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EXOSOMES: BIOGENESIS, ISOLATION TECHNIQUES, APPLICATIONS, AND THERAPEUTIC POTENTIAL

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Abstract

Exosomes are nanosized with extracellular vesicles (40–160 nm) that play a vital role in inter cellular communication by transporting proteins, lipids, and nucleic acids between cells. Once considered merely cellular waste products, they are now widely recognized for their significant involvement in both physiological and pathological processes, including cancer progression, neurodegenerative diseases, and immune system regulation. One of their most important features is their ability to reflect the molecular composition of their parent cells, making them highly valuable as potential biomarkers for disease diagnosis and prognosis.This review provides an overview of exosome bio-genesis, highlighting how they are formed and released from cells. It also examines their key advantages, such as stability, bio-compatibility, and the ability to cross biological barriers, along with their disadvantages and current limitations, including challenges in large-scale production and standardization.

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1. INTRODUCTION

Exosomes are tiny extracellular vesicles, measuring 40-160 nm, that are super important for inter cellular communication. They carry proteins, lipids, and nucleic acids, helping move bio active molecules around. They were first discovered back in 1981 by Trams Et Al., who collectively referred to plasma membrane-derived vesicles as exosomes & first proposed the concept of exosomes [1]. Later studies have shown how they play a part in things like cancer immune responses, and diseases that affect the Nerves [2].

Nowadays, people are really interested in diagnostics and treatments because they reflect the molecular signatures of their parent cell[3]. Initially, these entities were presumed to constitute waste products that are released through the shedding of the plasma membrane [4]. Subsequent studies demonstrated that the tracking of transferring-gold conjugates within the endocytic (ILVs) formed within multi vesicular bodies (MVB) may be released into the Extracellular space via fusion with the plasma membrane [5].

Consequently, exosome-based therapies are currently under investigation in anti-cancer clinical trials. Recent findings suggest that Taxol - loaded exosomes can effectively treat cancers in mice at doses that are 50 times lower than those required for conventional therapies, while also minimizing the elicitation of an immune response [6]. However, notwithstanding two decades of research, foundational knowledge regarding exosome biology remains in its nascent stages, and there exists a Limited Understanding of the roles that exosomes play in normal cellular physiology [7].

Exosome biogenesis

Exosomes are consistently produced from late endosomes, which originate from the inward budding of the limited Multi vesicular body (MYB) membrane. This invagination of late endosomal membranes leads to the development of intraluminal vesicles (ILVA) within Large MVBS [8]. During this process, specific proteins are incorporated into the invaginating membrane, while cytosolic components are engulfed and subsequently enclosed within the ILVs. Most ILVs are released into the Extracellular environment upon fusion with the plasma membrane, at which point they are designated as exosomes. Conversely, some components may be directed to lysosomes for degradation [9]. Exosomes possess a density ranging from 1.13 g/ml for B-cell-derived exosomes to 1.19 g/ml for epithelial cell-derived exosomes when analyzed via sucrose gradient centrifugation [10]. Empirical evidence has indicated that the formation of ILVs necessitates the function of the endosomal sorting complex required for transport [11].

These Micro domains are believed to be rich in Sphingomyelinases, which can generate ceramides through the hydrolytic removal of the phosphocholine moiety [12]. The cells produce various types of membrane vesicles, which include macro-vesicles that represent a heterogeneous population of membrane vesicles generated by outward budding from the plasma membrane, with sizes ranging from 100-1000nm and exhibiting various shapes [13].

1.1 Advantages of Exosomes

1.Nature-derived drug delivery Systems are increasingly utilizing exosomes due to their capacity to transport an array of therapeutic molecules, proteins, lipids, and nucleic acids. Specifically targeting cells, these characteristic positions make exosomes promising candidates for the development of advanced drug delivery systems.

2. In terms of immunogenicity, exosomes present a lower risk of immune rejection. This reduced immunogenicity arises from their origin in the patient's own cells, thereby minimizing the likelihood of adverse reactions. Such attributes enhance the safety profile of exosomes applications in personalized medicines regenerative therapeutic strategies.

3.The role of exosomes in tissue regeneration is marked by their capability to facilitate the transfer of growth factors and other bioactive molecules to damaged tissues. Evidence indicates their effectiveness in the treatment of various conditions, including cardiac diseases. Hepatic injuries and neurodegenerative disorders; thus, signifying their therapeutic potential in regenerative medicine.

1.2 Disadvantages of Exosomes

1. Exosome treatments can cause short-term side effects like redness, swelling, and minor irritation. In rare cases, these side effects can persist for a few days.
2. Some people may be allergic to substances in exosomes.
3. Exosome therapy may not be suitable for people with certain medical conditions, such as active cancer, autoimmune disorders, or uncontrolled infections.
4. The effects of exosome treatments are not always clear.
5. Exosome treatments can be expensive.
6. Exosomes have a short half-life, which can make it difficult to deliver them to injury sites.

1.3 Limitations of Exosomes

As the exosomes are isolated using different techniques, mostly by the ultracentrifugation method,it is a time-consuming process that requires a bulk sample, and it disrupts process leads to loss of biological activity of exosomes. First, exosomes tend to have a short lifespan in the body, meaning they can be quickly cleared away by macrophages.

MATERIALS AND METHODS

Exosomes are formed through the inward budding of endosomal membranes, leading to the formation of intraluminal vesicles within multivesicular bodies. These vesicles contain selected proteins and cytosolic components that are later released as exosomes. Their formation involves both ESCRT-dependent and ESCRT-independent pathways. The ESCRT system plays a key role in sorting cargo into vesicles, whereas lipid-mediated pathways involving ceramide and tetraspanins also contribute to exosome formation.

- Exosomes typically have a density ranging from 1.13 to 1.19 g/ml and are isolated using various techniques such as ultracentrifugation, ultrafiltration, polymer precipitation, and immunoaffinity chromatography.

2.1 Methods of Isolation of Exosomes

Exosomes isolation methods are used to separate and purify exsomes from biological fludis based on their size, density, surface markers. The following methods are involved[14].

2.1.1 Ultra Centrifugation Technique

Isolation of exosomes is done by ultracentrifugation having 2 steps. Before the isolation process, we can remove the fat globules, cream, cellular debris, and somatic cells at low-speed centrifugation.Requires 100 ml of bovine milk during the start of the processs and buffers must be ice-cooled, and centrifugation must be performed at 4°C (Super cooling)Ultracentrifugation (UC) is recognized as the predominant technique for isolation of exosomes [15].

The ultracentrifugation process is typically executed in 2 phases

The first involves a series of low to medium speeds to remove dead cells, cellular debris, etc. The second phase involves or entails high-speed centrifugation at forces reaching 1 lakh x g to isolate exosomes, which are then washed with phosphate-buffered saline (PBS) to eliminate any remaining proteins. The yield and purity of the isolated exosomes depend on centrifugal force, rotor type, and operational parameters. Density gradient centrifugation serves the purpose of further purifying exosomes. Ultracentrifugation to enhance exosome purity [16].

2.1.2 Ultra Filtration

- Ultrafiltration is a technique that involves the separation of a fluid across a semipermeable membrane, which selectively permits some substances.
- For isolation of extracellular vesicles, the porous membrane filter pore size must be 0.08-0.1mm.
- Based on the size, the membrane facilitates the concentration of the sample and the separation of molecules.

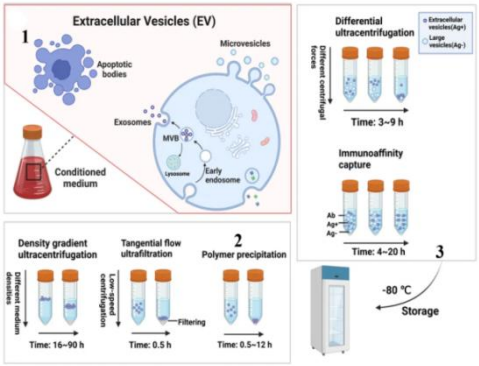


Figure 01: Different classes of Extracellular Vesicles and common methods used for Isolation and Separation

2.1.2.1 Extra Vesicles isolation:

1. Tangential flow filtration: Operates by directing the liquid horizontally across the membrane's surface, allowing the smaller particles to pass through the membrane.

2. Diafiltration: Involves the direct moment of mixture through membrane pores vertical direction.

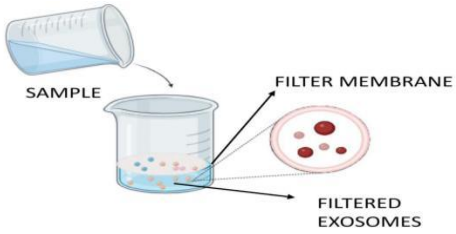


Figure 02: Process of Diafiltration

2.1.3 Isoelectric Precipitation

- Defatted milk was mixed with an equal volume of distilled water & pH was adjusted to 4.6 using 6N HCL to facilitate the aggregation of caseins.
- This mixture was subsequently subjected to centrifuge at 5100g for 20 min. at 25°C in a R14 A rotor (Hitachi Koki) by utilizing a Himac CR2OCT Centrifuge
- The isoelectric point of casein is 4.6, which leads to the formation of aggregates as a ppt at this PH. The resulting supernatant was filtered to eliminate any remaining milk fat globules and other particulate matter.

Evaluation of isolated exosomes is done by using nanoparticle analysis and western blotting analysis [17].

2.2 Isolation Techniques and Their Advantages and Disadvantages

2.2.1 Precipitation Technique

Firstly, it is characterized by a high yield of extracellular vesicles. Additionally, this method allows for the processing of numerous samples concurrently, thereby enhancing throughput. The process or procedure itself is relatively straightforward, which contributes to its accessibility. Moreover, the technique does not require specialized equipment, and it facilitates the maintenance of vesicle morphology, which is critical for subsequent analysis [18].

2.2.2 Ultra Centrifugation Technique

It facilitates the isolation of biomolecules from large volumes of solution without the need for additional chemical reagents, thereby preserving the integrity of the samples. Moreover, the widespread availability of ultracentrifugation equipment enhances its accessibility for use in various laboratory settings.

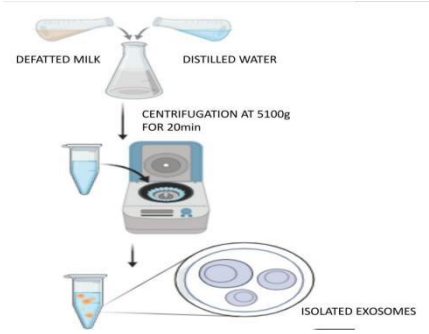


Figure 03: Exosome Isolation from milk, using a Centrifugation method

2.2.3 Size Exclusion Chromatography

This method provides results, enhancing consistency across multiple experiments. This technique preserves the integrity of vesicles, which is critical for applications involving EVS. It is easily scalable, allowing for adjustments to accommodate varying sample sizes and research requirements is method is characterized by extreme customizability, permitting modifications in terms of pore size, column volume, and specific experimental needs.

2.3 Disadvantages

1. The absence of specific markers for the effective isolation of EVS poses a significant challenge. The separation of EVS from antibodies or beads can be proved to be particularly difficult, potentially complicating the purification process. The required sample volume for effective immunoaffinity capture.
2. One significant constraint is the restriction on sample volume, which may hinder the analysis of larger quantities of material.
3. The resolution of similarly sized molecules is relatively low.
4. This method is inherently limited by the capacity to process only one sample at a time, which may restrict throughput in high demand scenarios [19].

APPLICATIONS

Emerging Applications of exosomes in Cancer Diagnosis and Therapy

3.1.1 Exosomes as diagnostic tools

Exosomes, which are nanoscale extracellular vesicles present in various biological fluids, offer significant potential for monitoring cellular states in both normal and pathological conditions. In the context of Biopsies, which may include liquid biopsies such as plasma, serum, urine, and saliva, exosomes have been employed to analyze and ascertain patient diagnosis, prognosis, disease progression, and chemoresistance status. Additionally, the secretion of exosomes has [20,21] been found to be upregulated in several complex Conditions, particularly Cancer. Under pathological circumstances, alterations in cellular behavior can be discerned by analyzing exosomes released by cells. The identification and examination of discrepancies in the levels of specific molecular Components can be achieved through transcriptomic, proteomic, and lipidomic analysis [22]. The application of exosomes for early diagnosis and prognosis of cancer and cardiovascular diseases[23,24] has been extensively investigated.

3.1.2 Exosomes in disease treatment

Their employment as drug Carriers for the encapsulation of Therapeutic agents; and [26]. They function as bioactive molecules, such as microRNAs (miRs),Empirical studies have demonstrated that the Application of exosomes as drug carriers can enhance drug efficacy and bio availability, while concurrently mitigating adverse side effects [27,28]. Associated with these pharmacological treatments. Specifically, RNA nanoparticles derived from exosomes have demonstrated enhanced targeting capabilities, facilitating cell entry. It has been observed that these RNA nanoparticles can be utilized to display various chemical ligands, including folic acid [29,30], as well as chemical drugs or RNA aptamers on the exosomal surface. Thereby achieving the objective of targeted cancer therapy[31], furthermore, nanoparticles that are modified on exosomes possess the capability to associate with small interfering RNA (siRNA), miRs, RNA aptamers, or ligands[32,33], thereby specifically inhibiting the proliferation of cancer cells

3.1.2.1 Exosomes in Cancer

In recent years, there has been an increase in Studies regarding tumors. They help treat various tumors like chronic myeloid Leukemia, gastric Cancer, colon tumors, Lung Cancer, and breast tumors. The antitumor effect is obtained from the plant source like ginger, lemon, tea, Ginseng, etc.

3.1.3 Clinical Applications

The Applications of exosomes in Clinical settings are extensive and diverse, encompassing a variety of therapeutic and diagnostic functionalities. A particularly promising domain is

regenerative medicine, wherein exosomes derived from stem cells have demonstrated efficacy in promoting tissue repair and regeneration. Specifically, in the Context of Cardiac diseases, such as Parkinson's disease and Alzheimer's disease, it has been actively investigated[34,35]. Beyond their therapeutic applications, exosomes have also garnered attention for their utility in diagnostics. A range of exosome biomarkers has been identified across various diseases, including cancer, thereby leading to the creation of non-invasive diagnostic tests[36,37]. Moreover, the capability of exosomes to serve as vehicles for targeted therapeutic delivery to specific cells or tissues has been explored, highlighting conventional delivery methods[38].This continuing investigation may yield significant contributions to both therapeutic and diagnostic strategies, enhancing the overall landscape of medical inventions [39,41].

The landscape of therapeutic intervention is anticipated to be significantly transformed by the evolution of precision gene editing technology, which facilitates the targeted modification of exosomes through genetic engineering approaches. Such advancements are expected to allow for more accurate regulation of biological processes and therapeutic outcomes [42].

3.1.4 Cell engineering technology:

This technology has been introduced in several studies, highlighting its capacity to modulate exosome secretion effectively. Investigation, human umbilical cord mesenchymal stem cell.

Exosomes were examined for their role in promoting angiogenesis via the wnt4/β-catenin signaling pathway, thereby illustrating the advantageous applications of cell engineering technology to the context of exosome regulation

Table 01: Anticancer Drug Loaded Exosomes

Agent	Origin of exosomes	Loading methodology	Type of cancer
Doxorubicin	Dendritic cell	Electroporation	Breast cancer
Paclitaxel	Macrophage	Sonication	Lung cancer
Cisplatin	Hepatocarcinoma cells	Diffusion	Ovarian and hepatocarcinoma
5 Floro uracil	Colorectal cancer cells	Electroporation	Colon cancer
Curcumin	Bovine milk	Agitation	Cervical and breast cancer
TRP 2	Serum	Electroporation	Any type of cancer
SIRP α	HEK293T cell	Transfection	Colon carcinoma
hMUC1	Colon cells	Transfection	Colon carcinoma
HER2-siRNA	HEKT293T and MCF-7 cells	Sonication	Breast cancer

CONCLUSION

Exosomes are the nanosized extra vesicular systems that provide a great drug delivery system into the cells, making a reliable delivery system. Plays a vital role in carrying the proteins, mRNA, and miRNA. There are various techniques applied for the isolation of exosomes, and they have their own advantages and disadvantages. They have a wide range of applications from the delivery of therapeutic agents, anticancer drugs, and promising a new way of immune and gene therapy without damaging the neighboring cells. Exosomes can deliver the drug to the targeted site as the bio compatible with human cells, resulting in less chance of rejection of therapy and good results. Currently, there are no FDA-approved exosomal drug-based therapies; they are undergoing clinical phase trials with various therapeutic agents, and they are not used as drug-carrying systems but also as diagnostic tools. Despite having a wide range of applications, it has certain drawbacks and many challenges, and it is a great field to work on. Exosomes are promising nanoscale vesicles with significant potential in drug delivery, diagnostics, and therapeutic applications. Their ability to transport bio active molecules and target specific cells makes them an effective tool in modern medicine. Despite their advantages, challenges such as isolation, stability, and regulatory approval remain. Continued research is necessary to fully exploit their potential in clinical applications.

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AUTHORSHIP CONTRIBUTIONS STATEMENT

Key contributions include conceptualizing the topic: B.Pujitha conducting comprehensive literature research: T.Vanitha synthesizing findings into a new framework: R.Kusuma drafting: P.Aparenjitha critically revising the manuscript: Mrs.A.Sasikala, Dr.M.Ramaiah.

Conflicts of Interest

The author declares no conflicts of interest.

Founding

Not applicable.

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