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BIOGENESIS, METHOD OF ISOLATION AND CHARACTERISTICS OF EXOSOMES

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ABSTRACT

Exosomes are small extracellular vesicles that are released by various cell types and play important roles in intercellular communication. Exosomes contain various biomolecules, such as proteins, lipids, and nucleic acids, that can be transferred between cells and modulate cellular processes. Exosomes have been implicated in various physiological and pathological processes, such as immune regulation, tissue repair, cancer progression, and neurodegeneration, and have potential applications as diagnostic or therapeutic agents. However, exosome research is plagued with the use of inconsistent isolation methodologies, nomenclature, and a lack of standardized data acquisition and analysis strategies. This limits the interpretation of the research conducted on exosomes. In this review, we explore the different methods for isolating exosomes and evaluate their advantages and disadvantages. We also discuss the challenges and opportunities in exosome research and highlight the need for standardized protocols and guidelines to ensure accurate and reproducible results.

KEYWORDS: Exosomes, Isolation, Biology, Characteristics, extracellular vesicles.

INTRODUCTION

Exosomes are extracellular micro sized vesicles and generated by cells. Exosome contains a set of neuclic acid, protein, lipids, metabolites, mRNA, DNA, enzymes, noncodingRNA.[1]

The word Exosomes was first discovered by Stahl and group in 1983 and Rose John stone in 1987 and the scientist found the exosomes from the sheep reticulocyte.[2]

Exosomes are small extracellular vesicles with size ranging between 30-150nm diameter and the source of the exosomes are endosomal system.[3]

The Exosomes are increasilly called as the significant mediators of cell to cell communication. The cells present in the body continuously produced large number of different types of micro vesicles like macromolecules and micro molecules into extracellular fluids one of them micro vesicles are Exosomes. biomedicine.[4]

Exosomes are nanosized vesicles. Natural exosomes are used as nanoparticles for the drug delivery system. Exosomes are found in the body fluid such as in saliva, blood, urine, breast milk, serum, cerebral spinal fluid, bile, lymph, tears, plasma and in semen.[3]

The Exosomes main role or use is to delivered signals from one cell to another cells and transmit information to targeting organ or cells.

Exosomes play important role in treatment of various types of cancer as macromolecules and as nano carrier.[5]

Exosomes can be classified based on their biogenesis, composition, and function. Based on biogenesis, exosomes can be classified as late endosome-derived exosomes or plasma membrane-derived exosomes. Late endosome-derived exosomes are generated through inward budding of the late endosome, while plasma membrane-derived exosomes are generated through outward budding of the plasma membrane. Based on composition, exosomes can be classified as containing a variety of proteins, lipids, and nucleic acids, including mRNA, miRNA, and other non-coding RNAs. The specific cargo of exosomes can vary depending on the cell type and physiological state of the cell.

Exosomes can be derived from both plant and animal cells, and they have some similarities and differences in their composition and function. Plant-derived exosomes are called exosome-like nanoparticles (ELNs) and are generated through inward budding of the plasma membrane. They are smaller in size than animal-derived exosomes, typically ranging from 30-100 nm in diameter. ELNs have been found in a variety of plant species and have been shown to contain a variety of proteins, lipids, and nucleic acids, including miRNAs and other non-coding RNAs. They are thought to play a role in intercellular communication and stress response in plants.

Animal-derived exosomes, on the other hand, are generated through inward budding of the late endosome and are typically larger in size than ELNs, ranging from 50-150 nm in diameter. They have been found in a variety of bodily fluids, including blood, urine, and saliva, and have been shown to contain a variety of proteins, lipids, and nucleic acids, including miRNAs and other non-coding RNAs. Animal-derived exosomes are thought to play a role in intercellular communication, immune regulation, and disease pathogenesis.[6]

Classification of exosomes

1.Natural exosomes

a.plant derived

b.Animal derived: 1.Normal exosomes.

2.Tumor exosomes.

2.Enginnered Exosomes :1.Cell derived nano vesicles(CDNs)

2.EVs inspired liposomes(EVLs)

[Exosomes Biology]

Origin: Endocytic pathway/Endosomes.Size: 30-15nm.Function: communication between cells. shape: cup shaped.mode of formulation: Endocytosis and Exocytosis. Diameter: 30 - 150nm. Lipids: Cholesterol, ceramide, Sphingomyelin, phosphatidylserine. Marker and surface receptors: Tetraspanins, Alix, Tsg 101, (CD9, CD63), ESCRT, Flotillin. Content: proteins, lipids, nucleic acid, non coding mRNA.Features: Biocompatibility, Low toxicity, Low immunogenicity. Examples: Tetraspanin, CD63, CD81, Antigen (MHC l and MHCll), Integrins, immunomodulatory protein.[7] route of administration: Intravenous injection (common), Subcutaneous injection, Intraperitoneal injection, Nasal administration, Oral administration, Intra tumoral injection. secreted by: neurons, oligodendroglial cells, microglia.[8]

Structure of Exosomes

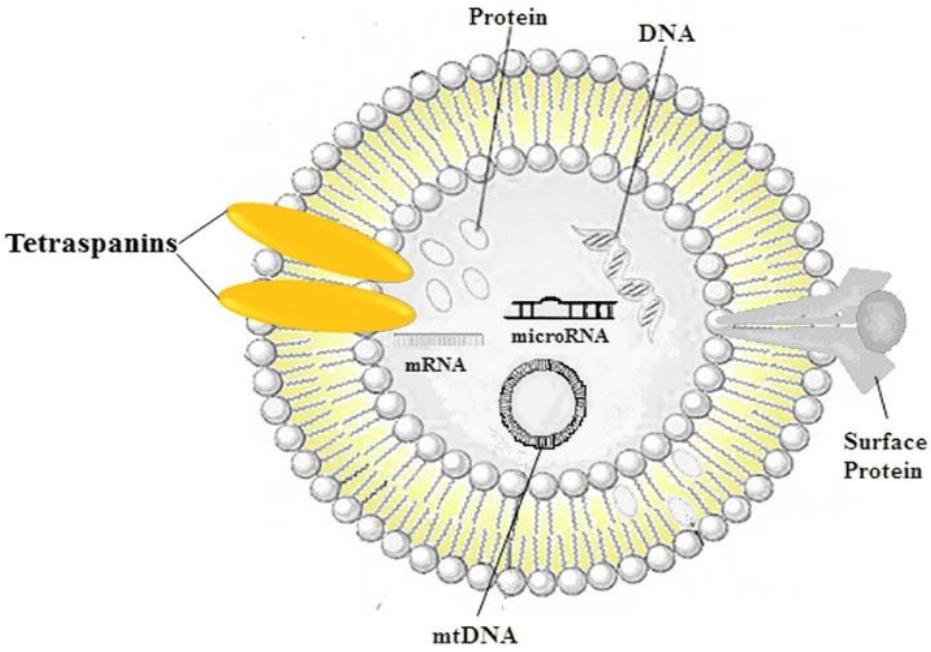

Figure1: structure of Exosomes.

Characteristics and properties

1. biocompatibility and safety.
2. physical stability factors.
3. Longitudinal body circulation
4. With a targeted supply chain.
5. material protection and encapsulation.
6. High repentance.
7. Enhanced cell segmentation.
8. Reducing toxicity.
9. low immunogenicity.
10. Organotropic properties.
11. Biocarrier permeability.[9]

Advantages of Exosomes

1. High peniteation is one of exosomes' benefits.
2. continuous blood flow.
3. Biocompatibility.
4. enhanced biological distribution.
5. least poisonous.

6. Increase blood flow.
7. carrier of medicine.
8. Immunocompatibility.
9. superior organotropism.[10]

Disadvantages of Exosomes

1. Low encapsulation agents as a result of low extraction yield efficiency.
2. rapid removal from the blood following in vivo injection.
3. No more manufacturing process.[10]

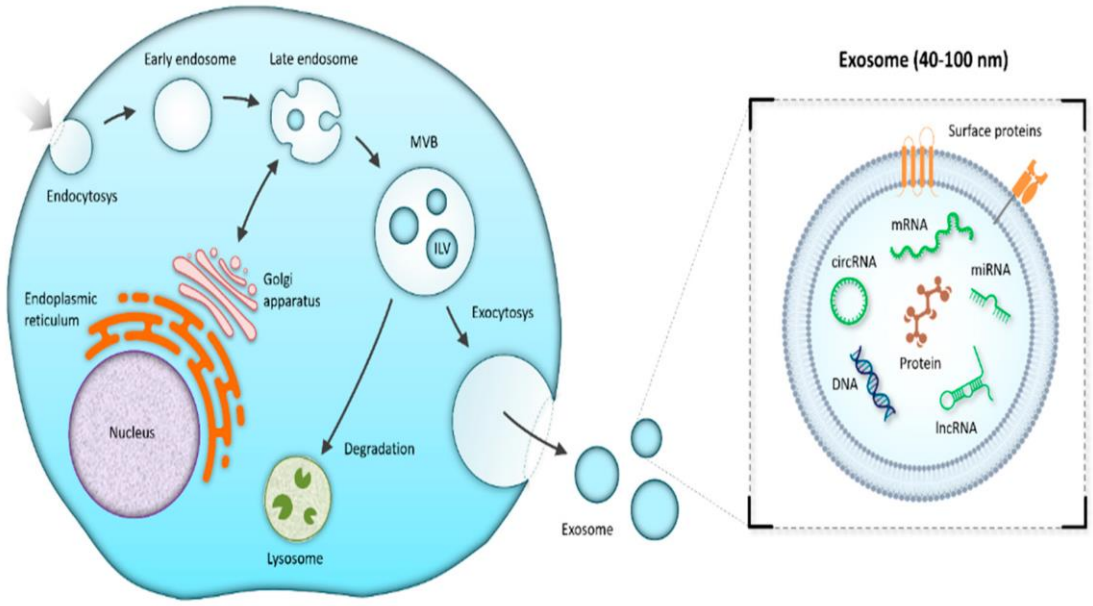

Figure 2: Biogenesis of Exosomes.

Biogenesis of Exosomes

Exosomes are formed through the endosomal pathway, which involves the inward budding of the plasma membrane to form early endosomes. These early endosomes mature into late endosomes, which contain intraluminal vesicles (ILVs) formed by the inward budding of the endosomal membrane. The late endosomes that contain ILVs are called multivesicular bodies (MVBs). MVBs can either fuse with lysosomes for degradation or with the plasma membrane for exosome release. The release of exosomes involves the fusion of MVBs with the plasma membrane, which results in the secretion of the ILVs as exosomes into the extracellular space. The exact mechanisms of exosome biogenesis and release are still not fully understood and are an active area of research.[11]

Biogenesis of Exosomes step wise

Regarding your question, the biogenesis of exosomes can be summarized in the following steps.

1. Inward budding of the plasma membrane: Exosomes are formed by the inward budding of the plasma membrane to form early endosomes.
2. Formation of multivesicular bodies (MVBs): The early endosomes mature into late endosomes or MVBs, which contain intraluminal vesicles (ILVs) that are formed by the inward budding of the endosomal membrane.
3. Sorting of cargo into ILVs: The cargo of exosomes can be selectively sorted and packaged into ILVs by various mechanisms, such as protein-protein interactions, RNA-protein interactions, and lipid-protein interactions.
4. Fusion of MVBs with lysosomes or plasma membrane: The MVBs can either fuse with lysosomes for degradation or fuse with the plasma membrane to release their ILVs as exosomes into the extracellular space.[12]

Role of Exosomes in Disease Progression

Exosomes have been extensively studied in the last several years for their potential as a diagnostic tool. It has been found that exosomes are present in nearly all body fluids, including milk, urine, saliva, and blood. Since Because Exosomes' distinct structure, which includes RNA, lipids, and proteins, may be helpful for diagnostic purposes.[2]

Explore the crucial role of exosomes in disease progression and their potential as biomarkers and therapeutic targets. Learn how exosomes contribute to the development and spread of various diseases and how studying their cargo can provide insights into disease mechanisms.

Diseases including cancer, neurological conditions, and cardiovascular conditions have all been linked to the advancement of exosomes. Exosomes have the ability to Diseases including cancer, neurological conditions, and cardiovascular conditions have all been linked to the advancement of exosomes. Exosomes have the ability to transport bioactive substances between cells, including proteins, lipids, and nucleic acids, which can alter gene expression and cellular signaling pathways. Tumor-derived exosomes, for instance, have the ability to influence the tumor microenvironment and transfer oncogenic chemicals to recipient cells, hence accelerating tumor development, angiogenesis, and metastasis in cancer. Exosomes have the ability to transfer misfolded proteins, such tau and amyloid-beta, between neurons in neurodegenerative diseases, which aids in the progression of the illness. Exosomes have the

ability to mediate communication between immune cells, smooth muscle cells, and endothelial cells inside cardiovascular disorders, hence regulating inflammation and vascular homeostasis. Consequently, exosomes have become attractive targets for the diagnosis, prognosis, and treatment of disease, ransport bioactive substances between cells, including proteins, lipids, and nucleic acids, which can alter gene expression and cellular signaling pathways. Tumor-derived exosomes, for instance, have the ability to influence the tumor microenvironment and transfer oncogenic chemicals to recipient cells, hence accelerating tumor development, angiogenesis, and metastasis in cancer. Exosomes have the ability to transfer misfolded proteins, such tau and amyloid-beta, between neurons in neurodegenerative diseases, which aids in the progression of the illness. Exosomes have the ability to mediate communication between immune cells, smooth muscle cells, and endothelial cells inside cardiovascular disorders, hence regulating inflammation and vascular homeostasis. Consequently, exosomes have become attractive targets for the diagnosis, prognosis, and treatment of disease.[14]

Exosomes have also been implicated in drug resistance and immune evasion in cancer. In addition, exosomes have been shown to play a role in the progression of neurodegenerative diseases, cardiovascular diseases, and infectious diseases. However, the exact mechanisms of exosome-mediated disease progression are still not fully understood and are an active area of research.[11]

Methods of Isolation of Exosomes

However, since exosomes are so tiny, isolating them can be difficult. But some laboratories have been successful in isolating exosomes utilizing strategies by using methods like ultracentrifugation, ultrafiltration, chromatography, polymer-based precipitation, immunomagnetic isolation and size based chromatography.[12-25]

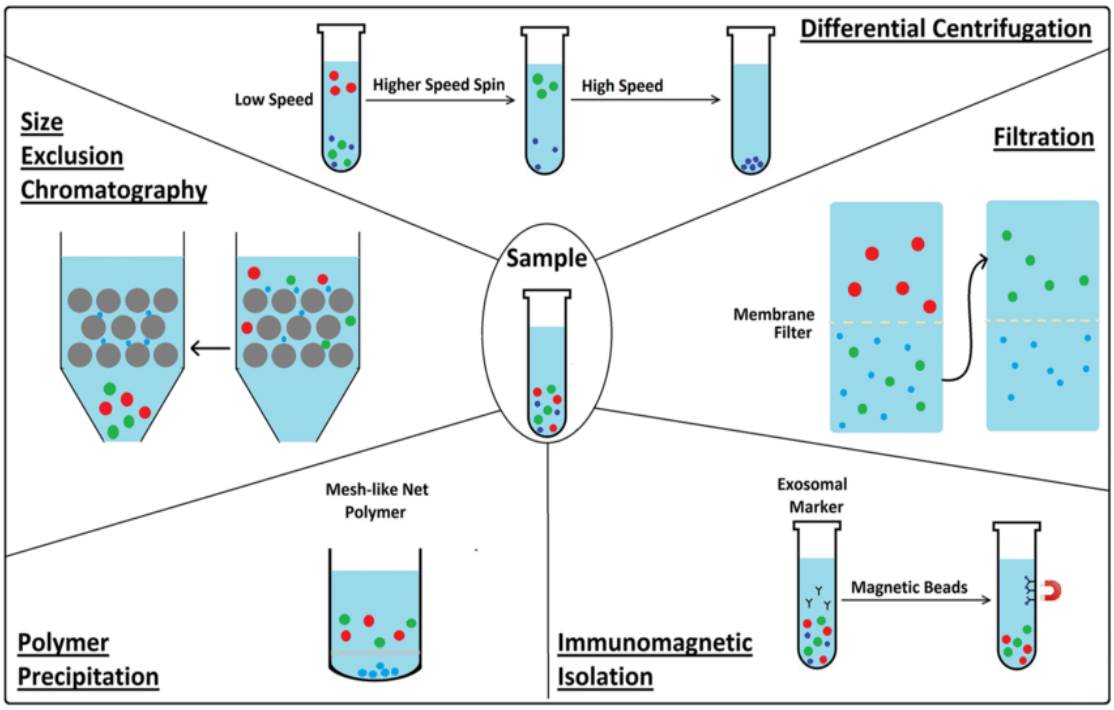

Figure 3: methods of isolation.

1. Ultracentrifugation

Exosomes are separated using the ultracentrifugation technique. Ultracentrifugation apply a high centrifugal force of a heterogeneous mixture. Exosomes isolation method is based on their size and density using high-speed centrifugation, Constituents present in the mixture sedimented according to density and size with large or densor particle sediment first. Which is one frequently technique used for exosome isolation.

The size of the exosomes is based for exosome isolation. extraction of exosomes from blood or cell-culture medium is challenging due to a significant there are lots of tiny particles in the media having a size range that is similar to that of exosomes.

Ultracentrifugation is a widely used technique for exosome isolation. It can be divided into two main types: differential ultracentrifugation (DUC) and density gradient ultracentrifugation.[12]

The first and most often documented exosomal isolation method is DUC. It can be applied to high-dose sample analysis because it is a straight forward procedure that does not introduce additional markers. DUC's fundamental idea is to sort particles according to their size, shape, and density, which determines their sedimentation coefficient.

Based on DUC, density gradient ultracentrifugation is an enhanced method. It makes use of discrepancies between the densities of the particles and the medium by adding sample particles to inert fluid having a density gradient (such sucrose and cesium chloride). Particles focus on their designated locations inside the gradient medium when subjected to a specified relative centrifugal force. In the end, distinct zones are created, allowing for the collection of separate high-purity particles from distinct zones. Particles with slightly different sedimentation coefficients can be isolated using this technique. Considering the natural disparity in density among various extracellular components, this technique can yield a more refined exosome.[13]

Method of Isolation

Ultracentrifugation is a commonly used method for the isolation of exosomes. It involves several steps.

Steps.

1. Collection of the biological sample, such as blood or cell culture media, containing exosomes.
2. Centrifugation at low speeds to remove cells and debris.
3. Further purification using a series of high-speed centrifugation steps.
4. Density gradient centrifugation to separate exosomes based on their density.
5. Final centrifugation to pellet the purified exosomes.[12-23]

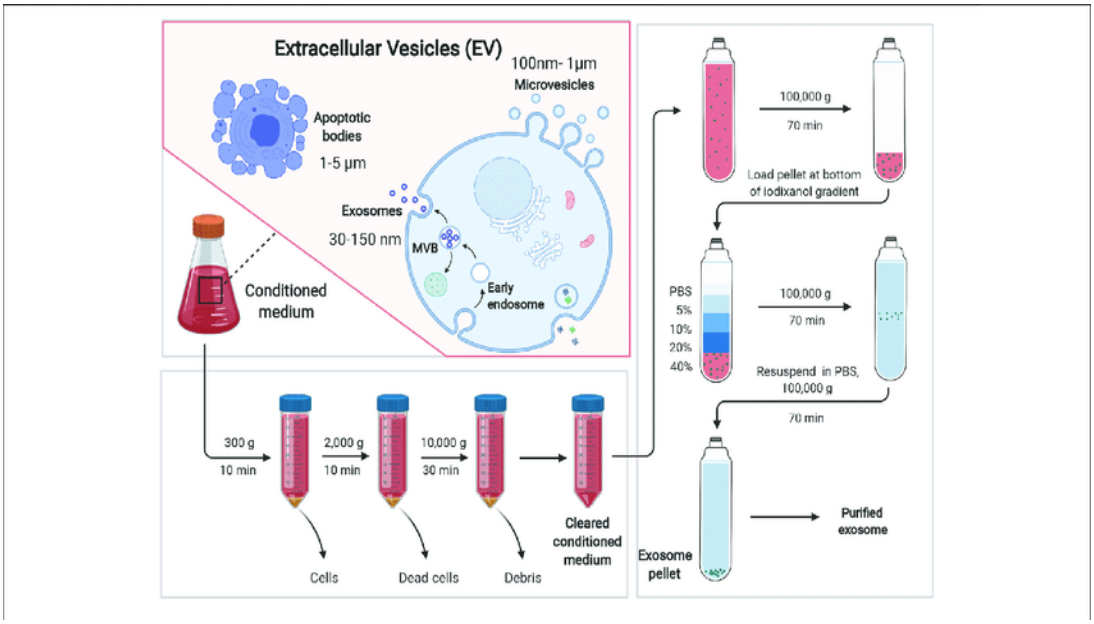

Figure 4: ultracentrifugation technique.

Advantages of Ultracentrifugation.

For the isolation of exosomes, ultracentrifugation offers a number of benefits, including.

1. High recovery: It has been demonstrated that ultracentrifugation, a well-researched and popular method for isolating exosomes, has high recovery rates.
2. High purity: High-purity exosome preparations can be obtained by efficiently separating exosomes from other extracellular vesicles and impurities using ultracentrifugation.
3. Versatility: Exosomes can be extracted via ultracentrifugation from a variety of biological materials, such as cell culture supernatants, blood, and urine.
4. Preserving the integrity of exosomes: Ultracentrifugation is a mild isolation technique that doesn't include strong chemicals or significant shear forces that might harm or change the exosomes.[13]
5. High sample capacity.
6. High seperation.[15]

Disadvantages of Ultracentrifugation.

1. Time consuming method.
2. Long running time.
3. Low portability.
4. High speed centrifugation can damage Exosomes.
5. Labour intensive.[15]

2. Ultra-filtration

One of the most popular size-based methods for isolating of exosomes is ultrafiltration this technique works on the same principle as traditional membrane filtration.[16]

Therefore, it is a size-based isolation technology.[19] Based on this principle, two types of ultrafiltration devices have been well developed: tandem-configured ultrafiltration and sequential ultrafiltration.[14]

Ultrafiltration separation technology is a novel method for the isolation of exosomes from samples. The technique is based on the principle that the pore size of the ultrafiltration membrane allows substances of specific relative molecular mass to pass through or be intercepted. Solvent and small molecules will be filtered through the membrane, while the molecules with higher relative molecular masses will be trapped in the ultrafiltration membrane, thereby achieving separation Stirred ultrafiltration is a type of ultrafiltration that

can be used for the separation of exosomes from samples. The use of this method for the separation of exosomes applies the same principles as centrifugal ultrafiltration, with the difference being that the latter relies on centrifugal force, rather than a membrane, to separate out the solvent and small molecules, which requires centrifugation at 100,000×g for 1-2 h to obtain exosomes. In stirred ultrafiltration, the pressure generated by the externally supplied nitrogen causes the sample to be passed through the ultrafiltration membrane to extract the exosomes.

However, there is a concern regarding the lack of purity of the end-product as there is with ultrafiltration. It is difficult to exclude those compounds or molecules with a similar diameter to exosomes from being coextracted, such as microbubbles and apoptotic bodies.[17]

Methods of isolation of Exosomes by using ultrafiltration

The following are the overall steps for exosome isolation via ultrafiltration.

1. Collect the organic pattern (including blood, urine, or mobile culture supernatant) and centrifuge it at low pace (e.g., three hundred × g) to eliminate cells and debris.
2. Transfer the supernatant to a new tube and filter out it via a 0.22-μm filter to put off larger particles.
3. Transfer the filtered supernatant to an ultrafiltration device with a membrane with a targeted pore diameter or molecular weight cut-off (MWCO) for isolating debris in a predetermined size variety.
4. Apply strain or centrifugal force to the pattern to pressure it thru the membrane and maintain the exosomes at the membrane.
5. Collect the filtrate containing non-exosomal debris and discard it.
6. Wash the membrane with the right buffer (which include phosphate-buffered saline or Tris-HCl) to get rid of any ultimate contaminants.
7. Elute the exosomes from the membrane the usage of the precise buffer.
8. Optionally, carry out a second ultrafiltration step to further purify the exosomes.
9. Characterize the remoted exosomes the usage of numerous methods including nanoparticle tracking analysis, transmission electron microscopy, and Western blotting.[12-24]

Digramatic illustration of ultrafiltration

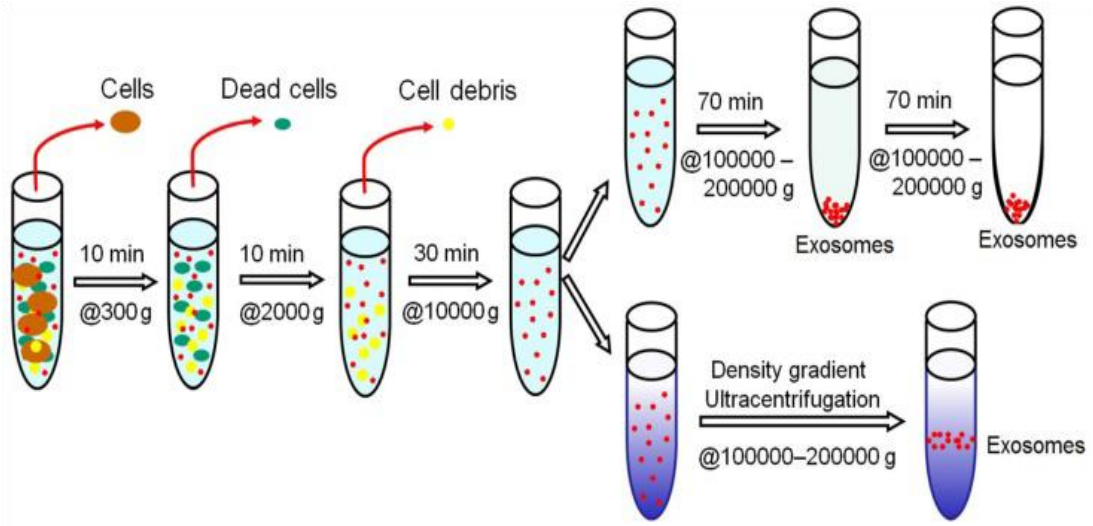

Figure 5: Ultrafiltration technique.

Advantages of ultrafiltration

Ultrafiltration has several advantages over exosome isolation,

1. Simple operation: Ultrafiltration is a simple and straightforward process that does not require specialized equipment or extensive training.

2. Flexibility of analysis in large biological samples: Large-scale ultrafiltration can be readily performed to process large numbers of biological samples, making it suitable for complex applications

3. Ability to separate exosomes of specific size: Ultrafiltration allows separation of exosomes based on their size, which is important for downstream applications requiring a specific size

4. Preservation of exosome integrity: Ultrafiltration is a gentle isolation technique that does not require strong chemicals or high tensile forces, which can damage or alter exosomes.[18]

Disadvantages of ultrafiltration

1. Use of force that might cause significant objects to distort and break up capsules.

2. Moderate purity of isolated Exosomes.

3. Shear stress induced deterioration.

4. Possibility of clogging and vesicles trapping.

5. Exosomes loss due to attaching to membranes.[18]

3. Size exclusion chromatography

Size exclusion chromatography (SEC) is a frequently employed method for isolating exosomes, which involves dividing the particles into different sizes. Larger particles flow

through the column more quickly in SEC, whereas smaller particles enter the pores and take longer to elute. The sample is run through the column made of porous resin. Smaller particles elute later than larger particles, resulting in the separation of particles based on size.[17]

Size exclusion chromatography (SEC) is a liquid chromatography technique that separates molecules based on their size. It uses a porous gel filtration polymer as the stationary phase and the starting biofluid as the mobile phase. The nature of the stationary phase allows differential elution: bigger particles elute first, followed by smaller vesicles, and then non-membrane-bound proteins. This is because the bigger the particle, the fewer pores it will be able to traverse, and thus will transverse a shorter path to the end of the column, making it elute faster in comparison to its smaller counterparts. The stationary phase or the chromatography column can be packed with a number of gel polymers including crosslinked dextrans (Sephadex), agarose (Sepharose), polyacrylamide (Biogelp), or allyldextran (Sephacryl).[19]

steps involved in the isolation of exosomes by size exclusion chromatography (SEC)

1. Prepare the SEC column:- Choose a suitable SEC column based on the sample volume and the desired resolution.- Equilibrate the column with the appropriate buffer (e.g., PBS) according to the manufacturer's instructions.
2. Load the sample:- Load the biofluid (e.g., blood plasma or serum) onto the SEC column.- Collect the fractions containing exosomes based on their elution profile.
3. Concentrate the exosomes:- Concentrate the exosome-containing fractions using a centrifugal filter device.- Wash the concentrate with PBS or another buffer to remove any contaminants.
4. Purify the exosomes:- Centrifuge the concentrate again to obtain a purified exosome pellet.- Resuspend the exosome pellet in PBS or another buffer for downstream applications.[12]

Diagram

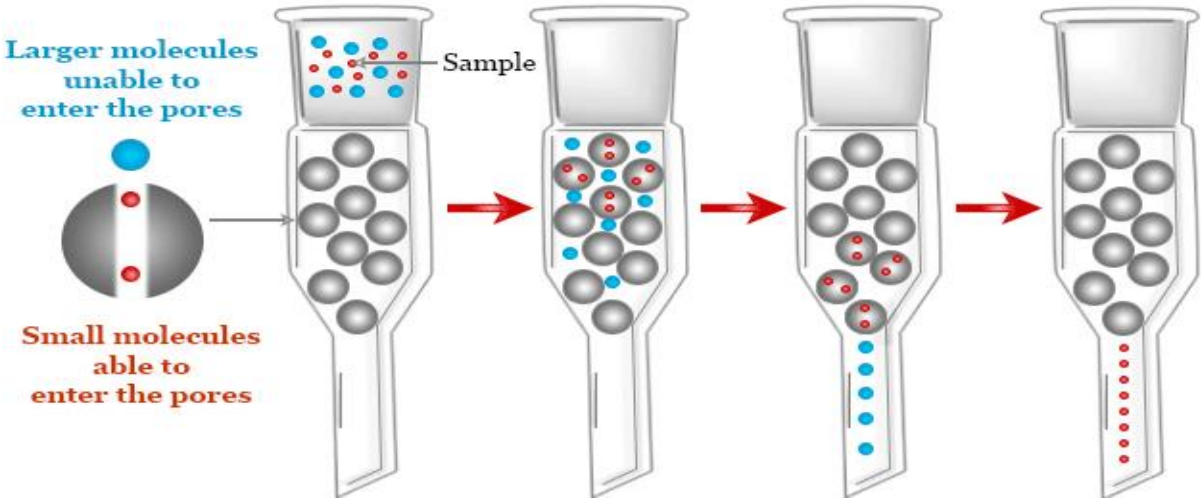

Figure 6: size exclusive chromatographic technique.

Advantages of size exclusion chromatography

1. High yield: SEC can isolate a large quantity of exosomes from a relatively small volume of biofluid, making it a suitable method for downstream applications that require a high yield of exosomes.
2. High purity: SEC can separate exosomes from other components based on their size, resulting in a highly purified exosome sample.
3. Gentle isolation: SEC does not require harsh chemicals or high shear forces, which can damage or alter the exosomes. This makes it a gentle isolation method that preserves the integrity of the exosomes.
4. Versatility: SEC can be used to isolate exosomes from a variety of biofluid, including blood plasma, serum, urine, and cerebrospinal fluid.
5. Compatibility with downstream applications: The isolated exosomes can be used for a variety of downstream applications, including proteomics, genomics, and drug delivery.[20]

Disadvantages of size exclusion chromatography

1. Low recovery: SEC can result in a lower recovery of exosomes compared to other methods, such as ultracentrifugation. This is because some exosomes may be trapped in the pores of the SEC column or may not be retained by the column due to their size.
2. Time-consuming: SEC can be a time-consuming method, as it requires several steps, including column equilibration, sample loading, and fraction collection. Additionally, the processing time per sample can be longer compared to other methods, such as ultracentrifugation.

3. Expensive: SEC can be an expensive method, as it requires specialized equipment and columns that can be costly.

4. Limited resolution: SEC can have limited resolution for exosomes that are similar in size to other components in the biofluid, such as lipoproteins. This can result in co-elution of exosomes with other components, leading to a less pure exosome sample.

5. Variability: The quality and reproducibility of SEC can be affected by several factors, including the type of column, buffer conditions, and sample preparation. This can result in variability in the isolated exosome sample.[20]

4. Polyethylene glycol (PEG)

The ExtraPEG method is a polyethylene glycol (PEG) precipitation-based technique for isolating exosomes and other extracellular vesicles.[21] SREP PDF. This method has been used for over 50 years to isolate viruses and macromolecules. PEG or other hydrophobic polymers precipitate exosomes through changing solubility and dispensability of the samples.[22]

PEG is a volume-excluding polymer that can be used to concentrate and purify viruses, and because exosomes and viruses have similar biophysical properties, the ExtraPEG method was developed to enrich and purify exosomes. Highly pure exosomes can be isolated with PEG, and previous reports have suggested that highly pure vesicle preparations have particle-to-protein ratios in excess of ten billion. The ExtraPEG method is a simple and cost-effective alternative to commercial kits, and it has been shown to yield high-purity exosome preparations with minimal protein contamination.[21]

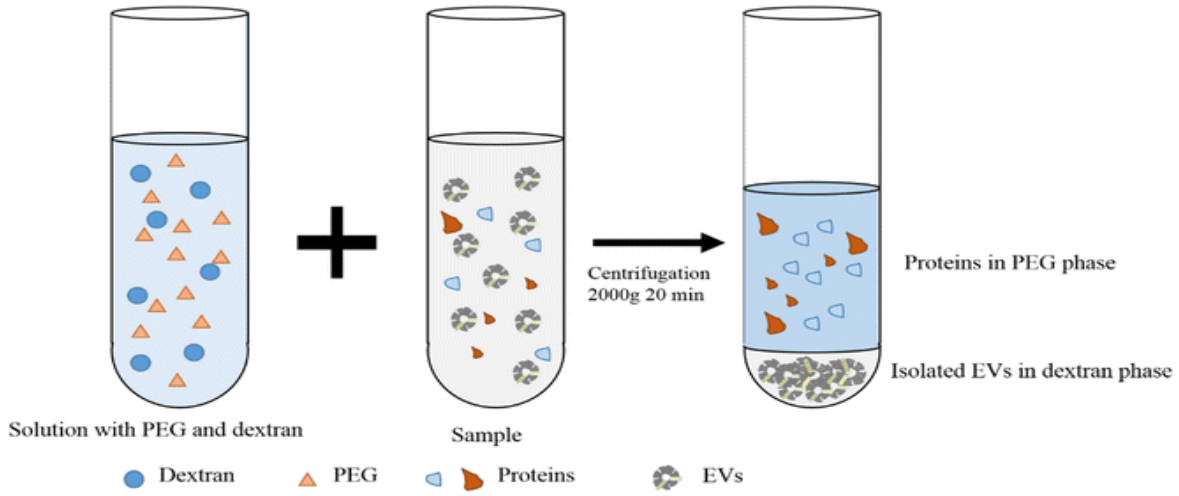

Figure 7: polyethylene glycol technique.

steps for isolating exosomes using polyethylene glycol (PEG) precipitation

1. Collect the biofluid containing exosomes (e.g. blood, urine, or cell culture media).
2. Centrifuge the biofluid at low speed (e.g. 300 x g) to remove cells and debris.
3. Transfer the supernatant to a new tube.
4. Add PEG solution to the supernatant and mix well.
5. Incubate the mixture at 4°C overnight to allow exosomes to precipitate.
6. Centrifuge the mixture at high speed (e.g. 10,000 x g) to pellet the exosomes.
7. Discard the supernatant and wash the exosome pellet with an appropriate buffer (e.g. PBS).
8. Centrifuge the mixture again to pellet the exosomes.
9. Discard the supernatant and resuspend the exosome pellet in an appropriate buffer for downstream applications.[22]

Advantages of polyethyl glycol precipitation

1. Cost-effectiveness: PEG is a relatively inexpensive reagent, and the ExtraPEG method is a simple and cost-effective alternative to commercial kits for exosome isolation.
2. High yield: PEG can concentrate and purify exosomes from large volumes of biological fluids, and the ExtraPEG method has been shown to yield high-purity exosome preparations with minimal protein contamination.
3. Versatility: The ExtraPEG method can be adapted to enrich for extracellular vesicles other than exosomes, and it can be used to isolate vesicles from a diversity of biological fluids
4. Ease of use: The ExtraPEG method is a simple and rapid technique that can be performed with standard laboratory equipment.
5. Compatibility with downstream applications: The ExtraPEG method yields exosomes that are compatible with downstream applications such as Western blotting, ELISA, and RNA sequencing.

Disadvantages of poly ethyl glycol precipitation

1. Low specificity: PEG can precipitate other proteins and contaminants in addition to exosomes, which can result in low specificity and purity of the isolated vesicles.
2. Variability: The efficiency of PEG precipitation can vary depending on the biological fluid and the concentration of PEG used, which can result in variability in the yield and purity of the isolated vesicles.
3. Potential damage to vesicles: The centrifugation steps involved in PEG precipitation can potentially damage the vesicles and affect their biological activity.

4. Limited scalability: PEG precipitation-based techniques may not be scalable for large-scale production of exosomes or other extracellular vesicles.

5. Limited characterization: PEG precipitation-based techniques may not provide detailed information on the size, shape, and composition of the isolated vesicles, which can limit their utility for certain applications.[21]

CONCLUSION

Exosomes are small extracellular vesicles that are released by cells and carry various biomolecules, such as proteins, lipids, and nucleic acids. They play important roles in intercellular communication and have been implicated in various physiological and pathological processes. However, exosome research is plagued with the use of inconsistent isolation methodologies, nomenclature, and a lack of standardized data acquisition and analysis strategies. Therefore, the development of more efficient and standardized methods for isolating and characterizing exosomes will be critical for advancing our understanding of their biological functions and potential clinical applications. Based on the efficiency, reliability, rigor, reproducibility, and easy of use, a SEC-coupled approach to exosome isolation for a high yield of homogenous, intact exosomes seems ideal. However, direct comparative studies are required to support this conclusion. We recommend that researchers validate the exosome isolation technique before beginning experiments, especially if using novel biofluid or samples.

Future perspectives

1. Development of More Efficient Isolation Techniques: Continued improvement and innovation of methods to enhance exosome isolation efficiency.

2. Exploration of Exosome Cargo for Therapeutic Purposes: Investigating exosome cargo for potential therapeutic applications in various diseases.

3. Better Understanding of Exosome Biology and Function: Gaining deeper insights into the role of exosomes in intercellular communication and disease pathogenesis.

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