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A Comprehensive Review on Exosomes Their Biology, Methods, and Applications

Swarupa Arvapalli[1*], Dr.N. Ravindra[2], B. Ruchitha[3], S.Hariharan[3], P.Soumya[3], P.Bhavani[3].

1.Assistant Professor, Joginpally B.R Pharmacy College, Moinabad, Hyderabad, 500090.
2. Principal, Joginpally B.R Pharmacy College, Moinabad, Hyderabad,500090.
3. Student, Joginpally B.R Pharmacy College, Moinabad, Hyderabad, 500090

ABSTRACT

Extracellular vesicles – more especially, exosomes – have been shown to effective at carrying biomarkers in extracellular environment. The lack of consistency in exosomes separation and analysis technique. limits the use of exosomes in therapeutic environment, despite their great promise. This review objectives are present in many forms of extracellular vesicles, highlight their distinctions and commonalities , go over the various techniques currently employed for exosome extraction and characterization. Exosomal research may become more standardized as a result of a full grasp of the isolation and analysis techniques already in use, making the use of exosomes in clinical settings a possibility.

Keywords: exosomes extracellular vesicles, biomarkers, immune modulation, engineered vesicles.

*Corresponding Author Name: Swarupa Arvapalli
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INTRODUCTION

Extracellular vesicles (EVs) are broadly defined as lipid bilayer-enclosed particles released by cells that cannot replicate and are not part of intact cells. EVs include multiple subtypes—exosomes (often ~30–150 nm), micro vesicles/ectosomes (typically ~100–1000 nm), apoptotic bodies, and other specialized vesicles—whose boundaries overlap in size, composition, and biogenesis. Because it is often difficult to prove the precise biogenesis route in routine experiments, ISEV recommends using operational terms such as "small EVs" (sEVs) rather than asserting "exosomes" unless endosomal origin is demonstrated [1–3]. Exosomes are explored as disease biomarkers (liquid biopsy), therapeutic carriers (for small molecules, RNA therapeutics, proteins, gene editors), and even as direct therapeutics particularly mesenchymal stromal/stem cell—MSC—derived EVs.

"Exosomes" refer to small EVs enriched in endosomal markers and recovered using common sEV workflows. These vesicles are released by immune cells, epithelial cells, stromal cells, neurons, cardiomyocytes, tumor cells, and stem/progenitor cells. They appear in plasma, serum, urine, saliva, cerebrospinal fluid, breast milk, synovial fluid, and bronchoalveolar lavage, among others. Exosomes selectively package proteins, RNAs, lipids, and other molecules depending on cell type, activation state, and environmental cues (hypoxia, inflammation, stress). Once released, exosomes can bind to target cells, fuse with membranes, or be endocytosed, delivering cargo and altering signaling pathways.

However, different isolation methods yield different particle mixtures; contaminants lipoproteins, protein aggregates can mimic EV signals; and many functional effects once attributed to exosomes can arise from co-isolated soluble factors. Therefore, rigorous methodology and transparent reporting are essential. [1,5,6].

Endosomal Pathway and Multivesicular Bodies

Exosomes arise from the endosomal system. The canonical model begins with endocytosis at the plasma membrane forming early endosomes. These mature into late endosomes and multivesicular bodies (MVB), characterized by intraluminal vesicles (ILVs) budding inward from the limiting membrane. When an MVB fuses with lysosomes, ILVs are degraded. When an MVB fuses with the plasma membrane, ILVs are released into the extracellular space as exosomes [2,7].

MVBSs are regulated by multiple factors: cellular stress, nutrient state, autophagy/lysosome function, Rab GTPases, cytoskeletal transport, and local calcium signaling. All ILVs do not become exosomes, so "exosome cargo" reflects selective sorting and the balance between secretion and degradation.

Even when produced by one cell line, small EVs are not uniform. Subpopulations differ by size, density, lipid composition, surface proteins, and cargo. Some ILVs may form at different endosomal stages; others may bud from plasma membrane microdomains but be recovered with "exosome" workflows. Exosomes are heterogenic: a therapeutic effect might be driven by a subset, and a biomarker might be enriched only in particular vesicle fractions.

Cargo Sorting

Exosomes are information carriers, and cargo selection is regulated rather than passive. Cargo can be grouped as proteins, lipids, nucleic acids, and metabolites.

Protein cargo

Protein content includes transmembrane proteins (tetraspanins, integrins), cytosolic proteins (heat shock proteins, cytoskeletal proteins), endosomal proteins (TSG101, ALIX), signaling proteins (kinases), and cell-type-specific markers. Tumor-derived EVs may carry oncogenic receptors or mutant proteins, while immune-cell EVs can carry MHC molecules and co-stimulatory proteins [2,4].

Protein sorting mechanisms include ubiquitination signals for ESCRT, tetraspanin-enriched microdomains, and lipid raft association. Cellular state strongly influences protein packaging: hypoxia can increase pro-angiogenic cargo; inflammatory stimulation can enrich immune mediators.

RNA cargo

Exosomes contain diverse RNAs: microRNAs, fragments of mRNA, long noncoding RNAs, circular RNAs, and small RNA species. Selective loading may involve RNA-binding proteins (RBPs) that recognize motifs or structures. Certain RBPs shuttle specific RNAs into ILVs, allowing exosomes to modulate gene expression in recipient cells. Although RNA transfer is well supported, careful controls are necessary to exclude external RNA adsorbed to vesicle surfaces or co-isolated ribonucleoprotein complexes [1,5].

DNA and mitochondrial components

Some EV preparations contain DNA (genomic DNA fragments, mitochondrial DNA). Whether DNA is inside true exosomes or associated with other EV types or contaminants remains context-dependent, and methodological rigor (DNase protection assays, density gradients, imaging) is important.

Lipid cargo and membrane composition

Exosome membranes are enriched in cholesterol, sphingomyelin, glycosphingolipids, and phosphatidylserine-related components, often resembling raft-like domains. These lipids affect vesicle stability, curvature, fusion propensity, and biodistribution. Bioactive lipids can also directly signal to recipient cells [1,5,6].

EXOSOME RELEASE, STABILITY AND UPTAKE MECHANISMS

MVB trafficking to the plasma membrane depends on Rab GTPases (e.g., Rab27a/b, Rab11, Rab35), actin remodeling, and SNARE-mediated fusion. Calcium signaling and stress pathways can increase secretion. Conversely, enhanced lysosomal activity may reduce secretion by diverting MVBs to degradation. Exosomes are relatively stable in circulation because their lipid bilayer protects cargo from enzymes. However, stability varies with temperature, freeze–thaw cycles, and storage buffers. Adsorption to tubes, aggregation, and co-precipitation with proteins can alter measurable yields. Standardized storage practices are therefore crucial for biomarker studies.

Exosome biodistribution is influenced by size, surface proteins, glycosylation, and opsonization. In systemic administration, EVs often accumulate in liver, spleen, and other reticuloendothelial organs. Surface integrins and tetraspanins can influence organ tropism and cell targeting. This is both a challenge (off-target uptake) and an opportunity (directing therapy to specific tissues). Recipient cells internalize exosomes via multiple routes: clathrin-mediated endocytosis, caveolin-dependent uptake, macropinocytosis, phagocytosis (especially by macrophages), and direct membrane fusion. Uptake can depend on ligand–receptor interactions (integrins, heparan sulfate proteoglycans) and local microenvironment pH. After internalization, cargo may escape endosomes to reach cytosol/nucleus or be degraded in lysosomes.

ISOLATION AND PURIFICATION

A central lesson from MISEV is that there is no universal “best” isolation method—only “fit-for-purpose” methods. [1,5,6].

Differential ultracentrifugation (dUC)

dUC is widely used: sequential spins remove cells/debris and larger vesicles, followed by high-speed spins to pellet sEVs. The advantage of this method is scalable, inexpensive once equipment exists, familiar and limitations include co-pelleting of protein aggregates and lipoproteins, vesicle deformation, variable recovery, and operator dependence.

Density gradient ultracentrifugation

Sucrose or iodixanol gradients separate vesicles by buoyant density, improving purity. This method is valuable when high specificity is required for proteomics or mechanistic studies. However, this method is time-consuming, lower throughput, and still not perfect separation from all non-EV particles.

Size-exclusion chromatography (SEC)

SEC separates particles by size, often yielding cleaner EV fractions than precipitation. It is increasingly favored for biomarker studies because it reduces soluble protein contamination.

The limitations include dilution of samples and imperfect separation from similarly sized lipoproteins in plasma.

Polymer-based precipitation

Commercial kits precipitate EVs using polymers (e.g., PEG). Advantages: convenience and yield. The major drawbacks include high contamination with proteins and lipoproteins; therefore, precipitation is usually discouraged for functional claims unless followed by further purification and strong controls [1,5].

Ultrafiltration and tangential flow filtration (TFF)

Filtration concentrates EVs based on size cutoffs. TFF is particularly useful for scalable manufacturing and can be combined with SEC for therapeutic-grade preparations. However, filter clogging, vesicle loss, and shear stress can occur.

Immunoaffinity capture

Antibodies against tetraspanins (CD9/CD63/CD81) or cell-specific markers capture EV subpopulations. This method has high specificity but bias toward selected markers, cost, and potential antibody carryover can occur. Useful for diagnostics when targeting tumor-specific EVs.

Microfluidic approaches

Microfluidic chips can isolate EVs using size, acoustics, electric fields, or affinity capture. These platforms offer promise for rapid clinical workflows but often require specialized validation and may struggle with standardization across labs.

CHARACTERIZATION AND QUALITY CONTROL [1,5,6].

Particle size and concentration is studied using Nanoparticle tracking analysis (NTA) which estimates size distribution and particle count; sensitive to settings and contaminants, Tunable resistive pulse sensing (TRPS) counts and sizes particles, Dynamic light scattering (DLS) can over-weight larger particles. The morphology of exosomes is studied using Transmission electron microscopy (TEM) / cryo-EM hich visualizes vesicles, cryo-EM preserves native structure and Atomic force microscopy (AFM) which provides topography but requires careful interpretation.

PHYSIOLOGICAL ROLES OF EXOSOMES

Immune Regulation

Immune-cell-derived EVs can carry MHC-peptide complexes and modulate antigen presentation, T-cell activation, and immune tolerance [4]. EVs from dendritic cells, B cells, T cells, and macrophages can either stimulate or suppress immune responses depending on cargo and context.

Tissue repair and regeneration

Exosomes from stromal and stem-like cells can promote angiogenesis, reduce apoptosis, and modulate inflammation. MSC-derived EVs are widely studied as candidates for cell-free regenerative therapy, partly because they may capture paracrine benefits of MSCs while reducing risks associated with live-cell infusion [11].

Neurobiology

Neurons and glia release EVs involved in synaptic function, myelination, and neuroimmune interactions. EV-mediated signaling may contribute to brain development and responses to injury.

Metabolic homeostasis

Adipose tissue, liver, muscle, and immune cells release EVs that influence insulin sensitivity, inflammation, and lipid metabolism. Exosomal microRNAs have been implicated as systemic metabolic regulators.

APPLICATIONS OF EXOSOMES

Cancer

Cancer is one of the most active areas of exosome research because tumors release abundant EVs that reshape their environment. Tumor EVs can Promote proliferation and survival by transferring growth signals or drug-resistance mediators. Remodel the tumor microenvironment by educating fibroblasts, endothelial cells, and immune cells to support tumor growth. Integrin patterns on tumor EVs have been associated with organotropism in some studies, suggesting a mechanism for selective metastasis. However, reproducibility can vary depending on isolation purity and model systems, reinforcing the need for standardized methodology [1,3,7].

Exosome cargo sorting is particularly relevant in cancer because oncogenic pathways can alter endosomal trafficking and loading, generating vesicles enriched in disease-driving signals. Recent reviews have highlighted regulated cargo selection mechanisms and how they affect tumor behavior and therapeutic opportunities [12].

Cardiovascular disease

Cardiomyocytes, endothelial cells, platelets, and immune cells exchange EVs that influence inflammation, fibrosis, angiogenesis, and remodeling after myocardial infarction. Endothelial EVs can modulate vascular tone and inflammatory recruitment. Platelet EVs can enhance coagulation and vascular inflammation. Stem cell-derived EVs have shown cardioprotective effects in preclinical models, often attributed to microRNAs and pro-survival proteins. Translation is promising but limited by challenges in dosing, targeting, and demonstrating consistent potency across EV batches.

Neurological disorders

EVs participate in neuroinflammation and may spread pathogenic proteins. In neurodegenerative diseases, EVs have been implicated in the trafficking of amyloid-beta, tau, and alpha-synuclein, potentially contributing to disease propagation. Conversely, EVs also offer a route for CNS biomarkers because some brain-derived EVs can be detected in peripheral blood, and engineered EVs may cross the blood–brain barrier under certain conditions.

Infectious disease

Pathogens can hijack EV pathways. In viral infections, EVs can carry viral RNAs/proteins, modulate innate immunity, and influence inflammation. EVs may either enhance antiviral defense (by delivering interferon-stimulated signals) or aid immune evasion (by presenting decoy receptors or suppressive cargo). In bacterial and parasitic infections, EV-like particles can deliver virulence factors and shape host responses.

Autoimmune and Inflammatory conditions

EVs can carry autoantigens, cytokine regulators, and microRNAs that skew immune balance. EV profiles are being explored for rheumatoid arthritis, lupus, inflammatory bowel disease, and asthma. A recurring challenge is specificity: inflammation from diverse causes may produce overlapping EV signatures.

Kidney disease and Urinary EVs

Urine contains EVs derived from multiple nephron segments, offering a noninvasive window into kidney physiology and pathology. Urinary EV biomarkers are investigated for acute kidney injury, diabetic nephropathy, glomerular diseases, and transplant monitoring. Because urine is less protein-rich than plasma, it may be analytically advantageous, but pre-analytical standardization remains crucial.

Exosomes as Biomarkers

Exosome-based diagnostics rely on the idea that EV cargo reflects the originating cells and is protected from degradation. Compared with circulating free DNA/RNA, EV-associated RNAs may be more stable and sometimes more tissue-informative [9,10].

Immunotherapy and Vaccines

Exosomes can present antigens via MHC molecules or display antigenic proteins, enabling vaccine designs. Early concepts used dendritic cell-derived exosomes to stimulate anti-tumor immunity [4]. Modern approaches include tumor antigen-loaded EVs, adjuvant incorporation, and combination with checkpoint inhibitors, though consistent clinical success remains challenging.

Storage and Stability

EV stability depends on buffer composition, freezing conditions, and cryoprotectants. Freeze–thaw cycles can reduce functional activity and alter measured particle counts. Lyophilization is explored but requires careful optimization. Because biomarker studies often store samples for long periods, stability testing and consistent handling protocols are essential.

CONCLUSION

Exosomes and small extracellular vesicles represent a powerful biological communication system with significant diagnostic and therapeutic potential. Their ability to package and protect complex molecular cargo makes them uniquely attractive for liquid biopsies and next-generation delivery platforms. At the same time, EV heterogeneity and methodological variability can easily lead to irreproducible findings and over-interpretation. With these standards, exosome research is positioned to deliver clinically useful biomarkers and safe, scalable therapeutics over the coming decade.

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