 (2023). https://doi.org/10.32604/or.2023.030401
132. J. Kim, H. Lee, K. Park, and S. Shin, *J. Clin. Med.*, **9**(3), 650 (2020). https://doi.org/10.3390/jcm9030650
133. A. Gámez-Valero, M. Monguió-Tortajada, L. Carreras-Planella, M. l. Franquesa, K. Beyer, and F. E. Borràs, *Scientific Reports*, **6**(1), 33641 (2016). https://doi.org/10.1038/srep33641
134. G. K. Patel, M. A. Khan, H. Zubair, S. K. Srivastava, M. d. Khushman, S. Singh, and A. P. Singh, *Scientific Reports*, **9**(1), 5335 (2019). https://doi.org/10.1038/s41598-019-41800-2
135. S. N. Neerukonda, N. A. Egan, J. Patria, I. Assakhi, P. Tavlarides-Hontz, S. Modla, E. R. Muñoz, M. B. Hudson, and M. S. Parcells, *Heliyon*, **6**(12), e05669 (2020). https://doi.org/10.1016/j.heliyon.2020.e05669