Potential use of Exosomes: A Type of Refined Biological Nano platform, for Delivering Therapeutic Drugs

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Abstracts

Exosomes are naturally occurring, nanosized (30–150 nm) vesicular vesicles that are released from cells by physiological or pathological events. Exosomes are becoming more and more popular because to their many advantages over traditional nano carriers, such as their capacity to avoid metabolic degradation or homing in the liver and their absence of unwanted accumulation before reaching their targeted destinations. Multiple studies have demonstrated the potential of exosomes in delivering therapeutic agents such as RNA molecules, proteins, small molecules, CNS acting drugs and chemotherapeutic drugs. By using exosomes as a drug delivery system, it is possible to enhance the BBB penetration and improve the pharmacokinetic and pharmacodynamic profiles of drugs, increase bioavailability, reduce toxicity and target specific cells or tissues. The use of exosomes also allows for the development of personalized medicine by using patient-derived exosomes for targeted therapy. Their biocompatibility and low immunogenicity make exosomes an attractive option for clinical use; however, there is still much research needed to optimize their therapeutic potential. Recent developments in the utilisation of exosomes as drug delivery systems were covered in this article. We looked at a thorough analysis of exosomes as therapeutic drug carriers and delivery systems across biological membranes, which included their structure, biogenesis, and importance as drug delivery vehicles. We also

highlighted modified and derived exosomes that have been developed for targeted drug delivery and cargo loading. We also concentrated on isolating, characterising, purifying, and producing large quantities of exosomes, and we noted the different formulations.

Key words: Exosomes, Drug carrier, Drug delivery system, BBB, Cell membrane, CNS, Cancer, Small molecules, Proteins and Biologics.

1. Background:

Exosomes are small, extracellular vesicles that are generated by several cell types, including neurons, and play a role in intercellular communication. Recent studies have shown that exosomes may also serve as promising drug-delivery vehicles for the treatment of various diseases. The biogenesis of exosomes involves the formation of multivesicular bodies that contain intraluminal vesicles, which are eventually released as exosomes (Rajput et al., 2022). Exosomes contain a diverse array of molecules, including proteins, lipids, and nucleic acids, which can be exploited for targeted drug delivery. The isolation and characterization of exosomes, coupled with their ability to cross the blood-brain barrier (BBB), make them an attractive option for the delivery of CNS drugs. By utilizing exosomes as drug carriers, researchers hope to improve the efficacy, bioavailability, and specificity of antitumour drugs, CNS drugs and also delivery of biologicals, proteins, enzymes and therapeutic monoclonal antibodies (Gayatri Patel et al., 2023).

An Innovative Approach for CNS drug delivery particularly, antiepileptic Drug Delivery Exosomes are small vesicles that are secreted by most cells and act as mediators of intercellular communication. They have recently emerged as an innovative approach for drug delivery, including their use as vehicles for antiepileptic drug delivery. Exosomes have a unique composition that allows them to be used for targeted drug delivery (Sophia G, et al., 2018). They are composed of lipids, proteins, and various types of RNA, including microRNA, which play an essential role in cellular signalling.

With the advent of nanotechnologies, nanoparticles have been proposed as an intriguing tool for potentially improving medication delivery via the BBB. Exosomes, which are nanosized vesicles released by diverse cells, are an important instrument for treatment approaches in this respect (Rezaie et al., 2022). Because exosomal carriers can benefit from both cell-based drug delivery and nanotechnology, interest in using exosomes for therapeutic applications has grown rapidly in recent years. Exosomes may move from cell to cell, easily transmit their contents across the cell membrane due to their unique characteristics, and deliver their contents in a biologically active state. Exosomes can cross past biological barriers such as the BBB. Exosomes have been used to penetrate the BBB using tiny chemicals, proteins, and nucleic acids (Suyash M, et al., 2020). The fundamental benefit of exosomes over other manufactured nanoparticles is that they are non-immunogenic, resulting in a long and enduring circulation. Exosomes as a therapeutic carrier, however, face significant challenges and barriers, including exosome donor cell selection, loading process optimisation, formulation purification, and toxicity and pharmacokinetic research.

The blood-brain barrier, a particular trait of the blood arteries that vascularize the CNS, allows these vessels to strictly control the transit of ions, chemicals, and cells between the blood and the brain (Lajoie et al., 2015). Most medications are unable to enter the brain because of the blood-brain barrier (BBB). The epithelial-like tight connections seen in the brain capillary endothelium are the source of this characteristic. Since the main function of the BBB is to prevent toxic substances from entering the brain, many substances particularly lipophilic drugs can passively defuse through the transcellular route, where nutrients are actively transported into

the brain and potentially toxic substances are expelled by active efflux pumps. The blood-brain barrier is a network of blood arteries and cells that protects the brain by filtering blood flowing to it. Some chemicals (such as anticancer medications) find it difficult to penetrate the brain due to this barrier. It hinders several chemotherapy medications from reaching brain cancer cells in sufficient quantities to kill them.

Diseases of the central nervous system (CNS) pose a hazard to people's health anywhere in the world. However, CNS-targeted medication development is quite difficult because of the unique anatomical and functional characteristics of the brain and spinal cord (Saraiva et al., 2016; He, Q., et al., 2018; Pehlivan et al., 2013). Exosomes are tiny lipid-bilayer-coated cellular vesicles that can be released by practically all cells and are crucial for intercellular communication. They stand out among CNS drug delivery techniques due to their advantages of low immunogenicity, the capacity to pass the blood-brain barrier, and the adaptability of drug encapsulation.

Exosomes, small extracellular vesicles (EVs) secreted by most cell types, have shown promising results as a drug delivery system. They are able to cross biological membranes and carry their cargo to specific target cells. Exosomes are quickly gaining interest as an alternative to traditional drug delivery methods such as liposomes or nanoparticles. Multiple studies have demonstrated the potential of exosomes in delivering therapeutic agents such as RNA molecules, proteins, and chemotherapeutic drugs. By using exosomes as a drug delivery system, it is possible to improve the pharmacokinetic and pharmacodynamic profiles of drugs, increase bioavailability, reduce toxicity and target specific cells or tissues (Zhang et al., 2019). The use of exosomes also allows for the development of personalized medicine by using patient-derived exosomes for targeted therapy. Their biocompatibility and low immunogenicity make exosomes an attractive option for clinical use; however, there is still much research needed to optimize their therapeutic potential.

In this article, we were discussed recent advances in the use of exosomes as drug delivery systems. We were explored the comprehensive review of exosomes as therapeutic drug carriers and delivery Systems across biological membranes which including structure, biogenesis and importance of exosomes as drug delivery vehicles and highlighted the modified and derived exosomes that have been developed for targeted drug delivery and cargo loading and also focused for isolation, characterization, purification and large production of exosomes and noted the various formulations of exosomes that have been developed for targeted drug delivery and highlight their potential advantages over traditional drug delivery methods. Additionally, we will delve into some of the challenges associated with exosome-based drug delivery and provide insights into how researchers are working to overcome them.

2. Blood-Brain Barrier: Biogenesis and Constituents

The blood-brain barrier (BBB) is a highly selective and dynamic physical barrier that separates the blood from the brain extracellular fluid **shown figure 1**. The BBB is composed of tightly packed endothelial cells that form continuous capillaries with no gaps (Abbott et al., 2010; Yang, T., et al., 2015). The tight junctions between these cells act as a barrier that restricts the passage of most molecules, including most drugs, from entering the brain.

The biogenesis of the BBB involves the regulation of gene transcription to form intercellular tight junctions and the expression of efflux transporters that actively extrude drugs from the brain. The composition of the BBB also includes

supporting cells, such as astrocytes and pericytes, which regulate the barrier properties and maintain the microenvironment of the brain.

To overcome the BBB penetration obstacle for antiepileptic drugs, researchers have explored exosomes drug delivery. Exosomes are small vesicles released from cells that can act as natural transport vehicles to penetrate the BBB. This approach has shown promise in preclinical studies for delivering antiepileptic drugs to the brain.

Overall, understanding the biogenesis and composition of the BBB is crucial for developing effective drug delivery strategies for treating neurological disorders such as epilepsy. So, the development of an efficient drug delivery system that can penetrate the BBB is essential for the effective treatment of epilepsy.

2.1. Blood brain barrier penetration: Principles for CNS Drug Delivery

The blood brain barrier (BBB) is a complex network of blood vessels that serves as a protective shield for the brain. It is composed of endothelial cells, pericytes, and astrocytes, all of which work together to regulate the exchange of substances between the bloodstream and the brain. It is important to keep in mind that not all types of drugs can easily penetrate through the BBB. Antiepileptic drugs, for instance, often face challenges in BBB penetration due to their chemical properties (Yang et al., 2015; Ceña, V et al., 2018). The principles of BBB penetration for antiepileptic drugs involve understanding the physiology of the BBB and finding ways to enhance drug delivery to the brain (Grabrucker et al., 2016; Gao et al., 2016; Leybaert, L., et al., 2007). One promising approach is through the use of exosomes, which are small extracellular vesicles that can carry drugs across the BBB. By utilizing such drug delivery methods, antiepileptic drugs can effectively and efficiently reach the brain to treat epilepsy.

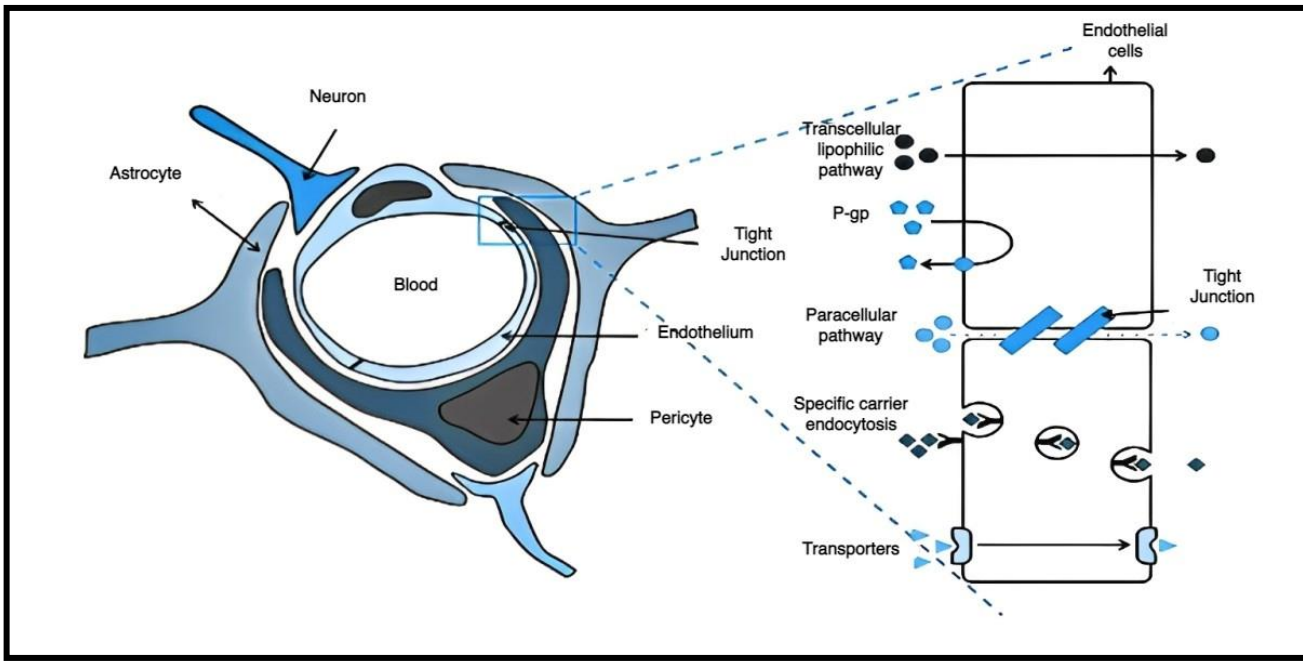

Figure 1: Schematic representations of the BBB and its transport system

3. Exosomes drug delivery for enhancement of BBB penetration for CNS drugs

The blood-brain barrier is a critical component that separates the brain from the circulating blood. It prevents potentially harmful substances from entering the brain but also restricts the delivery of therapeutic agents, including antiepileptic drugs. Knowledge of the biogenesis and composition of the blood-brain barrier is fundamental to understanding the principles of blood-brain barrier penetration. Researchers have developed various strategies to overcome this hurdle, including the use of exosomes for drug delivery. Exosomes are small membrane-bound vesicles capable of penetrating the blood-brain barrier, making them a promising vehicle for delivering antiepileptic drugs (Zhang et al., 2019). This approach holds great potential for the treatment of epilepsy and other neurological disorders.

Several ways for crossing the BBB have previously been used, including transcranial or nasal administration, infiltrating hypertonic factors, and lipidation of micro molecule medicines. Aside from their limitations, these methods are frequently invasive, increasing the risk of infection or brain injury. Furthermore, medicines are transported to the white matter via these mechanisms, which is undesirable [8]. The development of new technologies has resulted in increased BBB permeability and a reduction in negative effects. Physical, chemical, biological, and many nanoparticle processes are all involved in the operations [8].

3.1. Exosomes as a drug delivery vehicle

Exosomes are small, extracellular vesicles that are generated by several cell types, including neurons, and play a role in intercellular communication. Recent studies have shown that exosomes may also serve as promising drug-delivery vehicles for the treatment of CNS disorders particularly epileptic seizures and also cancer. The biogenesis of exosomes involves the formation of multivesicular bodies that contain intraluminal vesicles, which are eventually released as exosomes. Exosomes contain a diverse array of molecules, including proteins, lipids, and nucleic acids, which can be exploited for targeted drug delivery. The isolation and characterization of exosomes, coupled with their ability to cross the blood-brain barrier, make them an attractive option for the delivery of antiepileptic drugs. By utilizing exosomes as drug carriers, researchers hope to improve the efficacy, bioavailability, and specificity of antiepileptic drugs.

3.2. Biogenesis of exosomes

Biogenesis of Exosomes **shown figure 2**. Exosomes are small, membrane-bound vesicles that originate from the endosomal compartment of cells. They are increasingly becoming popular as drug delivery vehicles due to their numerous advantages such as their small size, biocompatibility, and ability to cross biological barriers. Exosomes are formed through the inward budding of multivesicular bodies which subsequently fuse with the plasma membrane resulting in the release of the exosomes into the extracellular fluid (Ren et al., 2016; Kourembanas et al., 2015; kotland et al., 2017; Ha, D et al., 2016; Wang et al., 2021).

Exosomes play a crucial role in intercellular communication and are involved in the transfer of various biomolecules such as proteins, lipids, and nucleic acids between cells. They have a unique composition which includes specific proteins such as tetraspanins, heat shock proteins, and cytoskeletal proteins, as well as lipids such

as cholesterol, sphingomyelin, and phosphatidylserine.

The isolation and characterization of exosomes are crucial for their use as drug delivery vehicles. Various techniques such as ultracentrifugation, filtration, and size exclusion chromatography have been used to isolate exosomes from different sources. The characterization of exosomes involves the use of techniques such as electron microscopy, dynamic light scattering, and Western blot to verify their size, shape, and protein content.

The use of exosomes as drug delivery vehicles for antiepileptic drugs holds great promise in the treatment of epilepsy. The ability of exosomes to cross the blood-brain barrier and target specific cells makes them an effective delivery system for antiepileptic drugs. By incorporating appropriate keywords such as exosomes and antiepileptic drug delivery, this article is optimized for search engines.

3.3. Compositions of exosomes

Exosomes are small, membrane-bound vesicles that have emerged as a promising tool for drug delivery **(Figures 2 and Figure 3).** One of the most exciting applications of exosomes is in the treatment of epilepsy. Exosomes are produced naturally by cells and are involved in a range of biological processes, including intercellular communication. They are highly adaptable, and researchers have been able to engineer them to target specific cells in the brain. Exosomes are composed of various molecules, including proteins, lipids, and nucleic acids, which can be manipulated to carry drugs. One of the benefits of this approach is that exosomes are biocompatible, which means they are well-tolerated by the body (Ren et al., 2016; Kourembanas et al., 2015; kotland et al., 2017; Ha, D et al., 2016; Wang et al., 2021). This makes them an attractive option for drug delivery in epilepsy, where there is a need for therapies that are both effective and safe. Isolating and characterizing exosomes is a complex process, but researchers are working hard to refine this technology. The potential of exosomes as drug delivery vehicles for antiepileptic drugs is exciting, and ongoing research is driving the development of innovative therapies for this condition.

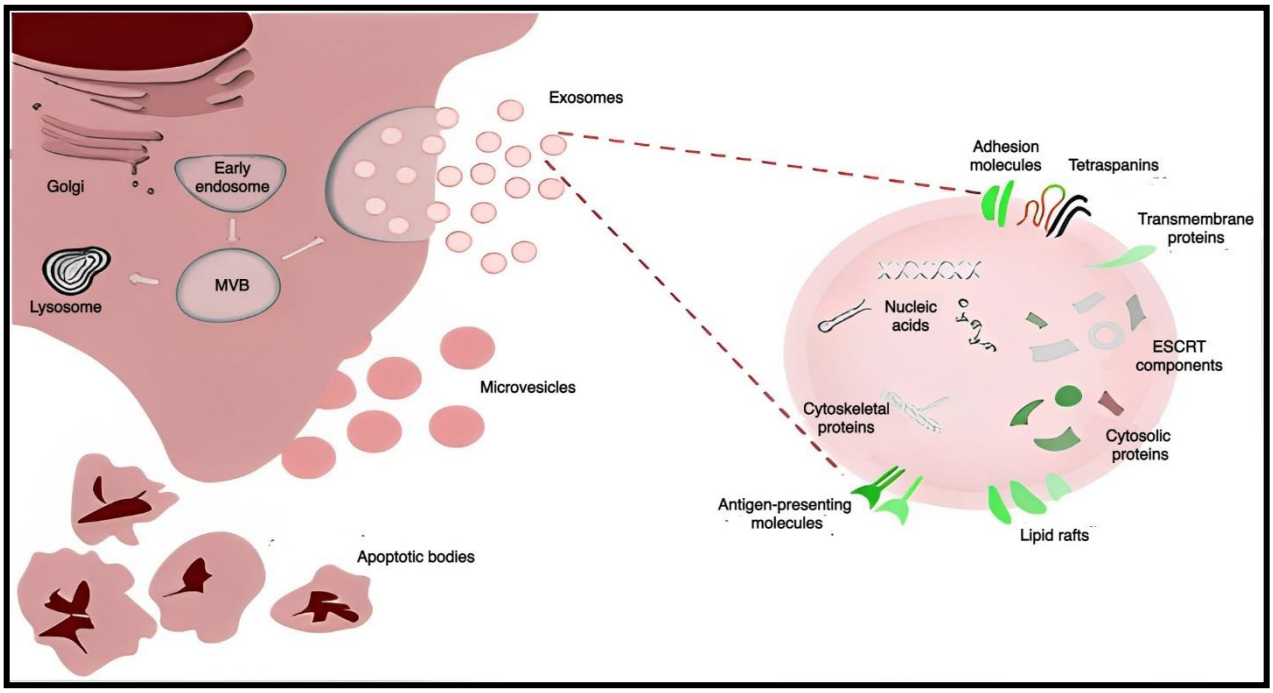

Figure 2 EVs' biogenesis and composition are represented.

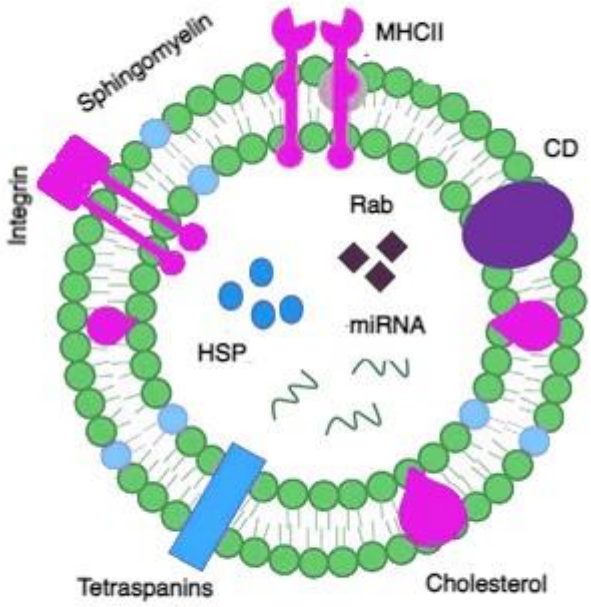

Figure-3: Shown the Exosome composition. Major Histocompatibility Complex (MHC)-II, integrin, cluster of differentiation (CD), tetraspanins, heat shock protein (Hsp), Ras-related protein (Rab), and other proteins are among the many types of proteins found in exosomes. Various lipids, including cholesterol and sphingomyelin, are also present in exosomes. Finally, it has been discovered that exosomes include nucleic acids, such as miRNA, mRNA, and non-coding RNAs [15].

3.4. Exosome structure and drug delivery significance

Exosomes, small vesicles secreted by cells, have recently emerged as promising drug delivery vehicles due to their unique features. They are membrane-bound organelles with a diameter of 30-150 nm and contain lipids, proteins, and RNA molecules. Exosomes are released into biological fluids such as blood, urine, and cerebrospinal fluid (Ren et al., 2016; Kourembanas et al., 2015; kotland et al., 2017; Ha, D et al., 2016; Wang et al., 2021). The use of exosomes for drug delivery has several advantages over other conventional drug delivery systems such as liposomes or nanoparticles. Exosomes have the ability to cross biological barriers such as the blood-brain barrier and target specific cells or tissues due to their surface protein composition. Furthermore, exosomes can protect the encapsulated drugs from degradation and clearance by the immune system.

The biogenesis of exosomes involves the inward budding of endosomal membranes, which subsequently form multivesicular bodies (MVBs). These MVBs can either fuse with lysosomes for degradation or be released extracellularly as exosomes. The unique structure of exosomes makes them highly attractive for drug delivery applications. Due to their small size and membrane-bound nature, they can

efficiently cross biological barriers such as the blood-brain barrier. Moreover, their cargo can be selectively targeted to specific cell types based on their surface markers. Exosomes are also immunologically inert due to the absence of major histocompatibility complex (MHC) class II molecules on their surface. Thus, they have low immunogenicity and do not elicit an immune response in the recipient organism.

Due to their endosomal origin, exosomes contain proteins involved in multivesicular biogenesis, heat shock proteins, tetraspanins, membrane transport proteins, and membrane fusion proteins (Vlassov et al. 2012). In addition, other proteins associated with exosomes are incorporated during exosome formation, acting as cargo during cell-cell communication (Ha et al. 2016).

Tetraspanins like CD9, CD63, CD81, and CD82, integrins, major histocompatibility complex class I and II proteins, and lysosomal proteins like Lamp-2b are examples of transmembrane proteins that are frequently abundant in exosomes (Conde-Vancells et al. 2008). Tetraspanins are often employed as positive indicators of exosomal presence (Johnsen et al. 2014), for targeted delivery of exosomal cargo, and for selective separation utilising affinity purification with antibodies to these proteins. Integrins and major histocompatibility complex (MHC) proteins are two additional transmembrane proteins that are frequently utilised as exosome detection markers (Ha et al. 2016, Frydrychowicz et al. 2015). Additionally, MHC proteins can be used to deliver highly targeted drugs to T-cells (Johnsen et al. 2014).

Heat shock proteins like Hsc70 and Hsp90, membrane transport and fusion proteins like GTPases, annexins and flotillin, proteins involved in multivesicular biogenesis like alix and TSG101, and Ras-related proteins are among the cytosolic proteins that are frequently found in exosomes (Conde-Vancells et al. 2008; Subra et al. 2010). These cytosolic proteins are employed as exosome detection and affinity-purification-based exosome separation indicators (Vlassov et al. 2012).

Mannose, polylactosamine, -2,6 sialic acid, and complex N-linked glycans are only a few of the saccharides that are abundant on the surface of exosomes (Batista et al. 2011). These saccharide residues are utilised in an approach developed by Aethlon-Medical-Inc (2020) to precisely isolate exosomes utilising affinity-capture techniques using lectins with high affinity to saccharide residues on the surface of exosomes.

Nucleic acids like DNA, miRNA, mRNA, rRNA, tRNA, snRNA, and other non-coding RNA species have been discovered in the cytosol of exosomes. Since the content of miRNA in exosomes differs greatly from that in parent cells or other microvesicles, it may serve as a signal for the existence of exosomes (Vlassov et al. 2012).

4. Exosome modification for improved target selectivity

One major challenge in using exosomes as therapeutics is their lack of specificity for target tissues. To address this issue, researchers are exploring various methods for modifying exosomes to increase their target specificity. One approach involves enriching the surface of exosomes with molecules that induce overexpression in the donor cell. Another approach is displaying targeting ligands by fusing them with exosome membrane proteins (Johnsen et al., 2014).

These modifications have shown promise in increasing the efficiency and specificity of exosome-based therapeutics. With further research and development, modified exosomes could potentially be used to treat a wide range of diseases with greater efficacy and fewer side effects than traditional treatment methods.

4.1. Enhancement of Surface Molecule to Promote Overexpression in Exosome Donor Cell

Exosomes are extracellular vesicles that are secreted by most cells. They play a vital role in intercellular communication, where they transfer bioactive molecules such as proteins, lipids, and nucleic acids between cells. The enrichment of exosomes with specific surface molecules enables the targeting of these exosomes to the desired cell types. One approach to enriching surface molecules in exosomes is through the overexpression of these molecules in the donor cell (Johnsen et al., 2014; Tian et al., 2018). This can be accomplished through different methods such as transfection, transduction, or electroporation. By increasing the expression level of a specific molecule on the surface of donor cells, there will be an increase in its incorporation into subsequent exosomes. The enrichment of specific surface molecules on exosomes can enhance their biological activity and targeting efficiency. This approach provides greater control over payload delivery and increased specificity towards target cells. Enrichment strategies also have implications for clinical applications such as targeted drug delivery and immunotherapy.

4.2. Targeting ligands are shown via fusing to the exosome membrane protein.

Exosomes have a natural ability to transport macromolecules between cells, which makes them an attractive candidate for drug delivery. However, to achieve the desired therapeutic effect, it is necessary to modify exosomes in such a way that they can specifically bind and deliver cargo to target cells. One strategy involves displaying targeting ligands on the surface of exosomes via fusion with membrane proteins (Chinnappan et al., 2020).

This approach requires identification of a suitable membrane protein that can tolerate the addition of foreign peptides without affecting its function and stability. Additionally, careful selection and design of the targeting ligand is crucial for achieving high specificity and efficacy. Numerous studies have demonstrated successful display of various ligands on exosome membranes, such as antibodies, peptides, and aptamers.

Theoretically, any transmembrane protein might fuse with a targeting ligand if it was concentrated in exosomes during biogenesis (Xitong & Xiaorong 2016). However, three transmembrane proteins that are often employed in exosome targeting include lactadherin, lysosome-associated membrane protein-2b (Lamp-2b), and platelet-derived growth factors (PDGFRs) (Xitong & Xiaorong 2016). According to research by Hung and Leonard (2015), lysosome-associated membrane protein-2b (Lamp-2b) is the protein that is most suitable for displaying a targeting ligand. Without any significant issues or difficulties, the protein has demonstrated effectiveness in improving targeted delivery to the brain, neurons (Alvarez-Erviti et al. 2011), breast cancer cells (Tian et al. 2014), and cardiomyocytes.

A brain-specific rabies virus glycoprotein (RVG) or an internalising RGD peptide can be added to Lamp-2b's N-terminus to improve targeting and medication delivery to the brain (Alvarez-Erviti et al. 2011). The same terminal of Lamp-2b was fused with a different RVG-protein, which led to exosome absorption through the nicotinic acetylcholine receptor and improved drug delivery to neurons (Hung & Leonard 2015). In order to target v3 integrins and improve exosome uptake to breast cancer cells, the internalising RGD peptide and Lamp-2b have been fused (Tian et al. 2014). Researchers have improved medication delivery to cardiomyocytes by genetically modifying cardiosphere-derived cells to produce Lamp-2b coupled with a cardiomyocyte-specific peptide (CMP).

Before isolating and purifying the vesicle, these targeting ligands are attached to the exosomal surface. Therefore, it is crucial to pick manufacturing techniques that do not have an adverse effect on these targeting ligands that reduces target specificity.

4.3. Surface modified exosomes for cerebral ischemia therapy

Cerebral ischemia, or the restriction of blood flow to the brain, is a condition that can have severe neurological consequences. Exosomes are small vesicles secreted by cells that have been shown to possess therapeutic properties. In recent years, interest in using exosomes for cerebral ischemia therapy has grown. One promising approach is the use of surface-modified exosomes.

The modification of exosome surfaces can serve several purposes. First and foremost, it can enhance their targeting ability and specificity. This is achieved by attaching molecules such as antibodies or aptamers to the surface that recognize and bind specifically to cells involved in cerebral ischemia. Additionally, surface modification can improve their stability and half-life in circulation by protecting them from clearance mechanisms (Johnsen et al., 2014; Tian et al., 2018).

4.4. Exosomes Combine with pH-sensitive Fusogenic Peptide and Cationic Lipid for Cytosolic Delivery

Scientists have discovered that exosomes combined with pH-sensitive fusogenic peptide and cationic lipid can be used for effective cytosolic drug delivery. The fusogenic peptide functions as a pH-sensitive trigger, allowing the exosome to enter cells effectively. The cationic lipid enables the exosome to fuse to the plasma membrane of the cell, allowing it to release its contents into the cytosol. This technique has been shown to be highly efficient in delivering drugs and other therapeutic agents into target cells (Tran, P. H et al., 2020).

Using this approach, researchers have been able to successfully deliver small interfering RNA (siRNA) molecules directly to tumor cells, inhibiting their growth and proliferation. Additionally, the use of exosomes for drug delivery is highly promising since they are non-toxic and biocompatible, making them ideal carriers for therapeutic agents.

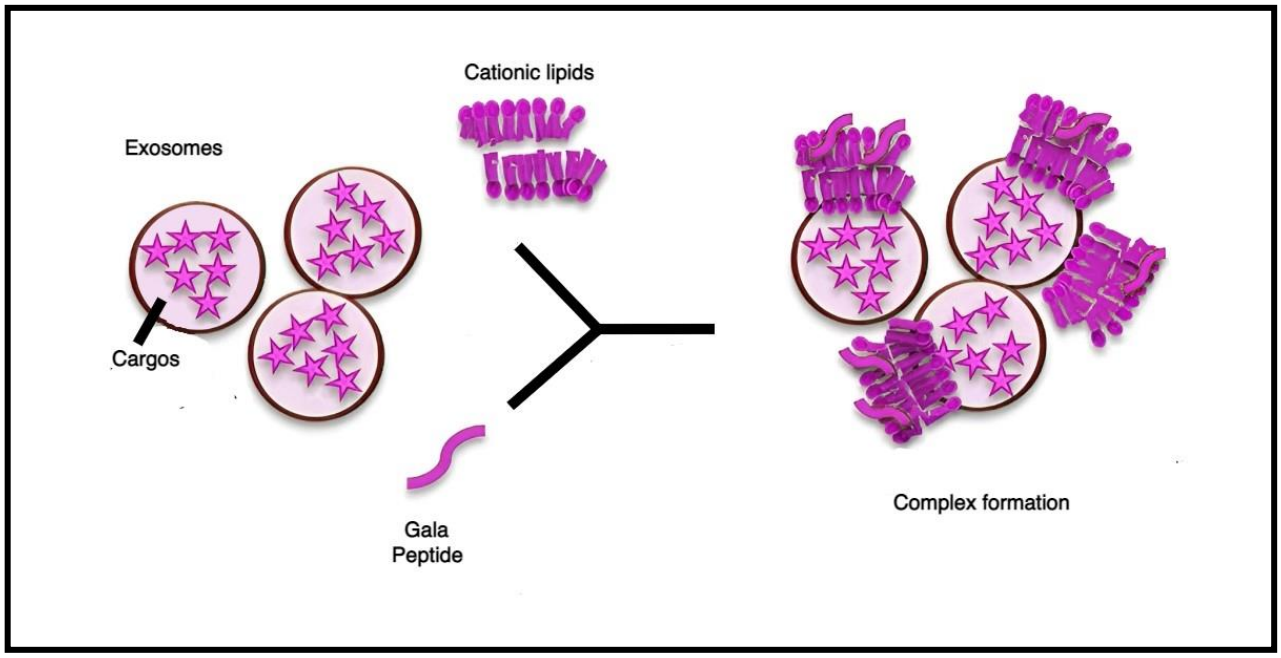

Figure-4: Modified Exosomes combined with GALA peptide and Cationic lipid.

4.5. Exosome Engineering with PEG and AA for Pulmonary Metastasis

Exosomes are emerging as promising drug delivery platforms. One of the challenges in using exosomes for drug delivery is to evade the immune system and prolong their circulation time in the bloodstream. To overcome this challenge, exosomes can be surface-engineered with polyethylene glycol (PEG) and amino acids (AA) that help to prevent recognition by the immune system and prolong their circulation time.

Surface modified exosomes have been shown to efficiently target primary tumors and their metastatic sites. In particular, exosome engineering with PEG has been effective in extending their half-life in vivo, leading to enhanced accumulation at targeted tissues. The addition of AA further enhances the circulation time by providing a protective barrier around the exosome surface, reducing clearance by macrophages.

The combination of PEG and AA has significant implications for pulmonary metastasis therapy as it allows prolonged retention of therapeutic exosomes in the lungs. Furthermore, this approach could potentially be used to target other organs affected by metastatic disease. These findings demonstrate great potential for surface-modified exosomes as a drug delivery platform for cancer therapy (Sander AA Kooijmans et al., 2012).

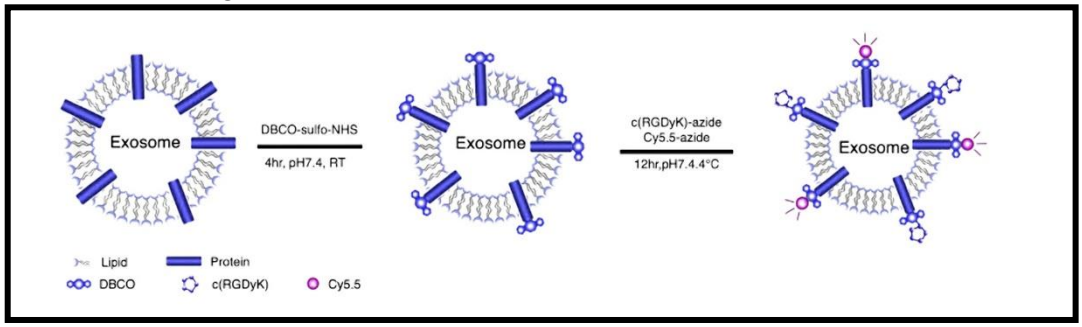

Figure-5: Exosomes modifed with DBCO, c(RGDyK), Cy5,5

4.6. Engineering exosomes by fusion with liposome

Exosomes are nanovesicles that can carry therapeutic molecules to target cells. However, the limited cargo capacity of exosomes is a challenge for drug delivery. To overcome this limitation, researchers have developed a method that fuses exosomes with liposomes to generate hybrid vesicles with enhanced drug loading capacity.

The engineering of exosomes by fusion with liposome has been shown to improve the stability and efficiency of drug delivery. The liposome membrane protects the exosome from degradation while facilitating targeted delivery to specific cell types. In addition, these hybrid vesicles can be engineered to express targeting peptides or antibodies on their surface for improved specificity and efficacy (Sander AA Kooijmans et al., 2012).

This approach offers a promising strategy for developing new nanocarriers for drug delivery applications. By combining the unique properties of both exosomes and liposomes, scientists can create hybrid vehicles that offer enhanced therapeutic benefits over traditional drug delivery methods. As research in this area continues to advance, we may see more effective and targeted treatments emerge for a wide range of diseases

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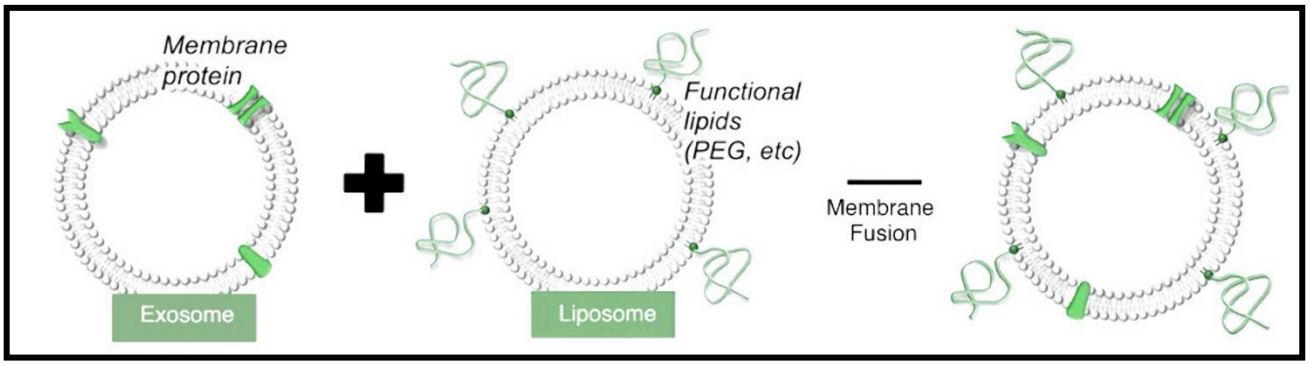

Figure-6: Exosomes Fused Liposomes for targeted Drug Delivery

4.7. Exosome-coated metal–organic framework nanoparticle

Recent advances have shown that metal-organic frameworks (MOFs) can be used in drug delivery due to their high loading capacity for drugs. However, MOFs have limited stability and biocompatibility when injected into the bloodstream. To overcome this limitation, researchers have developed a novel approach by coating the MOFs with exosomes, which are naturally occurring extracellular vesicles that can carry therapeutic molecules.

The exosome-coated MOFs offer several advantages over traditional drug delivery methods. First, they are highly biocompatible and can evade the body's immune system. Second, their small size allows them to penetrate deep into tumor tissues and release drugs directly into cancer cells. Finally, the exosome coating provides a stable platform for drug delivery over extended periods of time (Ha, D et al., 2016).

This innovative approach has promising implications for targeted cancer therapy and other diseases where traditional drug delivery methods have proven ineffective. By utilizing the natural properties of both exosomes and MOFs, researchers are working towards a more effective and personalized treatment approach that maximizes therapeutic benefits while minimizing side effects.

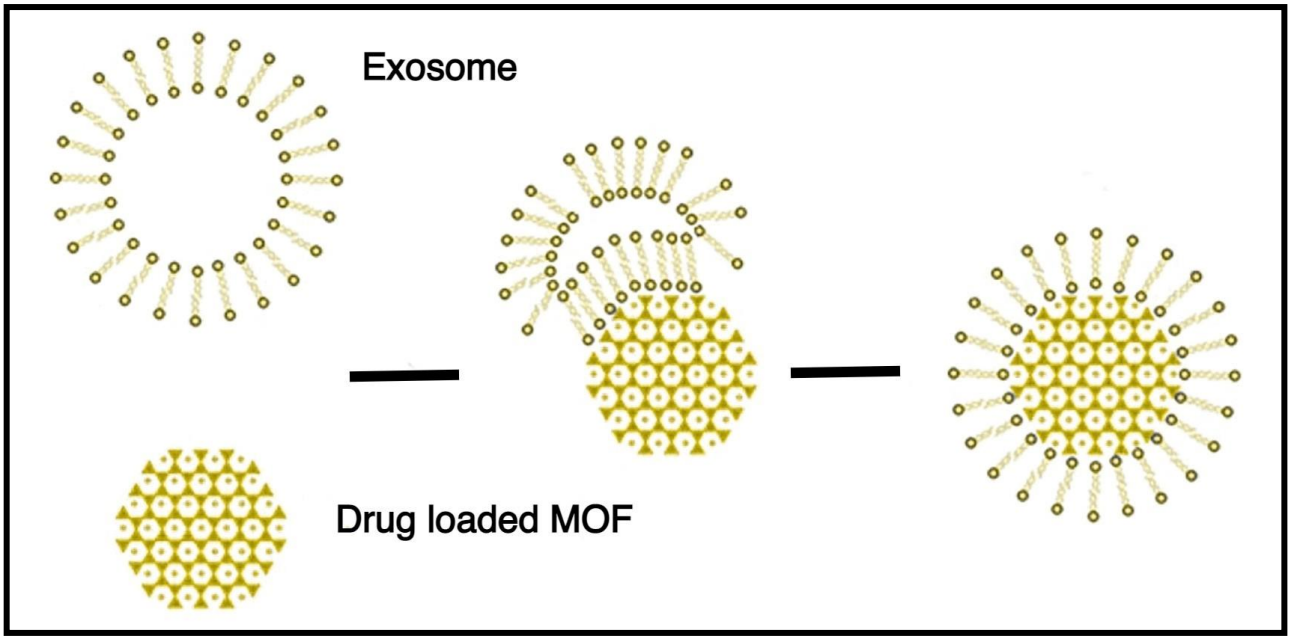

Figure-7: Eosomes Coated with Certain Metal-organic Framework

5. Derived exosomes

Exosomes have different roles which are depending on their progenitor cell. A few of research and development has been carried out in an attempt to identify the roles of varied and derived exosomes.

5.1. Macrophage-Derived Exosomes

Exosomes derived from macrophages are known to have immunomodulatory effects and regulate the immune response. These exosomes contain various immunoregulatory molecules, such as miRNAs, which play important roles in immune surveillance and host defense mechanisms. Macrophage-derived exosomes have been shown to modulate T cell activation, dendritic cell maturation, and natural killer cell function.

Furthermore, macrophage-derived exosomes have been implicated in a variety of pathological processes including cancer progression and inflammatory diseases. Studies have found that macrophages release exosomes that promote tumor growth and metastasis by inducing angiogenesis and extracellular matrix remodeling. However, recent research has also shown that these exosomes can be engineered to deliver anti-cancer drugs or other therapeutics directly to tumor cells (Ha, D et al., 2016).

5.2. Rhabdomyosarcoma (RMS)-derived exosomes

Rhabdomyosarcoma (RMS) is a rare and aggressive soft tissue cancer that primarily affects children. RMS-derived exosomes are known to regulate the tumor microenvironment and promote metastasis. These exosomes carry various bioactive molecules such as proteins, lipids, and nucleic acids that can alter the behavior of cells in the surrounding area.

Studies have shown that RMS-derived exosomes play a crucial role in promoting angiogenesis, which is the formation of new blood vessels. This process is essential for tumor growth and survival as it provides necessary nutrients and oxygen to the cancer cells. Additionally, RMS-derived exosomes can induce immune suppression by inhibiting T-cell function and promoting

regulatory T-cell activity. This creates an immunosuppressive environment that allows cancer cells to evade detection by the immune system (Chen H et al., 2021).

While the role of RMS-derived exosomes in promoting tumor progression is concerning, recent research has shown promise for their use as potential diagnostic or therapeutic tools. Researchers have found that these exosomes carry unique RNA profiles that can distinguish them from normal cells, making them a potential biomarker for early detection or monitoring of RMS. Furthermore, these exosomes could be utilized as delivery vehicles for targeted therapies due to their ability to enter specific cell types.

5.3. Metastatic Cancer Cell-Derived Exosomes

Exosomes derived from metastatic cancer cells have been found to play a crucial role in the progression of cancer. They are known to contain various bioactive molecules, including proteins, miRNAs, and mRNAs, which can modulate the behavior of recipient cells and promote tumorigenesis. The uptake of these exosomes by non-cancerous cells in the tumor microenvironment has been linked to several processes, including angiogenesis, immune suppression, and drug resistance.

Recent studies have shown that metastatic cancer cell-derived exosomes can also influence distant organs and pre-metastatic niches to prepare them for colonization by secondary tumors. This process is thought to involve the transfer of specific molecules that create a favorable environment for tumor growth. However, researchers are also exploring the potential therapeutic applications of these exosomes as carriers for targeted drug delivery (Chen H et al., 2021).

.This research highlights the importance of understanding how cancer cells communicate with their microenvironment through exosomal signaling and how this influences disease progression. By targeting these interactions and developing strategies to interfere with exosome-mediated communication between cancer cells and other cells in the body, we may be able to develop more effective treatments for metastatic cancer.

5.4. Malignant mesothelioma (MM) cell-derived exosomes

Malignant mesothelioma (MM) is an aggressive cancer that develops in the lining of the lungs, abdomen, or heart. The prognosis for MM patients is poor, with a median survival time of 12 to 21 months after diagnosis. Recent studies have shown that MM cells release exosomes that can promote tumor growth and metastasis.

MM-derived exosomes contain several types of proteins, including oncoproteins, growth factors, and cytokines. These proteins play a crucial role in the development of tumor microenvironment by promoting angiogenesis and inflammation. Moreover, MM-derived exosomes have been found to induce apoptosis resistance in recipient cells via transfer of anti-apoptotic proteins such as Bcl-2 and Mcl-1.

The discovery of MM-derived exosomes has opened up new avenues for targeted therapy against this deadly disease. By blocking the release or uptake of MM-derived exosomes, it may be possible to prevent tumor growth and improve patient outcomes. Future research should focus on identifying specific targets within these exosomes that can be used for therapeutic intervention (Parolini et al., 2009).

5.5. Osteoclast-derived exosomes

Osteoclasts and pancreatic cancer cells are two types of cells that have been found to release exosomes with unique properties.

Osteoclast-derived exosomes have been shown to play a key role in bone remodeling, as they contain specific proteins that regulate osteoblastic activity and bone formation. In addition, these exosomes are also involved in immune regulation, as they can stimulate the differentiation of regulatory T cells that suppress the immune response (Parolini et al., 2009).

5.6. **Pancreatic cancer cell (PCC)-derived exosomes**

Pancreatic cancer cell-derived exosomes, on the other hand, have been found to be involved in tumor progression and metastasis. These exosomes contain various molecules such as miRNAs, which can promote angiogenesis and enhance tumor cell proliferation and invasion. Furthermore, PCC-derived exosomes can modulate the tumor microenvironment by suppressing antitumor immune responses. However, these properties also make them attractive candidates for potential diagnostic biomarkers or therapeutic targets.

The research on osteoclast- and PCC-derived exosomes highlights the diverse functions of extracellular vesicles produced by different cell types. While osteoclast-derived exosomes may have potential therapeutic applications for diseases such as osteoporosis or autoimmune disorders, PCC-derived exosomes represent a promising avenue for understanding pancreatic cancer biology and developing novel therapies (Parolini et al., 2009).

5.7. **Bronchial Fibroblast-derived Exosomes**

Bronchial fibroblasts play a crucial role in the development of pulmonary diseases, such as asthma and chronic obstructive pulmonary disease (COPD). Recent studies have shown that bronchial fibroblast-derived exosomes can contribute to these diseases by modulating immune responses and promoting inflammation in lung tissues.

Exosomes derived from bronchial fibroblasts have been found to contain a diverse range of bioactive molecules, including cytokines, chemokines, growth factors, and microRNAs. These molecules are known to regulate important cellular processes such as cell growth, differentiation, apoptosis, and immune responses. Studies have shown that bronchial fibroblast-derived exosomes can promote the proliferation and migration of airway smooth muscle cells, which contribute to airway remodeling in asthma. Moreover, these exosomes can induce pro-inflammatory cytokine production in macrophages and enhance T-cell activation in lung tissues (Parolini et al., 2009).

5.8. **Mesenchymal Stem Cell (MSC)-Derived Exosomes**

Mesenchymal stem cell (MSC)-derived exosomes have been found to have a plethora of therapeutic applications. These small membrane-bound vesicles secreted by MSCs contain various bioactive molecules that can induce regenerative and anti-inflammatory effects in target tissues.

Studies have shown that MSC-derived exosomes can improve cardiac function in myocardial infarction, promote neuroregeneration in stroke, and help repair damaged lung tissue. Moreover, these exosomes have been found to possess immunomodulatory properties, making them a promising therapy for autoimmune diseases and organ transplantation (Rao, D et al., 2022).

The potential of MSC-derived exosomes has generated significant interest in the scientific community, with ongoing research aimed at understanding their therapeutic mechanisms and optimizing their production. As more preclinical and clinical studies are conducted, the use of

MSC-derived exosomes could revolutionize regenerative medicine as a safe and effective therapy for various diseases.

6. Isolation and characterization of exosomes

Exosomes are small vesicles that are secreted by most cells and act as mediators of intercellular communication. They have recently emerged as an innovative approach for drug delivery, including their use as vehicles for CNS and anticancer drug delivery. Exosomes have a unique composition that allows them to be used for targeted drug delivery. They are composed of lipids, proteins, and various types of RNA, including microRNA, which play an essential role in cellular signaling (Gandham et al., 2020).

Isolation and characterization of exosomes involves several steps, including the collection of the source material, isolation of the exosomes using various methods, and the characterization of the isolated exosomes. Some commonly used methods for exosome isolation are ultracentrifugation, size-exclusion chromatography, and precipitation methods such as polymer-based precipitation. Once isolated, the exosomes are characterized using various techniques, such as transmission electron microscopy, dynamic light scattering, and Western blotting.

The use of exosomes as drug delivery vehicles has the potential to revolutionize the treatment of epilepsy by providing targeted, long-lasting drug delivery. The inclusion of antiepileptic drugs in exosomes allows for slow and sustained release, making them a promising option for the treatment of epilepsy. By isolating and characterizing exosomes, researchers can develop more efficient ways to use them as drug delivery vehicles for antiepileptic drugs (Kalani et al., 2015).

Several different techniques, such as ultracentrifugation, immunoaffinity, ultrafiltration, SEC, and precipitation, are used to separate exosomes from cells and biological fluids. Each approach makes use of a unique exosome property, such as size, shape, density, or surface antigens, to facilitate isolation (Yi Zhang et al., 2020). The difficulty in using several techniques is getting a higher percentage of pure exosomes. The method for isolating exosomes from cells and biological fluids is the same, but some fluids need to be diluted due to their high viscosity, and in some samples, large particles need to be separated from them. Protease inhibitors should also be used to prevent the potential degradation of exosome proteins.

In addition to isolation and purification techniques, various methods have been developed for producing exosomes in vitro (Xin LUAN et al., 2017). For example, cells can be genetically engineered to overexpress certain components involved in the formation of exosomes resulting in increased production yields. Moreover, some studies suggest bioreactors can also be used for the large-scale production of exosomes under controlled conditions.

Differential ultracentrifugation is the most widely used method for isolating exosomes from various biological samples; it involves sequential centrifugation steps at increasing speeds to pelletize the different sized particles based on their buoyant densities. Density-gradient centrifugation involves layering a sample over a density gradient material such as sucrose or iodixanol followed by ultracentrifugation. The gradient will help to separate particles based on their buoyant densities. SEC uses a column packed with porous beads with defined pore sizes to separate particles based on size; this method is useful for eliminating contaminants such as protein aggregates but may also exclude smaller-sized particles such as small vesicles or protein complexes. Immunoaffinity purification employs specific antibodies

conjugated to beads that bind to antigens present on the surface of exosomes; this method can provide highly purified populations of exosomes but may suffer from batch-to-batch (Yi Zhang et al., 2020).

The production of exosomes is critical for various therapeutic applications. In addition to isolation and purification, numerous techniques have been developed to produce exosomes in large quantities. One such method is the use of mesenchymal stem cells (MSCs) as a source for exosome production. MSCs can be easily obtained from various tissues and can differentiate into multiple cell types, making them ideal candidates for producing tailored exosomes (Yi Zhang et al., 2020).

Another method for producing exosomes involves the use of genetically modified cells that overexpress specific proteins that facilitate exosome biogenesis. This approach has been successful in increasing the yield and purity of exosomes, while also allowing researchers to engineer specific cargo loading within the exosomes themselves. Future developments in this area hold great promise for generating larger quantities of high-quality exosomes that can be used across a wide range of therapeutic applications.

6.1. Large scale production of exosomes

With the increasing interest in exosomes for therapeutic applications, there is a need for large-scale production of these vesicles. Several methods have been developed to produce exosomes on a large scale, including ultracentrifugation, ultrafiltration, and tangential flow filtration.

Ultracentrifugation is one of the most commonly used methods for large-scale production of exosomes. This method involves pelleting exosomes from cell culture medium or biofluids using high-speed centrifugation. Ultrafiltration is another method that can be used to produce large quantities of exosomes. In this method, the cell culture medium or biofluids are passed through a membrane filter with a defined pore size to concentrate and collect the exosomes.

Tangential flow filtration (TFF) is also being increasingly used as an alternative to ultracentrifugation and ultrafiltration for the large-scale production of exosomes. TFF involves passing the cell culture medium or biofluids across a porous membrane under pressure. The pressure gradient across the membrane allows for efficient separation and collection of exosomes without compromising their integrity.

The successful development of these methods has led to increased availability of high-quality exosome preparations on a larger scale for further characterization and therapeutic applications (Yi Zhang et al., 2020).

6.1.1. Limitations in production of exosomes

Only when there will be optimised, standardised processes without departing from the regulatory criteria for large-scale exosome manufacturing will exosomes be accessible in global clinical platforms. For the development of scale-up, it is evident that all challenges relating to the transfer of contemporary technology would exist, including all of the process optimisation, validation, performance qualification activities, and implementation of a fileable quality control testing programme. The inability of scalable cell culture techniques to regulate environmental variables and reactor design or construction is the first obstacle in the product development cycle (Colao et al. 2018).

6.1.2. Limitations in Upstream Production of Exosomes

The production of exosomes is a complex process that involves several upstream steps. One of the primary challenges in this stage is obtaining a high yield of exosomes.

This can be achieved through effective isolation and purification methods, but precise control over multiple variables, such as temperature, pH, and oxygen levels, is required to ensure the quality and quantity of the final product.

An additional challenge in upstream production is the selection and maintenance of appropriate cell lines for exosome production. This involves finding cells that secrete high levels of exosomes while also being capable of sustained growth under laboratory conditions. Despite these challenges, advances in biotechnology offer new possibilities for optimizing upstream production methods and facilitating large-scale commercial production of exosomes (Kumeda et al. 2017).

6.1.3. Limtations in downstream processing for efficient purification of exosomes

Downstream processing is an essential step to purify exosomes from the initial cell culture supernatant. One of the main challenges is the lack of a universal and standardized protocol for exosome isolation, which often results in low yield and purity, as well as loss of bioactivity. Various methods such as ultracentrifugation, size-exclusion chromatography, and polymer-based precipitation have been used for exosome isolation. However, each method has its limitations and may require further optimization to achieve higher efficiency and reproducibility (Kumeda et al. 2017).

Another challenge in downstream processing is the heterogeneous composition of exosomes. Exosomes contain various biomolecules such as proteins, lipids, RNA, and DNA that can co-precipitate with them during purification. The presence of impurities can affect downstream applications such as proteomics analysis or drug delivery. Therefore it is necessary to develop strategies to selectively isolate pure exosomes from other vesicles or contaminants. Despite these challenges, ongoing research efforts are aimed at improving the purification techniques by combining multiple methods or developing novel approaches for specific applications.

6.1.4. Issues with in the preservation and stability of exosomes

Once exosomes are produced, their preservation and stability become crucial factors to ensure their therapeutic efficacy. One significant challenge is related to the instability of exosomes under various environmental conditions such as temperature, pH, and storage duration. Proper storage conditions must be maintained throughout the entire process to avoid degradation and loss of biological activity.

Another challenge is related to the impurities that can be present in the final product. Different purification methods can result in different levels of impurities, which may affect the stability and functionality of exosomes. To overcome this challenge, several techniques such as size exclusion chromatography (SEC), ultracentrifugation, and tangential flow filtration (TFF) have been developed for efficient purification.

In conclusion, maintaining the stability and purity of exosomes is crucial for ensuring their therapeutic efficacy. As more research is conducted on exosome production, new techniques will likely be developed to combat these challenges in preserving their storage properties (Kumeda et al. 2017).

6.1.5. Ingredient's used in the Formulation of Exosomes Drug Delivery

Exosomes are small vesicles that have gained increasing attention in recent years as promising drug delivery vehicles due to their unique properties, such as their ability to carry a wide range of biomolecules, low toxicity, and high biocompatibility. In order to utilize exosomes for drug delivery applications, it is necessary to formulate them with specific ingredients that can

enhance their stability, targeting efficacy, and therapeutic payload. Here we will discuss various ingredients used in the formulation of exosomes for drug delivery.

One key ingredient used in exosome formulations is lipids. Lipids are essential components of cell membranes and play a critical role in maintaining the structural integrity and stability of exosomes. Phosphatidylcholine (PC) is one commonly used lipid in exosome formulations due to its biocompatibility and ability to increase the stability and half-life of exosomes both in vitro and in vivo. Cholesterol is another important lipid component that can enhance the membrane rigidity and prevent fusion between exosomes and other cellular membranes during circulation (Kumeda et al. 2017).

6.1.6. Methods for exosomes formulations

Exosome formulation involves selecting appropriate methods for the isolation and purification of exosomes, as well as the incorporation of various ingredients for drug delivery. Ultracentrifugation remains the most commonly used method for isolating exosomes due to its ability to obtain high yields of pure exosomal fractions.

Other methods such as ultrafiltration, size-exclusion chromatography, and polymer-based precipitation have been developed to isolate and purify exosomes. For drug delivery purposes, ingredients such as lipids, polymers, and targeting moieties are incorporated into the formulation. The use of lipids in exosome formulations has been shown to improve their stability and increase cellular uptake, while targeting moieties can enhance their specificity towards particular cells or tissues.

Furthermore, recent advances in nanotechnology have allowed for the development of novel approaches to formulating exosomes with greater precision and control. These include microfluidic devices that can produce uniform-sized exosomes with varying cargo loads, as well as genetic engineering approaches that have enabled researchers to modify the contents of exosomes themselves.

The development of these new methods will undoubtedly lead to more effective drug delivery systems using exosomes. The potential benefits include improved bioavailability of drugs due to targeted delivery specifically toward affected areas and reduced systemic toxicity. There are promising outcomes regarding utilizing natural resources in medicinal technology advancements (Kumeda et al. 2017).

6.2. Characterization of Exosomes

Characterization of exosomes is crucial in understanding their biological functions and developing novel diagnostic and therapeutic approaches (Sokolova V et al., 2011). The characterization of exosomes provides information on their size distribution, morphology, surface markers, and cargo content. Electron microscopy, atomic force microscopy, and nanoparticle tracking analysis are commonly used methods to determine the size, morphology, and concentration of exosomes. Moreover, western blotting or enzyme-linked immunosorbent assay (ELISA) can be employed to detect specific proteins on the surface of exosomes such as tetraspanins (CD9, CD63, CD81) or tumor-specific antigens.

Besides physical characterization, lipidomics and proteomics analyses can reveal the lipid composition and protein cargo within exosomes. These analyses have shown that exosomes contain various classes of lipids such as phosphatidylserine, sphingomyelin, cholesterol, and gangliosides. Additionally ,the protein content of exosomes includes membrane fusion proteins (i.e., annexins), cytoskeletal proteins (e.g., actin), heat shock proteins (HSP70), enzymes (such as nucleases or hydrolases), growth

factors/receptors/proteins involved in metastasis/cancer signaling or immune regulation. Interestingly, the cargo composition is not static but varies depending on cell type/source/condition/stimulus (Kumeda et al. 2017).

6.3. Purification of exosomes

Exosomes are small vesicles secreted by cells that carry functional proteins, nucleic acids and lipids. Their purification is crucial for understanding their roles in cellular communication, disease diagnosis and therapeutic drug delivery. The most commonly used methods for exosome purification are ultracentrifugation, size exclusion chromatography (SEC), immunoaffinity capture and polymer-based precipitation (Sokolova V et al., 2011). Ultracentrifugation is the most widely used method for exosome purification due to its high yield and purity. However, its limitations include a long processing time, low reproducibility and requirement for specialized equipment. In contrast, SEC is a gentle technique that separates particles based on size. It allows the isolation of intact exosomes without denaturing them or compromising their biological activity. Immunoaffinity capture involves the use of antibodies specific to surface markers on exosomes to isolate them from complex biofluids such as blood or urine. Finally, polymer-based precipitation is a novel method that utilizes a chaotropic salt and a polyethylene glycol solution to precipitate exosomes from samples with high yield and purity. Purification of exosomes is an essential step in studying their biological functions and developing clinical applications. The choice of purification method depends on the sample source, desired yield and downstream application. Advances in technology have led to the development of more efficient methods with higher yield and purity while reducing processing time (Kumeda et al. 2017).

7. Cargo-Loading

Exosomes can also be loaded with various types of drugs, including chemotherapeutic agents, siRNA, and miRNA. The loading of drugs into exosomes is accomplished by co-incubation or electroporation. In co-incubation, the drug and exosome are mixed together and allowed to interact for a certain period before separation. Electroporation involves exposing the exosome to an electric field that creates transient pores in the membrane through which the drug can enter. One significant advantage of using exosomes as drug delivery vehicles is their ability to target specific cells and tissues. This targeted delivery ensures that drugs are delivered directly to diseased cells without affecting healthy cells (Hessvik et al., 2018). Additionally, the small size of exosomes allows them to penetrate deep into tissues that are difficult for other drug delivery systems to reach. With further research, scientists hope to develop more effective ways of loading drugs into exosomes, thereby enhancing their efficacy as a therapeutic tool (Fu S et al., 2020).

7.1. Exogenous protein loading to exosomes

Exogenous protein loading to exosomes is a promising strategy for therapeutic drug delivery. Exosomes are naturally occurring extracellular vesicles secreted by most cell types that can be harvested from biological fluids such as plasma or serum. They have gained attention as potential carriers for drug delivery due to their biocompatibility, low immunogenicity, and ability to cross biological barriers.

Several techniques have been developed for the loading of exogenous proteins into exosomes. One method involves co-incubation of proteins with purified exosomes in vitro, allowing time for protein uptake by exosomes. Another approach is the genetic modification of cells that secrete

specific proteins fused with an exosomal sorting signal, leading to their incorporation into the lumen or membrane of the secreted vesicles.

In conclusion, Exogenous protein loading to exosomes has tremendous potential for targeted therapeutic drug delivery. Developing efficient methods and exploring new strategies will enable researchers and clinicians to harness this potential for clinical applications (Yim, N et al., 2016).

7.2. Endogenous Protein Loading to Exosomes

Exosomes are small membrane-bound vesicles that play an important role in intercellular communication. They carry various molecules such as proteins, nucleic acids, and lipids, and transport them between cells. Endogenous loading of proteins into exosomes is a natural mechanism by which cells selectively sort proteins into exosomes for transport to recipient cells.

The endogenous protein loading process involves a specific pathway in the cell that sorts proteins into multivesicular bodies (MVBs), which then fuse with the plasma membrane to release exosomes. This process is regulated by several factors such as ubiquitination, ESCRT complex, and tetraspanins. Understanding the mechanisms of endogenous protein loading to exosomes can help us develop strategies for manipulating this process for targeted delivery of therapeutic proteins.

Research on endogenous protein loading to exosomes has shown promising results in the treatment of various diseases. For instance, researchers have demonstrated that engineered exosomes containing modified cargo proteins can be used for targeted drug delivery in cancer therapy. In addition, studies have shown that specific signaling pathways involved in endosomal sorting complexes required for transport (ESCRT) machinery could be targeted for therapeutic intervention (Yim, N et al., 2016).

7.3. siRNA and miRNA loading in to exosomes

Small non-coding RNAs, such as siRNAs and miRNAs, are powerful tools for gene silencing and regulation of gene expression. Exosomes are naturally involved in the transfer of RNA molecules between cells, making them a promising delivery vehicle for these small RNAs.

Various methods have been developed to load siRNAs and miRNAs into exosomes, including electroporation, sonication, and chemical transfection reagents. The choice of method depends on the type of RNA being loaded and the desired outcome. Once loaded into exosomes, these small RNAs can be delivered to target cells where they can regulate gene expression and potentially treat a variety of diseases.

The potential applications of exosome-mediated delivery of siRNA and miRNA are vast, ranging from cancer therapy to neurological disorders. By utilizing the natural properties of exosomes as intercellular messengers, researchers may be able to develop more effective therapies with fewer side effects than traditional drug delivery methods (O'Brien et al., 2020; Lu, M et al., 2018; Abels et al., 2016).

7.4. Loading of Nucleic Acids to Exosomes

Exosomes are known to play an important role in intercellular communication, and as such, they have become a focus of research for drug delivery. One important application of exosomes in drug delivery is the loading of nucleic acids such as mRNA, siRNA or miRNA. Nucleic acids can be loaded into exosomes through a variety of

methods including electroporation, sonication, and extrusion.

The use of exosomes for nucleic acid delivery has several advantages over traditional methods. First, exosomes can protect the nucleic acids from degradation by serum nucleases and other cellular enzymes. Second, exosomes have been shown to be taken up by target cells efficiently, increasing the effectiveness of the delivered nucleic acid molecules compared to other delivery vehicles. Finally, loading nucleic acids into exosomes reduces the potential for off-target effects because the cargo is specifically targeted to recipient cells.

Research continues to explore various loading methods for optimal efficacy and payload capacity. The potential for using exosome-based drug delivery is exciting as it offers a new avenue for treating diseases with minimal side effects (Piccin, A et al., 2007; Gibbings, D.J., et al., 2009; Jiang, X., & Gao, J., 2017).

7.5. Loading Small Molecules to Exosomes

Exosomes are small, membrane-bound vesicles that play an essential role in intercellular communication by transporting different biomolecules such as proteins, nucleic acids, and lipids. Loading small molecules such as drugs and metabolites into exosomes is a promising strategy for targeted drug delivery. Small molecules can be loaded into exosomes either by passive diffusion or active loading methods.

Passive diffusion is a simple method where small molecules can diffuse across the membrane of the exosome. However, this method has limitations in terms of the size and hydrophobicity of the molecule. Active loading methods involve incubation of exosomes with small molecules under specific conditions such as pH or temperature gradient to facilitate their uptake into the vesicle. Such active methods can ensure higher loading efficiency and specificity towards targeted cells.

The use of small molecule-loaded exosomes has shown promising results in pre-clinical studies for a variety of disease models such as cancer, inflammatory disorders, and neurodegenerative diseases. The potential benefits include reduced toxicity compared to traditional drug delivery systems and increased efficacy due to targeted delivery systems (Mehata et al., 2023; Schindler C et al., 2019).

7.6. Aspects of exosomes: Safety and Pharmacokinetics

One of the key factors affecting the biodistribution and toxicity of exosomes is their biological origin. It has been shown that tumor-derived exosomes may successfully convey anticancer treatments to their parental tumour (Yang et al. 2015). Although tumor-derived exosomes still have advantages for targeting tumours, systemic injection of them can raise safety issues: Exosomes from tumours may provide tumorigenic cues to healthy tissues while also encouraging tumour metastasis by starting the establishment of pre-metastatic niches in healthy tissue (Guo et al. 2019).

Mirzaaghasi et al. found that a significant percentage of exosomes were carried to the lung in sepsis-induced animals after intravenous infusion, with more than 30% moved there within 1 hour, whereas almost none were seen in the lung of healthy mice. Due to liver failure, exosomes were also demonstrated to be kept in the bloodstream for a long time (Mirzaaghasi et al. 2021). Fluorophore-labeled exosomes were cleared from blood in normal mice at a rate of 0.0054–0.0154 mL/min (Hwang et al. 2019), whereas in macrophage-depleted mice (Imai et al. 2015) and a mouse model of Parkinson's disease (Hwang et al. 2019),

the rates were 0.651 mL/h and 0.016 mL/min, respectively. After systemic administration, it has been demonstrated that the payload is distributed across a variety of major organs; even though the extent of the distribution varies considerably depending on the chemistry of some exosomes, its origin represents the molecular signature required for cellular interaction as well as the pathophysiological state of the subject. According to biodistribution studies, injecting more exosomes than 400 g causes unintended aggregation and accumulation in the lungs, which causes the test animal to suffocate (Fu et al. 2020). Either active or passive therapeutic exosome targeting strategies can be employed to deliver therapeutic exosomes to certain cells or regions. While active targeting achieves targeted distribution of exosomes by exosomal surface modification utilising a variety of technical approaches, passive targeting of exosomes makes advantage of exosomes' innate cellular tropism. However, as shown by a few instances in **Table 1,** the route of administration in both techniques significantly influences the desired outcomes. The most popular methods nowadays for assessing the in vivo behaviour of certain exosomes are bioluminescence and fluorescence imaging. Alternative clinical imaging modalities like magnetic resonance imaging, positron emission tomography, and single-photon emission computed tomography are now being used for exosome biodistribution and PK research in light of recent technical advancements in deep tissue penetration imaging (Sokolova et al. 2011).

Exosomes are directly or indirectly marked with various colours, such as fluorescent proteins—RFP (red fluorescent proteins) and GFP (green fluorescent proteins), for the purpose of visualisation. Luminogens, radiolabeling agents like 99mTc-HMPAO and 111-indium/125-iodine, bioluminescent imaging labelling agents like Gaussia luciferase, immunofluorescent molecules like antibody + dye, and magnetic contrast agents like superparamagnetic iron oxide nanoparticles (SPIONs) and gold-iron nanoparticles (GIONs) are some examples of lipophilic fluorescent dyes that are used in imaging. For computed tomography, even gold nanoparticles are utilised as a labelling agent (Sokolova et al. 2011). Fig. 3 depicts the current in vivo noninvasive imaging techniques that may be used to monitor exosome biodistribution and metabolic transfer.

The majority of ADME case studies that are now available are based on exosome injection given systemically. Both healthy and tumor-bearing animals showed quick systemic clearance of intravenously administered unmodified exosomes from PC3 and MCF-7 cell origin, which is attributable to the slower absorption of the exosomes by the reticuloendothelial system and may also

Table-1 Routes for ingesting exosomes and their importance in drug biodistribution

Exosome source	Cargo	Routes of administration	Biodistribution
Macrophage	Catalase	Intranasal, intravenous	When injected intravenously, more exosomes are dispersed throughout the inflamed brain of the Parkinson's disease

			C57BL/6 mouse model.
MDA-MB-231 and HCT-116 cell lines	Doxorubicin	Intraperitoneal	The colon, liver, heart, and spleen get both exosomal medicines (to a smaller extent) and free medications (to a substantially higher level). Exosomal formulation of the medicine accumulates in cardiac tissue 40% less than free drug, minimising cardiotoxicity.
TUBO breast cancer cell lines	Doxorubicin	Intravenous	After 60 minutes following injection, increased quantities of bioaccumulation in the liver and lungs had no effect on the heart tissue.
Raw bovine milk	Curcumin	Oral	The bioaccumulation of curcumin in the brain is six times larger than that of free curcumin. However, there are also more medicines present in the liver.
Embryonic stem cells	Curcumin	Intranasal	Treatment for the neuroglial-vascular

			condition involves distributing the cargo to astrocytes, neurons, arteries, and other cellular compartments of the brain.
Murine macrophage cell line	Curcumin	Intravenous	Exosomal curcumin is more concentrated in the infarcted areas of the brain than free curcumin is.

8. Recent Advances in the Use of Exosomes as Drug Delivery Systems

Exosomes have emerged as a promising tool for drug delivery due to their innate ability to cross biological barriers, including the blood-brain barrier. Their small size, ranging from 30 to 150 nm, and lipid bilayer membrane composition make them ideal candidates for carrying therapeutic payloads. In addition, exosomes are naturally produced by cells in the body and are involved in various cellular processes such as immune response and cell-to-cell communication. Recent studies have demonstrated that exosomes can be engineered to express specific surface proteins or contain specific molecules, allowing targeted delivery of drugs to specific cells or tissues. This has led to significant advancements in the field of personalized medicine as it allows for targeted drug delivery based on an individual's genetic makeup and disease state. Furthermore, exosomes can also be used for sustained drug release which has shown increased efficacy compared to traditional drug delivery methods. The use of exosomes as drug delivery systems holds great promise for the treatment of a wide range of diseases including cancer, neurological disorders, and infectious diseases.

Exosomes have been found to be promising vehicles for drug delivery due to their ability to cross biological barriers and selectively target specific cells. Researchers have developed various methods to load exosomes with therapeutic payloads, including small molecule drugs, siRNA, miRNA, and proteins. One such example is the use of exosomes derived from mesenchymal stem cells (MSCs) to deliver anti-inflammatory drugs for the treatment of inflammatory diseases. In another example, exosomes isolated from dendritic cells have been loaded with tumor antigens to stimulate an immune response against cancer cells. These exosomes have shown promise in preclinical studies as a potential cancer vaccine. Additionally, exosome-based therapies are being investigated for the treatment of neurodegenerative diseases such as Alzheimer's and Parkinson's disease. Exosomes derived from neural stem cells have been used to deliver therapeutic proteins and nucleic acids across the blood-brain barrier and target neurons in the brain. Overall, these

examples demonstrate the diverse potential applications of exosome-based drug delivery and highlight their potential as a new class of therapeutics in a wide range of diseases.

Exosomes have been found to be effective in various drug delivery applications due to their stability, biocompatibility, low toxicity, and ability to cross biological barriers. They are being investigated for use in delivering a wide range of therapeutic agents such as miRNA, siRNA, chemotherapeutic agents, peptides, proteins, and nucleic acids. Researchers are constantly exploring novel formulations that can be used to enhance the effectiveness of exosome-based drug delivery systems. One example of an exosome formulation is the use of dendritic cell-derived exosomes (Dex). Dex have been shown to effectively deliver tumor antigens to dendritic cells in vitro and activate T lymphocytes. Another example is the use of mesenchymal stem cell-derived exosomes (MEX) that can target specific cells such as cancer stem cells. MEX have also been shown to enhance angiogenesis by carrying angiogenic factors and promoting tissue regeneration. Furthermore, exosomes derived from macrophages can be used for delivering anti-inflammatory drugs and cytokines for treating inflammatory diseases such as arthritis. These examples illustrate the diverse potential applications of exosome-based formulations in drug delivery systems.

9. Current scenario and future prospect of exosomes

The field of exosome research has seen tremendous growth in the last decade. Exosomes have been extensively studied for their potential as diagnostic and therapeutic tools. They have been found to contain a wide range of bioactive molecules, including proteins, nucleic acids, lipids, and metabolites. This diverse payload makes exosomes an attractive candidate for various applications, such as drug delivery and biomarker discovery.

The future prospects of exosomes look very promising. With increasing research activities and technological advancements, are discovering new ways to harness the potential of exosomes. Researchers are exploring the use of exosomes in a variety of clinical applications, such as cancer therapy, regenerative medicine, and immunotherapy. Additionally, exosome-based diagnostic tools are being developed that can detect diseases at an early stage with high accuracy.

Overall, the current scenario for exosome research is exciting with many opportunities to explore in both basic science and translational research. Although there are still several challenges that need to be addressed concerning production methods and purification techniques to make them more efficient and cost-effective. But with continued efforts in this area by researchers across the globe, we can expect significant progress in the field of exosome-based therapeutics and diagnostics.

10. Conclusion

Exosomes are small extracellular vesicles that are secreted by almost every cell type in the human body. They play a crucial role in intercellular communication and have been identified as a promising tool for drug delivery due to their ability to cross biological barriers. The use of exosomes as drug delivery vehicles provides several advantages over traditional methods, including improved stability, reduced toxicity and increased specificity. One current strategy for using exosomes as a drug delivery vehicle is through the modification of their surface proteins. This modification allows for the targeted delivery of drugs to specific cells or tissues, reducing off-target effects and increasing

efficacy. Additionally, the use of engineering techniques such as bioconjugation has been shown to improve the loading capacity of exosomes with therapeutic molecules. The combination of these strategies provides an effective means for delivering drugs directly to diseased cells or tissues while minimizing systemic exposure and associated side effects.

In conclusion, exosomes have emerged as a promising drug delivery vehicle due to their ability to cross biological barriers and deliver therapeutic cargo directly to target cells. The unique properties of exosomes, including their natural biocompatibility and low immunogenicity, make them attractive candidates for future clinical applications. While there are still challenges to be addressed in terms of scalability and reproducibility, the potential benefits of exosome-based therapies cannot be ignored. As research in this field continues, we can remain optimistic about the many possibilities that exosomes may bring to the table in the realm of drug delivery.

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