寄稿論文

Exosomes: From Basic Biology to Clinical Utilization

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Exosomes were first recognized in the early 1980s during studies of reticulocyte maturation, where small vesicles were shown to remove unwanted membrane proteins such as the transferrin receptor via multivesicular body (MVB)-mediated release.[1,2] Johnstone and colleagues later coined the term “exosome” (1987), initially viewing them as a cellular disposal pathway.[3] In the mid-late 1990s, landmark immunology studies revealed that exosomes from antigen-presenting cells carry functional MHC molecules and can activate T cells, reframing exosomes as active mediators of intercellular communication rather than cellular waste. In this article, we will further summarize the recent progress on basic biology of exosomes as well as their clinical applications and considerations.

Exosome Biogenesis

Exosomes are a subtype of extracellular vesicles (EVs). They are nanoscale, phospholipid bilayer enclosed vesicles typically ~30-200 nm in diameter that are released by almost of all cell types.[4,5] Exosomes originate from the endocytic system. Following endocytosis, early endosomes mature and interact with intracellular compartments (e.g., the trans-Golgi network and endoplasmic reticulum), during which specific biomolecules are sorted into the endosomal lumen. Inward budding of the endosomal membrane generates intraluminal vesicles (ILVs) within multivesicular bodies (MVBs). When MVBs fuse with the plasma membrane, ILVs are released into the extracellular space; these released ILVs are commonly referred to as exosomes.[5,6]

Exosome-Mediated Cell–Cell Communication

As shown in **figure 1**, exosomes can act as carriers of biological information across short and long distances through biofluids (e.g., blood, urine, saliva, milk).[7,8,9] Recipient cells may interact with exosomes through (i) receptor–ligand interactions at the cell surface, (ii) multiple endocytic routes (including clathrin-mediated uptake, caveolin-mediated uptake, macropinocytosis, and phagocytosis), and (iii) in some contexts, direct membrane fusion.[4,10] Beyond direct cellular uptake, exosomes may also remodel local microenvironments by depositing cargo in the extracellular space, contributing to sustained signaling effects.[11]

Exosomes contain diverse cargo, including proteins (such as enzymes, signal proteins, transcriptional factors

etc.), lipids (e.g. ceramides), and nucleic acids (mRNA as microRNA, long-non-coding RNA as lncRNA, circular RNA as circRNA, and —occasionally DNA), as well as bioactive molecules such as cytokines and growth factors.[4,5,7] Delivery of these cargo molecules can alter signaling pathways and gene expression in recipient cells, leading to functional or phenotypic changes. Exosomes are therefore implicated in a broad range of physiological and pathological processes, including immune regulation, tissue repair, cancer progression, cardiovascular disease, and central nervous system disorders.[4,5] Because exosomes can protect cargo within a lipid bilayer and facilitate delivery to target cells, they have attracted significant interest as potential therapeutic agents and drug delivery vehicles.[12]

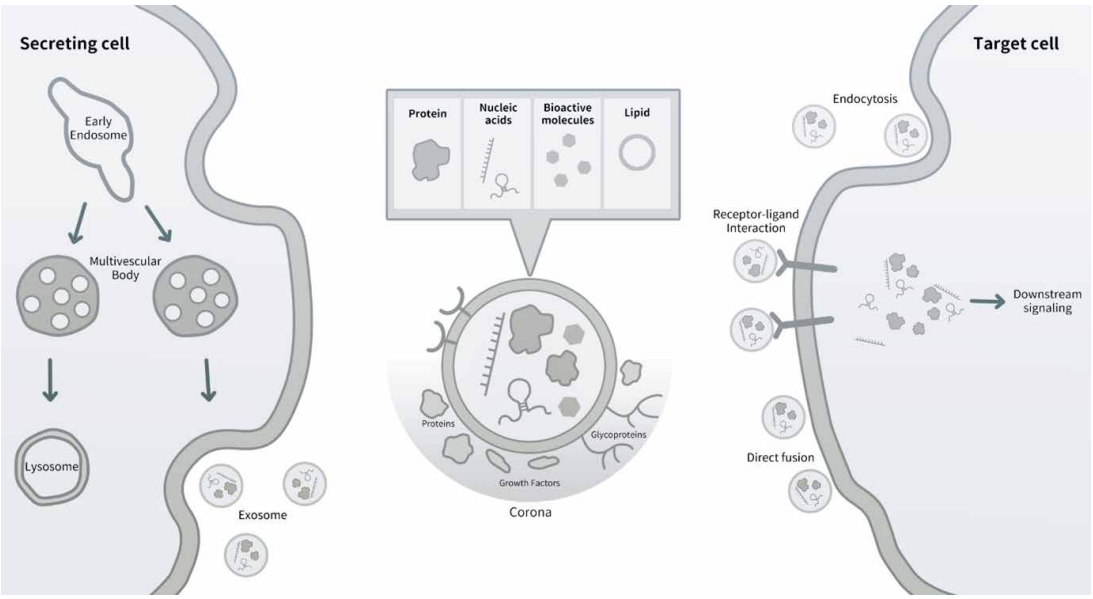


Figure 1. Exosome biogenesis, cargo composition, and uptake mechanisms driving intercellular signaling

Schematic overview of exosome-mediated cell-cell communication. In the secreting cell (left), early endosomes mature into multivesicular bodies (MVBs) containing intraluminal vesicles; MVBs either fuse with lysosomes for degradation or with the plasma membrane to release exosomes into the extracellular space. Released exosomes carry diverse cargo classes—including proteins, nucleic acids, bioactive molecules, and lipids (top inset). Upon release, exosomes can adsorb extracellular components (e.g., proteins, glycoproteins, growth factors), forming a biomolecular "corona" that may influence stability and interactions with recipient cells (center). In target cells (right), exosomes elicit downstream signaling via multiple routes, including endocytosis, receptor–ligand interactions at the cell surface, and direct membrane fusion, leading to delivery of cargo and modulation of cellular responses.

Sources of Exosomes and Functional Diversity

Exosomes (and other EVs) are produced by many organisms and cell types. EV-like particles have been reported from mammalian cells, plants, and microbes, and EVs can be isolated from many biofluids and artificial culture systems. Importantly, exosomes from different sources can differ substantially in composition and function.

For example, stem cell-derived exosomes have been widely investigated for regenerative and immunomodulatory properties, with reported effects in contexts such as wound healing and tissue repair.[13] Blood- and/or Urine-derived exosomes have been explored as non-invasive biomarker sources, since their cargo

may reflect physiological or disease states.[14] Plant-derived EVs have been reported to exhibit antioxidant and anti-inflammatory activities in some experimental settings.[15] Immune cell–derived exosomes, such as those from dendritic cells, have been studied for their ability to modulate immune responses, including potential anti-tumor immunity.[16] Milk-derived exosomes (MDEs) have also been examined for roles in intestinal homeostasis and nutritional health,[5] with studies suggesting protective effects against certain dietary or environmental stressors.

Exosome Isolation and Purification

A key practical question is how to isolate exosomes with sufficient yield and purity. Several commonly used approaches include[8] as well as summarized in **figure 2**:

1. Differential ultracentrifugation
   Sequential centrifugation steps at increasing speeds are used to pellet larger debris first and enrich smaller vesicles later. This method is widely used but may yield preparations with variable purity due to co-isolation of non-vesicular components.
2. Density gradient ultracentrifugation
   Gradients (e.g., sucrose or iodixanol) separate particles by buoyant density, improving purity compared with differential ultracentrifugation, though the method can be time-consuming.
3. Ultrafiltration (e.g., tangential flow filtration, TFF)
   Size-based membrane filtration retains particles above a defined pore size or molecular weight cutoff. TFF is often used for scalable processing and can be compatible with large-volume production.
4. Polymer-based precipitation (e.g., PEG precipitation)
   Polyethylene glycol reduces solubility and promotes precipitation of extracellular particles under lower-speed centrifugation. This approach is convenient but can co-precipitate proteins and other contaminants, often requiring downstream cleanup.

In practice, isolation strategies are frequently combined (e.g., filtration plus chromatography or density-based steps) to balance functionality, yield, purity, scalability, and cost.

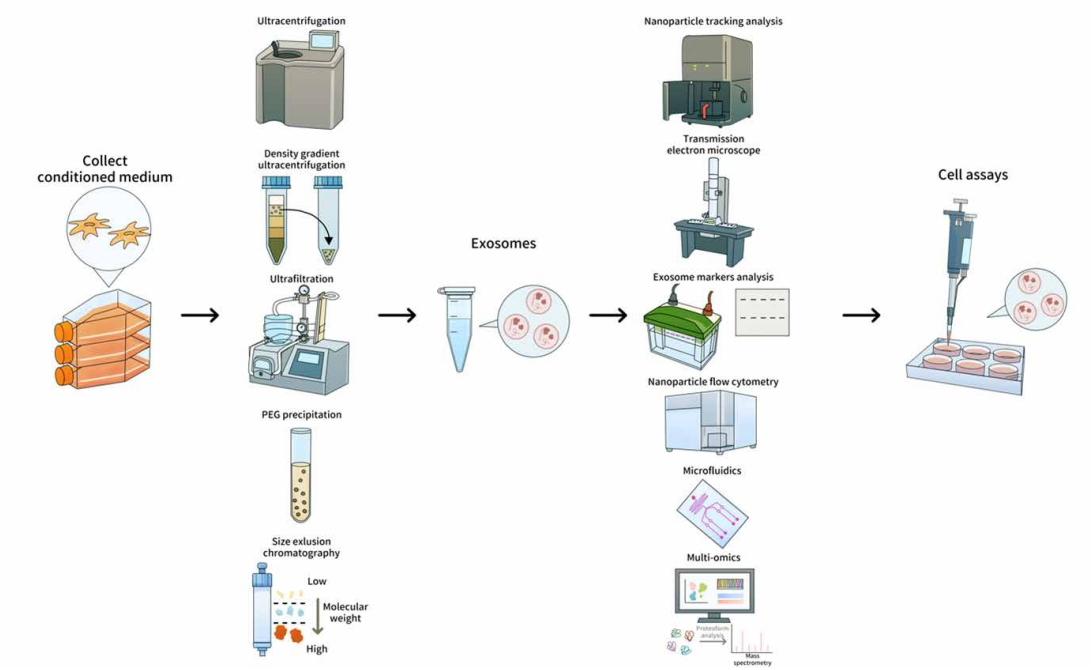


Figure 2. Workflow for exosome isolation, characterization, and functional evaluation from conditioned media

Schematic pipeline illustrating common steps used to obtain and evaluate exosomes from cell culture conditioned medium. Conditioned medium is first collected from cultured cells (left) and subjected to exosome isolation/enrichment using one or more approaches, including differential ultracentrifugation, density gradient ultracentrifugation, ultrafiltration (e.g., tangential flow filtration–based concentration), polymer-based precipitation (e.g., PEG), and size exclusion chromatography (middle). The resulting exosome preparation (center) is then characterized using complementary analytical methods (right), such as nanoparticle tracking analysis (NTA) for particle size and concentration, transmission electron microscopy (TEM) for vesicle morphology, and exosome marker analysis (e.g., immunoblot or immunoassays) to support identity assessment. Additional platforms—including nanoparticle flow cytometry, microfluidic-based enrichment/analysis, and multi-omics profiling (proteomics, transcriptomics, lipidomics)—can further define vesicle subpopulations and cargo composition. Finally, functional activity is evaluated in downstream cell-based assays (far right) to link exosome properties to biological effects in a fit-for-purpose manner.

Multi-Omics Profiling of Exosomes

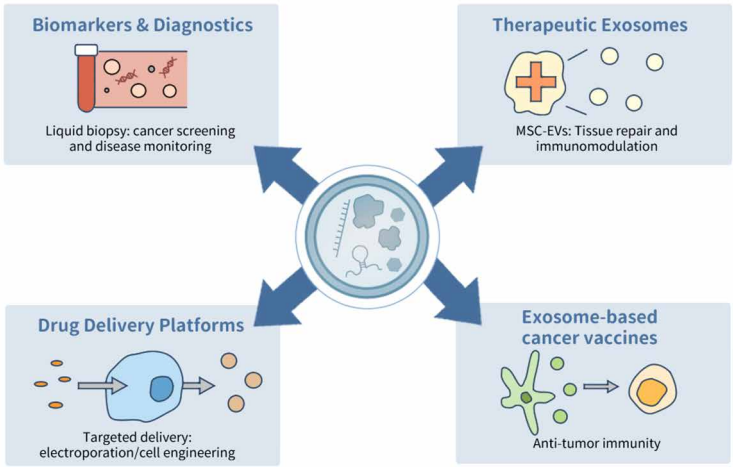
After isolation, exosomes can be characterized using multi-omics approaches to define their cargo and infer functional roles. Proteomics can identify functional protein components, reveal cargo-sorting patterns, and suggest candidate biomarkers. Transcriptomics can profile RNA species within exosomes—including miRNAs and other non-coding RNAs—many of which are relatively stable due to protection by the lipid bilayer. Lipidomics and metabolomics can further characterize bioactive lipid species and metabolic signatures.[11]

Compared with single-analyte measurements, multi-omics provides a systems-level view of exosome biology. Integrating omics data with pathway analysis and curated databases can help predict interactions between exosomes and recipient cells and identify diagnostic or therapeutic opportunities. In oncology in particular, exosome-derived biomarkers have been actively explored for early detection, prognosis, and treatment monitoring.

Clinical and Translational Applications

Exosomes have several major translational directions[11] as shown in **figure 3** and below:

1. Biomarkers and diagnostics
   Because exosomes are present in many biofluids and can protect molecular cargo from degradation, they are attractive sources for minimally invasive biomarkers. Exosomal RNA, DNA-associated signals, and protein markers have all been investigated, especially for cancer diagnosis and prognosis.
2. Therapeutic exosomes
   Increasing interest has focused on mesenchymal stromal cell–derived extracellular vesicles (MSC-EVs) due to reported immunomodulatory and regenerative properties. Clinical research has explored MSC-EVs in areas such as tissue repair and inflammatory conditions, among others.
3. Drug delivery platforms
   Exosomes can potentially act as delivery vehicles due to their membrane structure and cell interaction mechanisms. Cargo loading strategies are often described as: (1) Direct loading, in which therapeutic agents are introduced into isolated exosomes (e.g., by electroporation or co-incubation), and (2) Indirect loading, in which parent cells are engineered or treated so that secreted exosomes contain desired cargo.[9]
4. Exosome-based cancer vaccines
   Exosomes from immune cells or tumor-associated sources have been studied for their potential to stimulate anti-tumor immune responses. While promising, this area still requires extensive validation and safety assessment.[9]



Comparisons of Exosome Loading Strategies

Strategy	Pros	Cons
Direct Loading Post-isolation processing, high cargo capacity, broad cargo range (drugs, genes)	High loading efficiency, controllable release, versatility in cargos, enhanced therapeutic effect	Membrane damage, inconsistency, strict conditions
Indirect Loading Maintain cell integrity, scalable by cell culture	High biocompatibility, crossing biological barriers, cargo protection, ideal for loading miRNA and proteins	Production challenges (purity and yield), release control

Figure 3. Different clinical and translational applications of exosomes

The schematic illustration (top) highlights four primary domains of exosome application in clinical applications: (1) Biomarkers and diagnostics: utilized in liquid biopsy for cancer screening and disease monitoring; (2) Therapeutic exosomes: specifically mesenchymal stem cell-derived extracellular vesicles (MSC-EVs) for tissue repair and immunomodulation; (3) Drug Delivery Platforms: exosomes can act as delivery vehicle, by employing targeted delivery techniques such as electroporation and cell engineering; and (4) Exosome-based cancer vaccines: designed to elicit anti-tumor immunity. The table summarizes the advantages and limitations of two distinct exosome cargo loading approaches: Direct loading and indirect loading, comparing the advantages and disadvantages of the two different loading strategies.

Manufacturing process and considerations

For industrial translation, exosome development should be framed as a manufacturing and quality system rather than a purely biological phenomenon. A practical end-to-end workflow begins with **source definition and controlled collection**, in which the origin (e.g., producer cell line, milk, biofluid, or plant material) is clearly specified with traceability and acceptance criteria, and harvest variables such as temperature, time-to-processing, and storage/hold conditions are standardized; for culture-derived products, upstream controls further include media composition, culture duration, and stress conditions that can shift EV yield and cargo profiles. The process then proceeds through clarification, typically using low-speed centrifugation and/or depth filtration to remove cells and debris, often coupled with pre-filtration to minimize fouling of downstream membranes or chromatographic media. Concentration and buffer exchange are commonly implemented by ultrafiltration or tangential flow filtration (TFF), enabling scale-up while exchanging into formulation-compatible buffers; at this stage, recovery should be tracked and processing stresses (e.g., high shear or transmembrane

pressure) minimized to preserve vesicle integrity.[17] Downstream purification/polishing is selected based on the source matrix and is directed toward reducing free proteins, aggregates, lipoproteins, and other co-isolated nanoparticles; importantly, improvements in purity should be confirmed using orthogonal analytical methods rather than relying on a single marker readout.[18] Finally, formulation and fill/finish require defining a pH and ionic-strength window that maintains vesicle stability, establishing packaging and cold-chain requirements when necessary, and implementing a stability program;[19] conventional 0.22 μm sterile filtration is often unsuitable because it can remove or damage vesicles, so microbial control is typically achieved through low-bioburden processing, closed handling where appropriate, and fit-for-purpose microbiological testing (particularly critical for therapeutic platforms). Across these steps, industrialization depends on a predefined set of critical quality attributes (CQAs) spanning identity (particle size distribution and concentration measured by tools such as NTA, complemented by a multi-assay marker strategy), purity (total protein and a protein-to-particle metric as an operational purity index, plus source-specific contaminant monitoring such as lipoproteins or matrix proteins), potency (a fit-for-purpose bioassay aligned to the intended use—e.g., barrier support or stress-response endpoints for topical applications—anchored to an internal reference batch to detect drift), safety (bioburden/sterility strategy appropriate to product class, residual process reagents, and mycoplasma/endotoxin where relevant), and stability (real-time and accelerated plans with trending of size, concentration, and potency surrogates over time). These elements should culminate in batch-by-batch release documentation via a Certificate of Analysis (COA) that records lot information and storage conditions, reports quantitative release tests (size, concentration, protein, purity proxy, and identity marker panel), includes potency relative to a reference, and documents safety tests and final disposition (released, conditional release, or rejected). Taken together, this manufacture architecture operationalizes exosomes as a manufacturable bioactive ingredient, reduces batch disputes, strengthens partner confidence, and provides a foundation for credible commercialization.

Current Challenges and Future Directions

Despite their potential, several challenges remain before exosome-based applications become routine: [6,7,9,13]

1. Standardization and reproducibility
   Exosome preparations can be heterogeneous in size, cargo, and biological activity. Establishing standardized manufacturing and quality control (QC) criteria is essential.
2. Incomplete understanding of basic biology
   While broad mechanisms of biogenesis and uptake are known, many details of cargo selection, trafficking, and functional delivery remain unclear.
3. Purity and co-isolated contaminants
   Exosomes often co-exist with non-vesicular extracellular nanoparticles, protein aggregates, and lipoproteins in biofluids. Many isolation methods co-enrich these components, complicating interpretation and downstream use. Moreover, commonly used markers such as CD9, CD63, and CD81 do not capture all exosome subpopulations, so robust characterization typically requires multiple orthogonal assays.
4. Scalable manufacturing and GMP compliance
   Achieving high-yield, large-scale production under standardized conditions remains challenging. For drug delivery applications, loading methods may affect vesicle integrity or alter exosome properties, creating additional QC burdens.
5. In vivo tracking, biodistribution, and pharmacokinetics
   Methods to track exosomes and quantify delivery efficiency in vivo are still developing. Exosomes may be cleared rapidly by the mononuclear phagocyte system, reducing effective delivery to target tissues.
6. Safety considerations
   Although exosomes are often perceived as “natural,” their safety profiles must be evaluated carefully. For example, MSC-EVs may have context-dependent effects, and some studies suggest possible pro-tumorigenic risks under certain conditions. Rigorous safety testing is therefore essential.

Conclusion

Exosomes represent a rapidly evolving class of biological nanoparticles with promising applications in diagnostics, therapeutics, and drug delivery. However, translation requires continued advances in mechanistic understanding, isolation and manufacturing technologies, potency assays, and standardized QC frameworks. With careful development and evidence-based evaluation, exosomes may become an important component of future biomedical and biotechnology innovations.

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執筆者紹介

Zhen-Yi Li was born in 2001 in Taipei, Taiwan. She completed her undergraduate studies at National Taiwan University. She is now studying for a master's degree in the laboratory of Professor Tang-long Shen (National Taiwan University), where she is doing research in the field of cancer-related exosomes.

Jiamei Liu received her Master degree from the Institute of Microbiology and Immunology, National Yang Ming Chiao Tung University (Taiwan) in 2023, under the supervision of Professor Chuen-Miin Leu. Since 2025, she has been working in the laboratory of Professor Tang-Long Shen, Department of Plant Pathology and Microbiology, National Taiwan University (Taiwan).

Tang-Long Shen is a Professor in the Department of Plant Pathology and Microbiology, National Taiwan University (NTU). He earned his B.S. (1987–1991) and M.S. (1992–1994) at NTU, and completed his Ph.D. in Molecular Medicine at Cornell University (1997–2002). After returning to NTU, he joined the faculty in 2004, serving as Assistant Professor (2004–2011) and Associate Professor (2011–2016), and has since held senior leadership roles including Director of the Center for Biotechnology (from 2016) and Director of the NTU GIP-TRIAD International Joint Degree Master's Program in Agro-biomedicine in Food and Health (from 2021). Prof. Shen's research centers on cell signaling and cancer biology, with emphasis on integrin and growth factor signaling, cancer cell biology (including tumor exosomes), molecular plant–microbe interactions such as RNA silencing and viroid biology, and secondary metabolites of medicinal fungi such as Cordyceps spp. In addition to his academic work, he has been active in Taiwan's extracellular vesicle community, including leadership roles associated with the Taiwan Society for Extracellular Vesicles (TSEV).

関連製品

Exosome Isolation Reagent [for Serum]	1.5mL	15,000円	E1553
Exosome Isolation Reagent [for Cell Culture Media]	25mL	18,000円	E1601