

Exosomes: Nature's Smart Nanocarriers For Drug Delivery-Biogenesis, Harvesting, And Challenges

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Abstract

Exosomes are the communication channels between the cells. They are the small nanovesicle release by cells to influence and adjust regeneration processes in our body. When cells want to communicate they just can't send a message because we have a lot of enzymes in our body that designed to breakdown those messages. The messages that are important for our body we're able to package them in a sort of protective shield known as exosomes and they're liberated or released out of the cell and they're protected from the environment so the enzymes can't access the RNA, peptides or proteins that are inside those packages. The stem cells are the cells that try to communicate to the surrounding tissue they package the biological material in the exosomes . The exosomes move around the body and they find the target cells and release the message or biological material and the targeted cells then respond to the message by modulating, regenerating or doing something different. Due to their size and ability to transport biological contents to target cells, exosomes have been exploited as possible drug delivery vehicles and diagnostic indicators since their discovery. This article highlights the biogenesis , harvesting of exosomes to modify into drug delivery system and why is it considered potent for drug delivery system along with it applications and in pharmaceuticals , challenges faced when using exosomes as a drug delivery vesicles.

Keywords- Exosomes, nanovesicle, Protective shield, stem cells, biological materials, Drug delivery.

1. Introduction

Exosomes are naturally occurring nanovesicles, that has size range within 30-150nm. These are the naturally occurring extracellular vesicles released from various types of cells.[1] Almost all cells produce exosomes but there is a specific type of cell that has a special regenerative potential. It's the mesenchymal cells. Among their many properties mesenchymal cells have a modulatory and anti inflammatory and regenerative effect on damage tissues. This is why mesenchymal cells are main source of exogenic product.[2] Exosomes are tiny, round vesicles that carry a variety of biomolecules (proteins, RNA, and lipids) that can be used as therapeutic purposes such as gene editing and protein delivery as cargo for cell-to-cell communication. Their lipid bilayer is rich in tetraspanins and adhesion proteins. Under transmission electron microscopy, it seems to be spherical or cup-shaped.[3] Exosomes are encapsulated in a lipid bilayer membrane which protects their contents during transport this membrane structure is what makes exosomes ideal for drug delivery.[4] Although the structure of exosome is similar to liposomes but the primary difference between them are exosomes are the endogenous molecules which means they are produced naturally due to which there are identification proteins present in their hydrophilic surface which is a unique characteristic because of the presence of identification proteins the body thinks that exosomes are the part of the body . so, the body produce less immune response against them . Therefore, we can include therapeutic agents such as genetic material and protein drug which is more prone to degradation in liposome .[5] 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, biocompatibility, favored tumor homing, and changeable targeting efficiency.[6] It has high biocompatibility and low immunogenicity due to which the it has longer circulation time.[7] They are derived from different regions from the body they may be tumor derived, stem cell derived or immune cell derived.[8,9] For example, if the exosomes are derived from tumor cells then later we can encapsulate anti tumor drugs and introduce into the body so that the therapeutic material are not degraded and show their action into the targeted site efficiently. [10] Based on their function they can be diagnostic , therapeutic or pathological. Exosomes can be isolated by different methods such as filtration, centrifugation, size exclusion

chromatography and polymer precipitation.[11] with the help of exosome we can provide therapy to the own cells by modulating the cellular activity. The overview of exosomes along with its key characteristics, advantages and applications is mentioned in Figure 1.

Escudier et al. (2005) conducted the first known human clinical trial, a Phase I study in which they used autologous dendritic cell-derived exosomes (Dex) loaded with tumor peptides to vaccine patients with metastatic melanoma. This experiment proved that producing and administering exosomes to humans is both feasible and safe.[12] In 2008, a Phase I trial using autologous ascites-derived exosomes (Aex) in combination with GM-CSF for colorectal cancer was conducted as a follow-up early clinical investigation that also proved to be safe and immunogenic, including T-cell responses in several patients.[13] In around 2011-2015 exosomes were being used in an increasing number of registered clinical trials, primarily in the early stages and particularly in oncology. [14] Exosomes for wound healing and macular hole repair are among the regenerative medicine trials were started in 2017-2020. Tianjin medical university in china used MSC derived exosomes for large idiopathic macular holes also conducted trials for cutaneous ulcers.[15,16] During the pandemic around 2020-2022, MSC-derived exosomes are being tested for SARS-CoV-2 pneumonia, musculoskeletal disorders, and COVID-19 and respiratory diseases. [17] In 2023-2024 EV/exosomes were being used in more and more current trials (>200+); these trials were conducted in a wider range of therapeutic domains (oncology, CNS, infectious, immunology). According to the market, hundreds of trials are in progress.[18] Recent developments in the product and regulatory pipeline (such IND clearances) in 2025; according to some estimates, the first exosome-based therapies will hit the market in 2029. Beginning Phase 1b/2a in 2024, Aruna Bio, Inc. is clearing an IND for AB126 (neural-derived exosome) for acute ischemic stroke.[19,20]

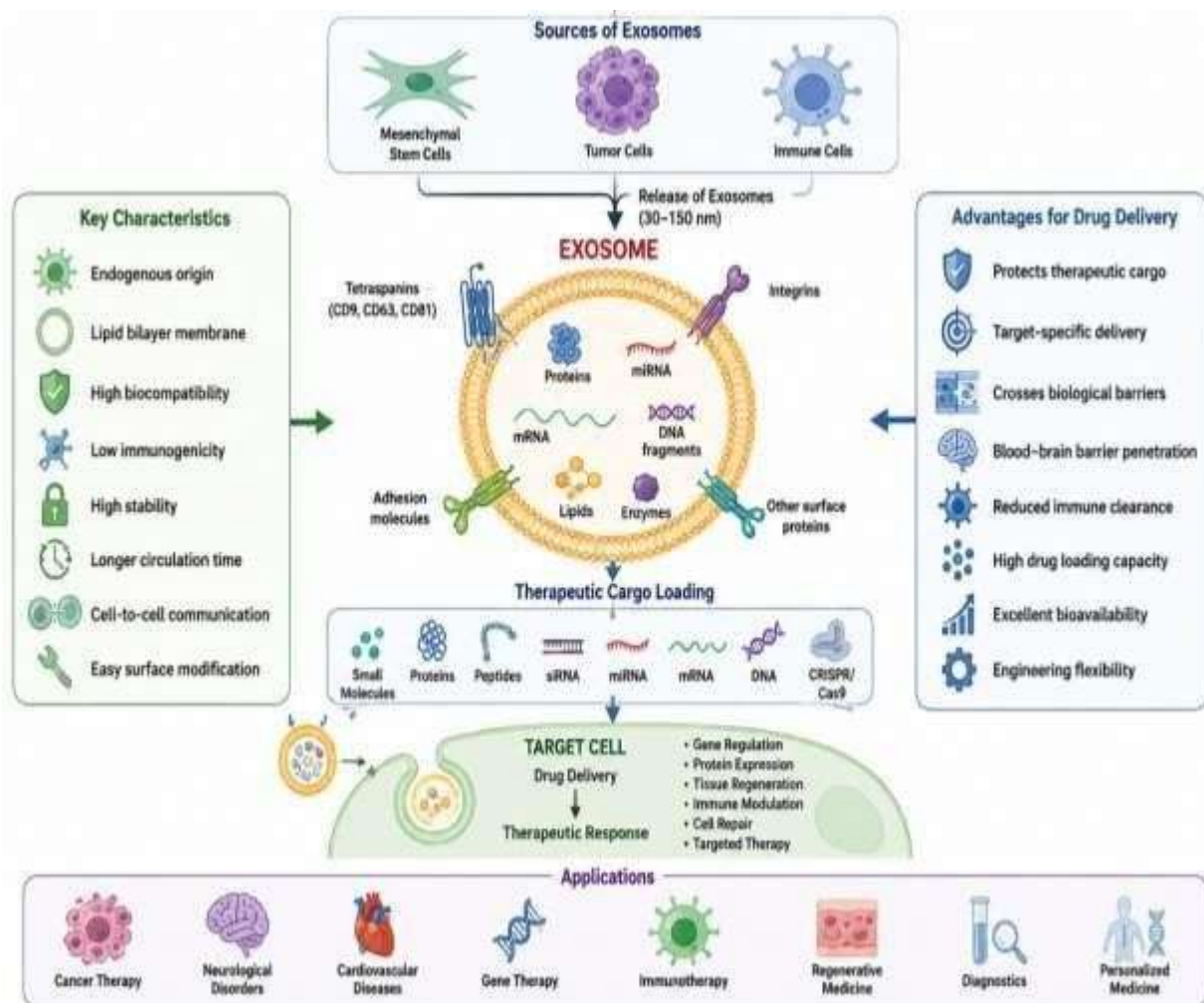


Figure 1: Overview of exosomes as endogenous nanocarriers for drug delivery.

2. Biogenesis of exosome

Formation of exosomes, initiated by formation of early endosomes which is formed by endocytosis of plasma membrane components such as lipids, proteins, receptors these endocytic vesicles comprise to form early endosome. These early endosome mature to late endosomes which is also termed as Multivesicular bodies (MVBs).[21] During the period of maturation the endosomal membrane inside the MVB, intraluminal vesicles (ILVs) are created when the endosomal membrane undergoes inward budding. These ILVs are later released as exosomes.[22] ILVs are selectively packed with cargo (proteins, lipids, and RNAs) using two primary processes, ESCRT dependent pathway (Endosomal Sorting Complex Required for Transport) and ESCRT independent pathway. In ESCRT-Dependent Pathway Ubiquitinated membrane proteins are recognized by ESCRT-0, ESCRT-I and II promote invagination of the membrane[23], ESCRT-III encourages vesicle scission to discharge ILVs into the MVB lumen ,Accessory proteins (like VPS4 and ALIX) facilitate recycling and deconstruction. However , in ESCRT- independent pathway Lipids and tetraspanins are the driving forces where Ceramide causes membrane budding through sphingomyelinase and Tetraspanins (CD9, CD63, and CD81) choose cargo and organize microdomains.[24] After sorting cargos into ILVs the MVBs have two possible fates either ILVs and their cargo degrade as a result of fusion with lysosomes or ILVs fuse with the plasma membrane to release what are now known as exosomes into the extracellular space. The fusion of MVBs with the plasma membrane is controlled by SNARE proteins and Rab GTPases (Rab27a/b, Rab11, and Rab35).Exosomes can carry their contents to close or distant cells once they are released.[25] The biogenesis is illustrated in Figure 2.

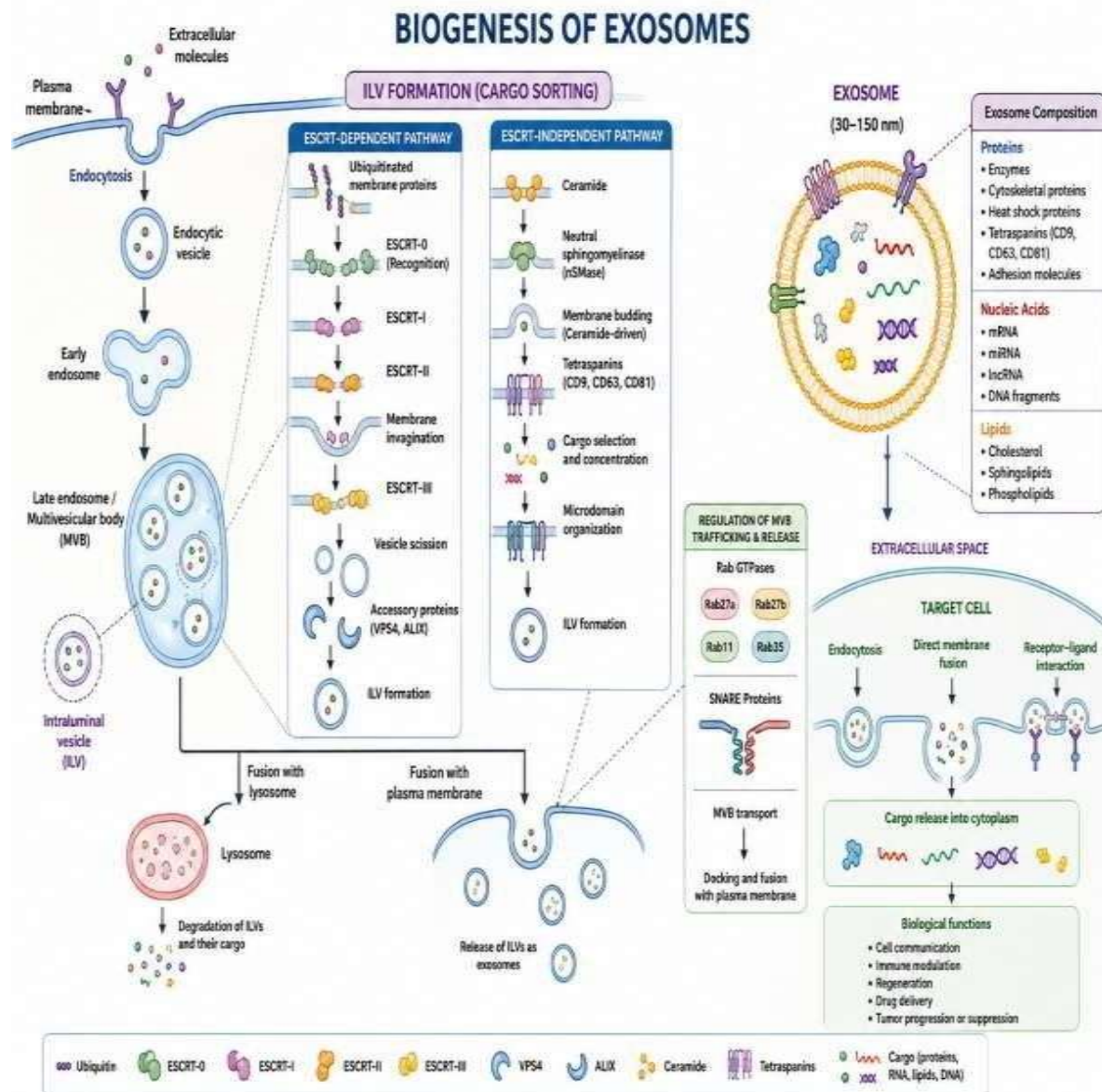


Figure 2: Biogenesis of Exosome

3. Harvesting of exosome

Harvesting of exosomes refers to process of isolating and purifying exosomes from various biological sources such as saliva, urine, cell culture media, blood and other body fluids. Main aim is to separate vesicles intact, biologically active, and free from contaminants like proteins or lipoproteins. Usually, the process starts with a pre-clearing stage in which materials are filtered and centrifuged at a low speed to get rid of bigger vesicles, debris, and cells. The application of various isolation techniques, including as size exclusion chromatography (SEC), differential ultracentrifugation, density gradient centrifugation, ultrafiltration, precipitation-based approaches, and immunoaffinity capture, comes next. The type of biological sample and the intended downstream use determine which approach is best.[26]

3.1 Differential Ultracentrifugation

Differential ultracentrifugation is a technique that is used to separate tiny biological particles such as exosomes, microvesicles, organelles and protein complexes from a mixture like cell culture media, blood or tissue extracts. [27] It operates on the concept of applying increasing centrifugal forces on particles in order to separate them according to their size and density. The procedure for how exosomes are isolated through differential ultracentrifugation are illustrated in Table 1. [28,29]

Table 1. Procedure for isolation of exosomes using differential ultracentrifugation

Step 1	Collect the liquid
Step 2	Take the culture medium or body fluid such as blood plasma, urine in which our cells grew, that contains exosomes.
Step 3	Keep everything cold ice or 4 °C to inhibit the disintegration of vesicles.
Step 4	First, remove the larger items (debris and cells).
Step 5	Spin once again at a slightly higher speed (2,000 × g for 15–20 minutes). Larger cell fragments and dead cells are pulled down as a result. Take out the pellet.
Step 6	Take out the medium-sized particles.
Step 7	Spin for about half an hour at 10,000 × g. Larger vesicles and apoptotic bodies—which are larger than exosomes—are brought down by this. Throw away the pellet. (to ensure that only tiny particles are left, pass the liquid through a 0.22 µm filter).
Step 8	Capture the exosomes
Step 9	Now, spin in an ultracentrifuge for one to two hours at a very high speed (100,000 × g).
Step 10	Small exosomes (30–150 nm) drop to the bottom at this speed. A tiny, occasionally undetectable pellet develops.
Step 11	Clean the exosomes by washing them.
Step 12	To re-suspend the pellet, add a little amount of sterile PBS(phosphate buffered saline) and stir gently.
Step 13	For about an hour, spin once more at 100,000 × g. This makes it easier in removing contaminants and residual proteins.
Step 14	Finally collect the exosomes. Be careful not to lose the pellet as you remove the liquid on top.
Step 15	The clean exosome pellet should be re-suspended in a very modest amount of PBS (between 50 and 200 µL).
Step 16	Small portions should be stored at -80 °C to prevent frequent freezing and thawing.

Note - Spin slowly → remove big things

Spin faster → remove medium things

Spin very fast → catch the exosomes

3.2 Size Exclusion chromatography

Size-exclusion chromatography (SEC) is a technique for isolating exosomes that separates particles according to size. Smaller proteins and molecules flow down the column later, while exosomes (30–200 nm) elute in the early fractions. It preserves the structure and function of exosomes while purifying them in a gentle and repeatable manner.[30,31] The procedure for how exosomes are isolated through size exclusion chromatography are illustrated in Table 2.

Table 2. Procedure for isolation of exosomes using Size Exclusion chromatography

Step 1	Collection of sample it may be cell culture media, blood/serum, urine. Process rapidly and keep on ice.
Step 2	Firstly clear the debris by centrifugation, if we centrifuge 300 × g, 10 min — remove cells (discard pellet), 2,000 × g, 20 min — remove dead cells/large debris, 10,000 × g, 30 min — remove large vesicles. Filtering the supernatant with a 0.22 µm syringe filter is optional.
Step 3	Use a 100 kDa MWCO centrifugal concentrator to concentrate to about 0.5–2 mL if the initial volume is large(optional).
Step 4	Prepare the SEC column by using 10–20 column volumes of 0.22 µm-filtered PBS at room temperature to equilibrate the column also eliminate air bubbles.
Step 5	Now load the sample by applying the sample gently into the column bed gently, by not disturbing the frit also note the loading time and volume.
Step 6	Elute and gather fractions. Elute using PBS and gather small fractions consistently (0.5-1.0mL each fraction for small columns). Exosome-rich fractions are found in the early void volume of typical commercial qEV-type columns (for small columns, the manufacturer's example is fractions ~7–10). Refer to the instructions for your column.
Step 7	Identify & pool EV fractions, determine which fractions contain particles by NTA (Nanoparticle tracking analysis), protein assay, or by following manufacturer guidance. Pool EV-rich fractions only.
Step 8	Concentrate & exchange buffer. Concentrate pooled fractions with a 100 kDa MWCO concentrator to desired volume for downstream use. If required, perform buffer exchange into your assay buffer.
Step 9	Quality checks are carried out such as particle size determination, Protein concentration determination, marker check that is western blot for CD9/CD63/CD81 and negative marker for example calnexin.
Step 10	For short term store at 4°C for few days for long term store measured sub-portion of a sample at -80°C also prevent frequent freezing and thawing.

3.3 Ultrafiltration

Ultrafiltration is a membrane-based method that separates and concentrates exosomes as per their size and molecular weight. It uses semipermeable membranes that only allows smaller molecules like salts and proteins flow through and limit the passage of particles having molecular weight of 100-300kDa. This method is simple, rapid, and easily scalable for processing large sample volumes. Tangential flow filtration (TFF) systems are used for large-scale or clinical-grade isolations because of their improved vesicle recovery and less membrane fouling, whereas centrifugal ultrafiltration units are frequently utilized for small-scale preparations.[32]

3.4 Tangential flow filtration

TFF offers a scalable and gentle way to concentrate huge amounts of plasma or culture supernatant. For UF, choose MWCO(Molecular Weight Cut-Off) that retains EVs; hollow-fiber modules marketed for "EV concentration" are the best. For pre-clear, use a microfiltration or ultrafiltration hollow-fiber or cassette that will retain vesicles (pore diameters ~0.1 to 0.22 µm). Modules designed for EV work typically have nominal pore diameters that hold nanoparticles between 30 and 200 nm; if MWCO style (kDa) is being used, select modules that are 100 kDa to 300 kDa equivalent (they keep exosomes while let proteins/small solutes pass through). EV modules from manufacturers are best.[33,34] The procedure for isolation of exosomes via TFF is elaborated in Table 3.

Few operating conditions should be maintained such as:

- 4°C (cold room or jacketed lines) is the temperature.
- Start low and ramp up to a gradual linear velocity for the crossflow or feed flow. Depending on the membrane area, an example small-lab hollow fiber can flow between 10 and 50 mL/min. Adjust to the module's size.
- In order to minimize fouling and vesicle deformation, the transmembrane pressure (TMP) should be kept low. Many labs run between 0.05 and 0.2 bar (about 3 psi) for EVs.

- Gather the permeate, which includes tiny compounds and soluble proteins. EV-enriched retentate will be used.
- Depending on downstream requirements, concentrate to a tolerable retentate volume (for example, 500 mL → 5–10 mL).
- Diafiltration (buffer exchange): do three to five diavolumes (add buffer while removing equal permeate volume) to remove serum proteins or exchange into PBS; track conductivity and protein content to determine completion.[35]

Table 3. Procedure for isolation of exosomes using Tangential flow filtration.

Step 1	Use sterile PBS (approximately 5–10 times the membrane volume) to thoroughly rinse the filter module. Rinse it again with sterile PBS after sanitizing it according to the manufacturer's recommendations.
Step 2	Fill the tubing and system with PBS to remove any trapped air bubble ensure that the flow is smooth.
Step 3	Add the pre-cleared (centrifuged and filtered) sample into the feed reservoir. Keep everything cold — around 4 °C — to protect the exosomes.
Step 4	Begin recirculating the sample slowly. Gradually increase the crossflow rate while keeping the transmembrane pressure (TMP) low to prevent damaging vesicles.
Step 5	Watch the flux and pressure readings. Avoid any sudden pressure spikes that could stress the membrane or the vesicles.
Step 6	Filtering should continue until the retentate, or final volume, is reached. If diafiltration is being done, add sterile PBS while filtering until the buffer exchange is finished.
Step 7	Gently flush the retentate into a sterile, low-binding tube. Try to minimize the time the sample spends at room temperature.
Step 8	To preserve performance for upcoming runs, clean the membrane and tubing according to the manufacturer's instructions.

3.5 Centrifugal Ultrafiltration

One of the simple & effective membrane based technique for concentration & purification of exosome is centrifugal Ultrafiltration. The term itself describes that the technique relies on centrifugal force, that pushes liquid sample through semi permeable membrane with a defined molecular weight cut off (MWCO) that allows smaller molecules i.e. salts, proteins & buffer components to pass through and restricts the passage of large vesicles like exosomes in upper chamber (retentate). Comparatively to conventional ultracentrifugation, centrifugal ultrafiltration offers several advantage because it is faster, less labor-intensive, requires smaller sample volumes, and reduces the risk of vesicle damage. The process is typically performed using commercial centrifugal filter units e.g., Amicon® Ultra, Vivaspin® that are operated at low to moderate speeds (2,000–5,000 × g) at 4 °C to preserve vesicle integrity. This technique is frequently employed for buffer exchange and desalting, or for concentrating exosome-rich supernatants following pre-clearing procedures (such as low-speed centrifugation and filtering).[36,37] The procedure for isolation of exosomes via centrifugal Ultrafiltration is elaborated in Table 4.

Materials Required [38]

- Pre-cleared sample such as cell culture supernatant, plasma, serum, or urine can be taken.
- Centrifugal ultrafiltration units for example , Amicon® Ultra, Vivaspin®
 - Membrane type can be regenerated cellulose or PES (polyethersulfone)
 - MWCO is typically for molecules having molecular weight of 100 kDa or 300 kDa
- Refrigerated centrifuge (swing-bucket rotor preferred)
- Sterile PBS or desired buffer (0.22 µm filtered)
- Sterile, low-binding collection tubes

Table 4: Procedure for isolation of exosomes using centrifugal ultrafiltration.

Step 1	To get rid of cells and debris, centrifuge the biological sample in steps: After 10 minutes of 300 × g, remove the cells. 20 minutes at 2,000 × g to eliminate apoptotic bodies 30 minutes at 10,000 × g to eliminate bigger vesicles and debris For further clarity, you can choose to filter via a 0.22 µm filter.
Step 2	The centrifugal filter unit should be Prepared .To get rid of preservatives, rinse the membrane with sterile PBS first. After the initial washing, discard the filtrate.
Step 3	To load the sample.Avoid going above the device's maximum volume when transferring the previously cleansed supernatant into the top chamber. Before centrifugation properly balance the tubes.
Step 4	Centrifuge at 3,000–4,000 × g for 15–30 min at 4 °C to Avoid totally drying the membrane by routinely checking the retained volume.
Step 5	Continue centrifugation until the retentate reaches the desired final volume (e.g., 200–500 µL). If performing buffer exchange/diafiltration, add sterile PBS to the retentate and repeat centrifugation.
Step 6	To collect the exosomes. Gently pipette out the retentate (upper chamber) — this contains concentrated exosomes. Place in a low-binding, sterilized microtube and store at 4 °C or on ice until needed.
Step 7	To store for Short-term store at 4 °C (up to 24–48 h) and for Long-term aliquot and store at –80 °C to avoid repeated freeze–thaw cycles.
Step 8	For Cleaning and disposal.dispose of used filter units according to biosafety protocols. If reusable, clean and sanitize per manufacturer’s instructions.

4. Why exosomes are considered potent for drug delivery systems?

Exosomes have proved to be a highly potential nanocarriers for drug delivery due to their inherent biocompatibility, stability in circulation, and natural cell-to-cell communication capacities. As they are produced from endogenous biological processes. Therefore, exosomes possess low immunogenicity and low toxicity, that allow them to escape through immune clearance and circulate longer than many fabricated nanoparticles. It contains a lipid bilayer membrane that offers extra protection for encapsulated therapeutic cargo such as small molecules, siRNA, miRNA, proteins, and CRISPR components.[39]

Their natural ability to target is a significant advantage. Tetraspanins, integrins, and adhesion molecules are surface proteins that allow exosomes to home to particular tissues according to their parent cell origin. This natural tropism can be used or modified for targeted therapy. They are able to get across biological barriers, such as the blood–brain barrier, which is a major obstacle for traditional medication carriers.[40]

Additionally, exosomes display great loading capacity and maintain medication stability by protecting bioactive molecules from enzyme destruction. Several engineering solutions (e.g., electroporation, sonication, membrane extrusion, surface modification) offer further enhancement of targeting and cargo loading efficiency. Their ability to control immune responses and deliver therapeutic molecules to difficult-to-reach tissues positions them as a diverse platform for cancer therapy, neurological illnesses, cardiovascular diseases, and gene therapy.[41]

Exosomes are among the most effective and prepared options for cutting-edge drug delivery systems due to their biocompatibility, natural targeting, barrier-penetration capacity, and engineering flexibility.[42]

5. Applications of Exosomes

5.1 Diagnostic and Biopsy

Exosomes are released into nearly every body fluids that includes blood, urine, saliva and cerebrospinal fluid . Their molecular cargo includes proteins, lipids, RNAs, and DNA fragments that reflects the physiological or pathological status of the cell or tissue from which they are originated. Therefore, they are considered suitable for non-invasive diagnosis and prediction. Cancer-specific proteins and nucleic acids are conveyed by tumor-derived exosomes in cancer therapy, which permits the diagnosis of the tumor, staging, monitoring of therapy response, and even detection of minimal residual disease.[43] Exosome profiles, for example, miRNAs, protein markers, offer possible early diagnostic or prognostic biomarkers for disorders like kidney injury, neurological problems, and cardiovascular disease.[44]

5.2 Drug and Nucleic Acid Delivery

Exosomes act as natural nanocarriers because their lipid bilayer protects encapsulated cargo (small molecules, RNAs, proteins) from degradation, and their biocompatibility and low immunogenicity reduce the risk of adverse immune responses. They can be designed or “loaded” with therapeutic cargos like chemotherapeutics, siRNA/miRNA, proteins, gene-editing tools to deliver them to target cells. Owing to innate surface indicators, exosomes frequently have targeting power – they can carry cargo preferentially to certain cell types or areas. [45]

5.3 Regenerative Medicine and Tissue Repair

Without the risks of whole-cell therapies, exosomes generated from stem cells, or other regenerative cells such as mesenchymal stem cells or endothelial progenitor cells, have been shown to promote tissue repair, angiogenesis, extracellular matrix remodeling, and wound healing. They can modulate inflammation, support cell survival, and aid in the repair of organs such as the kidney, heart, liver, and skin, thus representing a promising cell-free alternative for regenerative therapies. [46,47]

5.4 Immunotherapy

Exosomes can transport antigens or immuno-modulatory substances and impact immune responses. This has been investigated for the creation of “cell-free vaccines” or immunological treatments, such as antigen-loaded exosomes made from tumor or dendritic cells to boost anti-tumor immunity. Similarly, exosomes from immunological or regulatory cells may modulate inflammation or autoimmunity, giving therapeutic possibilities in inflammatory, autoimmune or degenerative disorders. [48,49]

6. Challenges

6.1 Heterogeneity and characterization

Exosome stuff is super mixed – different sizes, cargo, markers, where they come from. That makes it tricky to figure out what's going on, get the same results twice, and understand what they do. It's still hard to tell real exosomes apart from other stuff floating around, like other vesicles, lipoproteins, and globs of protein. You really need to use a bunch of ways to check them out (like NTA/TRPS, EM/cryo-EM, proteomics, and look for specific markers) to be sure, but not everyone does it. [50, 51]

6.2 Isolation, purity and yield trade-offs

Getting a good amount of pure substance that you can actually use is difficult because no single method is perfect. Things like ultracentrifugation and precipitation each have their own positive and negatives- you might end up with other substances which are not required, lose some of what is actually desired, or just not be able to process enough of it. This can cause problems and hamper other experiments you want to do later, like proteomics or functional assays. [52]

6.3 Scalability and GMP manufacture

Going from small lab experiments to manufacturing products that's good enough for patients and following all the rules can be a significant challenge. Some problems are that cellular production of the necessary substance is inadequate, there's variation between different donors, selecting appropriate equipment and processes can be challenging (like TFF, chromatography), and figuring out the right tests to make sure a complicated medicine is safe to use is hard. [53]

6.4 Cargo loading efficiency and stability

Getting meds or gene editors into tiny bubbles called exosomes can be tricky. Doing it right so they still do their job is hard. Usual loading methods, like using electricity, sound, or just mixing them, can make the stuff clump, break, or even ruin the bubbles. Additionally, keeping the substance stable in the body and giving the correct dose each time isn't easy. [54]

6.5 Targeting specificity and biodistribution

Exosome targeting occurs naturally but will vary by cell source and surface composition when measuring the exosome in vivo; many administered exosomes distributed throughout the body but collected predominantly in the liver, lungs, and spleen. Delivery of exosomes to target tissue (brain and tumours) will therefore be limited. Engineered surface ligands increase specificity to target sites however, they also create complications of immunogenicity and complexity due to additional modifications. [55,56]

7. Conclusion

Exosomes have emerged as highly promising natural nanocarriers, mainly because of their biological origin they come from, their inner durability, and the ability to transport functional biomolecules between cells. If we try to map out how they form, like the biogenesis pathways, it gives a better picture of what's happening

when these little vesicles sort and then deliver therapeutic cargo. That kind of understanding is basically what helps people design engineered exosomes, with stronger targeting, and more efficient loading. Also, harvesting and purification is getting better, with ultracentrifugation, size-exclusion chromatography, ultrafiltration, and affinity based methods, which has boosted both preparation quality and overall yield, although issues of purity, scalability, and standardization still persists. The wide range of applications of exosomes from drug and gene transfer to cancer therapy, neuroregeneration, immunomodulation, diagnostics, and regenerative medicine. This whole spread makes it feel like exosomes could be transformative across biomedical science. Still, progress is there but it's not the end of it. Key issues keep showing up, like heterogeneity, cargo loading that's not very efficient, limited large scale production, targeting that can be inconsistent, storage stability that sometimes drops, and regulatory rules that keep evolving. Fixing these weak spots will likely require better isolation protocols, smarter engineering approaches, tight quality control rules, and collaboration across disciplines. Overall, exosomes represent a powerful and versatile platform for next-generation drug delivery and therapeutics. Continued research into their biology, improved manufacturing approaches, and clearer clinical guidelines will pave the way for their safe, reproducible, and widespread clinical application in the future.

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