International Journal of Pharmacy and Pharmaceutical Research (IJPPR)
Volume 31, Issue 1, January 2025 **ijppr.humanjournals.com** ISSN: 2349-7203

The Exosome: A Review on Isolation, Characterization and Future Aspects in Drug Delivery

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Received: 2025-1-07 Revised: 2025-1-19 Accepted: 2025-1-25

ABSTRACT

Exosomes, type of extracellular vesicles (EVs), are membranous structures that have a variety of compositions and are implicated in both pathological and biological processes. Because of their size and ability to transport biological components to target cells, exosomes have been exploited as possible drug delivery vehicles and diagnostic indicators since their discovery. Exosomes are remarkable and great drug delivery vehicles for application in a variety of diseases and cancer therapy because of their characteristics, which include stability, favored tumor homing, biocompatibility, and changeable targeting efficacy. This page offers a concise synopsis of exosome biogenesis, functions, and contents in addition to separation and characterisation methods. Newer exosome studies combined immunoaffinity and microfluidic system approaches for more efficient exosome collection. Further, we discuss the future perspectives of exosomes as a drug delivery vehicle.

Keywords: Exosomes, Drug loading, Isolation, Drug delivery vehicle

1. INTRODUCTION:

Exosomes are consistently rounded, membrane-covered entities that range in diameter from 30 to 150 nm (Figure 1) [1]. Multivesicular bodies form exosomes, which are then released into the extracellular environment following their incorporation into the plasma membrane [2]. Exosomal membranes are composed of sphingomyelin, cholesterol, phosphatidylinositol, ceramide, phosphatidylethanolamine, phosphatidylserine, etc.[3].

They can deliver and carry intracellular information by mixing with cells that receive it. As a result, they represent a viable method for developing drug delivery systems that transport a payload to specific cells, tissues, and organs[4].In addition to their function in intercellular communication, exosomes have the ability to transport proteins, bioactive lipids, and mRNA and control the expression of genes and proteins[5]. Exosomes transfer chemicals that can give recipient cells new traits and/or promote cell division upon contact [6].

They are primarily found in several biological fluids, such as urine, saliva, serum, semen, blood, plasma, breast milk, tears, amniotic fluid and cerebrospinal fluid (CSF) [7]. They are also found in various types of cells like platelets, dendritic cells, macrophages, B cells and T cells as well as mesenchymal stem cells, endothelial and epithelial cells [8]. They are also secreted by variety of cancer cells. Earlier studies suggested that exosomes play a crucial role in multiple biological activities such as inflammation, apoptosis, coagulation, intracellular signalling, antigen presentation and cellular homeostasis [9]. They may be utilized as biomarkers and for therapeutic purposes in diseases [10].

Exosomes have been found to contain 1116 lipids, 3408 mRNAs, 2838 miRNAs, and 9769 proteins [11,12]. Certain lipids, proteins, DNA, mRNA, and noncoding RNAs are among its constituents that can act as both autocrine and paracrine agents. Exosomes contents can be used as a grading system for the development of cancer and as prognostic indicators. Additionally, it regulates tumor cell angiogenesis, metastasis, and tumor growth [13]. In recent years, exosomes have gained a lot of attention as a possible drug delivery vehicle due to their groundbreaking preclinical success as a solution for these unresolved clinical difficulties [14]. To guarantee the clinical development of exosome-based medicinal solutions, a thorough understanding of exosome biology is necessary.

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2. Source and classification of exosomes:

Currently, exosomes are mainly classified according to the type of their parental cells. Almost all types of human cells can produce exosomes. Exosomes are membrane derived, homogenous vesicles having multiple origins and can be classified as ectosomes (exosomes secreted by neutrophils or monocytes) or cardiosomes (exosomes produced by cardio myocytes) [15].Depending on whether exosomes have been artificially modified, they are broadly classified into natural exosomes and engineered exosomes. Depending on the species of origin, exosomes are divided into animal-derived and plant-derived exosomes. The exosome subpopulation further has large exosomes (Exo-L; 90–120 nm), small exosomes (Exo-S; 60–80 nm), and exomeres (~35 nm) [16].

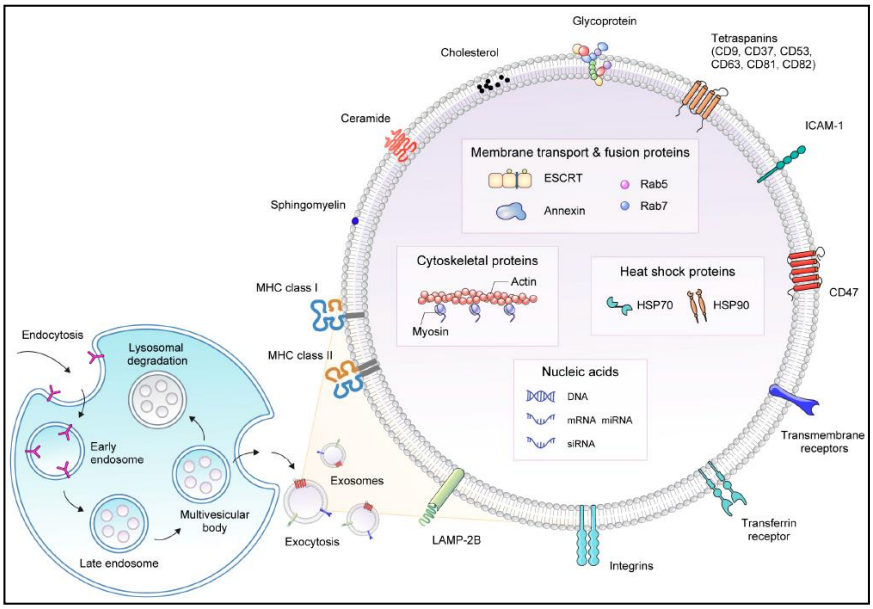

Figure 1. Structure and biogenesis of exosome [17]

3. Biogenesis

Exosome biogenesis is associated with the endosomal system and proceeds along the endosomal trafficking pathway. Primary endocytic vesicles, multivesicular bodies, and early endosomes make up the endosomal system [18].

When intracellular fluid is absorbed, the plasma membrane invaginates, resulting in the formation of early endosomes. The late endosome is formed as a result of the early endosome maturation and expansion; intraluminal vesicles (ILV) are then produced by the internal budding of the endosomal membrane in multivesicular bodies (MVBs). Once the MVBs have fused to the cell membrane, they are discharged into the extracellular microenvironment [19]. Exosomes are the current name for the vesicles. Exosomes have a density of 1.13 to 1.19 g/mL in a sucrose gradient. Without the use of dangerous cryoprotectants, they can be separated by centrifugation at 100,000g and maintained functionally intact for over six months when stored at 80 °C [20].

3.1. Mechanism:

One of the key mechanisms of exosomes is their ability to deliver cargo to other cells [21]. Exosomes can interact with target cells in three main ways:

(1) They can bind to the cell membrane of the target cell through receptor–ligand interactions, which can lead to the activation of specific signalling pathways;

(2) They can be taken up by the cell through endocytosis, allowing their contents to be released inside the cell; and

(3) They can bind directly to the cell membrane of the target cell, allowing their contents to be transferred directly into the cytoplasm of the cell [22, 23].

4. Isolation and separation:

It is essential to isolate them in order to comprehend their mechanism and potential uses in biologics. Numerous techniques have been created to make it easier to separate exosomes from bodily fluids. Exosomes are challenging to isolate because to their overlapping size range, comparable shape to other extracellular vesicles, and significantly smaller size. Researchers have used a variety of techniques to attain high recovery rates, high purity, and high throughput separation; each technique has pros and cons [24].

4.1. Ultracentrifugation:

Centrifugation is the most often used isolation method. The sedimentation coefficient difference serves as the foundation for ultracentrifugation between extracellular material and exosomes. Differential ultracentrifugation and gradient density ultracentrifugation are two further classifications for ultracentrifugation. Sequential differential velocity centrifugation at low, high, and ultracentrifugation speeds are used in differential ultracentrifugation. The cells are separated and the cell detritus is eliminated using low-speed centrifugation. Large extracellular vesicles, apoptotic bodies, and microvesicles are isolated and eliminated using high-speed centrifugation. Exosomes are then collected and sedimented using ultracentrifugation. However, gradient ultracentrifugation can overcome this method's low separation purity. Two or more distinct gels or solutions with varying densities are used in this procedure. By separating the EVs based on their densities, a high separation rate is attained. However, because it takes some time to achieve solution equilibrium, this approach is time-consuming [25, 26].

4.2. Immunological separation:

The antigen-antibody response is the basis for the immunological separation principle, which captures exosomes. This technique takes advantage of the existence of different proteins on the membrane of exosomes in order to catch them.

Beads, chromatography matrices, and antibody-coated plates are used in recent research to achieve highly pure and quick immunological separation. It is a costly technique that is limited in its use to large-scale samples and requires specialized chemicals and cell-free samples [26, 27].

4.3. Ultrafiltration:

The membrane separation method known as ultrafiltration operates on the basis of size and molecular weight-based separation of exosomes as well as other substances. Exosomes can be isolated from macromolecules by utilizing membranes with pores that are the same size as an exosome (100 nm), allowing the exosome to pass through while the membrane retains other components. To shorten process times and make operations easier, ultrafiltration and ultra-centrifugation are frequently used together. However, the utilization of this procedure is limited by the potential for membrane pore blockage and the large increase in process time caused by repeated membrane washing operations [28].

4.4. Polymer- Based Precipitation Separation:

This method uses hydrophilic polymers such as polyethylene glycol (PEG) to precipitate exosomes based on charge. By trapping water molecules and causing exosomes to sink with a comparatively modest centrifugal force, PEG decreases the solubility of exosomes. When samples containing exosomes co-incubate with PEG solution (MW: 8000 Da), exosomes are precipitated. Filtering or centrifugation can also be used to collect or isolate the deposited exosome following an overnight incubation at 4 C. The following method is rather easy to apply and has little downtime, and it doesn't require sophisticated equipment [29, 30].

4.5. Magnetic Separation:

Exosomes must be captured and separated by magnetic separation using antibody-modified magnetic beads, also known as magnetic labelling. This approach is primarily utilized because to its high-throughput, contactless, and specialized separation capabilities. Immunomagnetic beads hold onto the exosomes, but PBS removes the other contents. The chamber lyses, captures, and analyzes the antibody-labelled magnetic beads [31].

4.6. Acoustic fluid separation:

Because of its great scalability, acoustic fluidic separation enables the manipulation of bioparticles at the nano and microscales. An effective way to produce acoustic waves is with transducers made of piezoelectric materials. Electrical polarization can be produced by piezoelectric materials under mechanical tension, or electrical polarization can result in mechanical deformation [32].

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Extracellular vesicles were separated by Lee et al. using a standing surface acoustic wave separation device running at 38.6 MHz. Using a cut-off size of 300 nm, exosomes were separated from other extracellular vesicles. The acoustic fluidic separation approach has enormous promise due to its biological acceptability and contactless separation benefits. The maximum process output is 0.40–0.43 L/min [33].

4.7. Dielectrophoretic Separation (DEP)

The basis for dielectrophoretic separation is the idea that polarized particles in a non-uniform electric field experience dielectric force. The size and inherent dielectric characteristics of the cells, in addition to the applied electric field's magnitude and frequency, determine how much force is given to the particles by DEP. Larger particles move toward low electric fields, whereas exosomes, which are nanoparticles, are drawn to areas with high electric fields. Although this method's utilization is limited by the need for electrothermal heating, its advantages of quick and high-throughput qualities can be investigated [34, 35].

4.8. Deterministic Lateral Displacement separation (DLD)

Certain necessary sizes for particle separation are present in devices or chips that support DLD separation. The principle is that the course of flow will remain unchanged, but particles larger than the critical size will change. Despite its simplicity and lack of labels, researchers are having trouble with separation and clogging [36].

4.9. Microfluidics Technologies:

Even while microfluidic-based methods for isolating exosomes are still in their early stage, they have a lot of potential for clinical use because they usually demand for lesser amounts of raw materials and yield extremely pure exosome preparations in a short amount of processing time. Because of their great sensitivity and low yield, microfluidic technologies for exosome isolation are usually employed for diagnostic applications. Using microfluidics, exosomes or microvesicles can be isolated using three primary methods: (A) immunoaffinity, (B) sieving, and (C) trapping exosomes on porous surfaces [37].

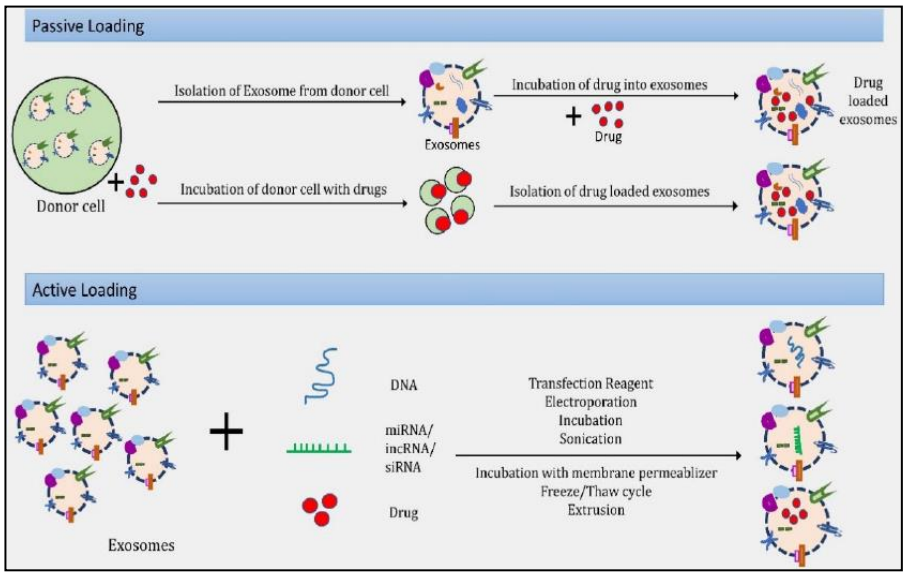

Figure 2.The drug-loading methods (passive and active) of exosomes [38]

5. Characterization techniques of exosomes [38]:

Table 1.Summary of characterization techniques:

S. No.	Characterization Technique	Principle	Application
1	Atomic Force Microscopy (AFM)	Surface sensing, detection, and imaging	3D geometry, size, and other biophysical characteristics. Mechanical properties.
2	Colorimetric detection	Determines the particles in calorimetric detection, quantified using ELISA.	Utilized to detect exosomes from cancer cells.
3	Dynamic Light Scattering (DLS)	Particle light scattering and optical signal	Determine particle size and dispersion.
4	Fluorescence Correlation Microscopy (FCM)	Antibody tagged with a fluorescent dye and measured by a plate reader in microfluidic-dependent FCM.	Immunocapture and quantitative analysis
5	Enzyme-linked Immunosorbent Assay (ELISA)	Plate-based enzyme-linked immunosorbent test	Identifies and measures proteins, peptides, hormones, and antibodies; also used to determine exosomes from plasma, serum, and urine using different precise antibodies.
6	Nanoparticle Tracking Analysis (NTA)	Particles' light scattering and Brownian motion	Quantify particle diameter Estimate the presence of antigens on exosomes.
7	Microscopy study Transmission Electron Microscopy (TEM) Scanning Electron Microscopy (SEM)	Accelerated electron beam Low-energy electrons are ejected from only form proximity to the sample surface.	3D form, size and structure of particles. Surface characteristics comprising size, shape and morphology.
8	Nuclear Magnetic Resonance (NMR) detection	Micro-NMR technique	Assess the number and presence of proteins in exosomes. Detect exosomes after concentrating microvesicles containing immunogenic nanoparticles via filtering.
9	Surface Plasmon Resonance (SPR) detection	Microfluidic-based SPR device.	Improve detection performance by nano-plasmonic exosome (nPLEX) created by modifying a nanosubstrate.

6. Exosome storage:

The three main preservation techniques now employed for the long-term storage of exosomes are lyophilization, cryopreservation, and drying with a spray [39]. Temperature and antifreeze are the two most important components for cryopreservation. Storage at 4 °C might weaken the biological activity and diminish the protein cargo of exosomes, whereas −80 °C is considered the ideal temperature causing the least impact on exosome form and content [40, 41].

The best option is non-permeable disaccharide antifreeze, particularly trehalose, which stops exosome aggregation and cryodamage [42]. Heat-sensitive items that have been lyophilized or freeze-dried, such as exosomes and vaccines, can be readily stored and reconstituted with the addition of water. According to a recent study, cryoprotectant lyophilization could preserve exosomal protein and RNA activity for about four weeks even after storage at room temperature. Lastly, because spray-drying is a one-step procedure as opposed to freeze-drying, it eliminates the need for costly machinery and drawn-out multi-step grinding.

Nonetheless, the fundamentals of spray drying factors that can impact exosome stability and cargo integrity, including exosome feeding rate, atomization pressure, and outlet temperature [43].

7. Therapeutic applications of Exosomes:

Exosomes have a wide range of diverse clinical uses. Exosomes made from stem cells have been demonstrated to support tissue regeneration and repair, making regenerative medicine one of the most exciting study fields. For instance, by encouraging angiogenesis and enhancing cardiac function, exosomes have been utilized to treat heart conditions including myocardial infarction. Exosomes have been investigated as a therapeutic agent for the management of neurological conditions such as Alzheimer's and Parkinson's [44].

Exosomes have been investigated for their diagnostic potential in addition to their therapeutic promise. Non-invasive diagnostic procedures have been made possible by the discovery of exosomal biomarkers for a number of illnesses, including cancer [45, 46]. Exosomes have also been investigated as a way to get around the drawbacks of conventional delivery techniques by delivering targeted therapies to particular cells or tissues [47].

Table 2.Use of exosomes as drug carriers in various diseases: [38]

Drug	Type of Drug	Disease Model	Therapeutic Effect	Exosomes Origin	Drug Loading Method
Curcumin	Small molecule drug	Brain tumor and autoimmune encephalitis	Inhibited brain inflammation and delayed brain tumor growth	Tumor cells (GL26-Luc, BV2, 3T3L1, 4T1, CT26, A20 and EL-4)	Direct mixing
miRNA	Genetic substances	Glioblastoma Tumor	Provide diagnostic information	Glioblastoma cells	Transfection
Signal regulatory protein _	Protein	Tumor	Enhanced phagocytosis of tumor cells	HEK293T cells	Transfection

8. Challenges:

Exosomes provide numerous advantages as drug delivery systems, but there are some obstacles that need to be addressed. Exosomes' low capacity for loading and encapsulation is the first major drawback of employing them as a medication. Exosomes are quickly eliminated from the systemic circulation upon in vivo introduction, even though they have a unique lipid and protein makeup. Similar to phosphatidylcholine/cholesterol liposomes, less than 5% of the injected exosomes are still in the bloodstream three hours after injection. The faster exosome clearance in vivo is explained by macrophages' ability to capture exosomes. Exosomes have the ability to deliver payload directly into the cytoplasm for characterisation, bypassing the endosomal and lysosomal pathways, in contrast to polymeric nanoparticles or liposomes. Yet another challenge arises due to the heterogeneity of exosomes due to their varied size, composition, function and cellular origin, which adds complexity to their characterization [38].

9. Conclusion:

Exosomes could be a better drug delivery vehicle surpassing the side effects of other synthetic nanoparicles. Specifically, plant-based exosomes are non-immunogenic in their use. Novel technologies like microfluidics can be applied for improved isolation and makes the process easier. Also, Scientists recently employed Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/CRISPR Associated Protein 9 (Cas9) System to cure numerous hereditary disorders like cancer by repairing, eliminating, or suppressing disease-related genomic abnormalities. The researchers employed a Liposome-exosome hybrid nano system to introduce the CRISPR/Cas9 system within mesenchymal stem cells (MSCs). As well as AI could play a key role in predicting the optimal genetic modifications to enhance exosome functionality. Thus, development in these areas will lead to a promising avenue for drug delivery results in the future. We suggest that exosomes with modified surface will be acting as a high affinity substrate for pathogenenic viruses than somatic cells and forming an inactive complex which is similar to one of the strategies of inverse targeting.

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How to cite this article:
Abinaya S et al. Ijppr.Human, 2025; Vol. 31 (1): 197-204.

Conflict of Interest Statement: All authors have nothing else to disclose.