Extracellular Vesicle 3 (2024) 100032

Contents lists available at ScienceDirect

Extracellular Vesicle

journal homepage: www.elsevier.com/locate/vesic

Exosome as drug delivery system: Current advancements

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ARTICLE INFO

Keywords:
Therapeutic
Cargo
Exosomes
Drug delivery
Blood-brain barrier
Nanovesicles

ABSTRACT

Exosomes are nanovesicles released from cells due to pathophysiological events. These nanoparticles are resistant to metabolic destruction and can transcend the blood-brain barrier. Exosome therapy could be employed as precision medicine by targeting the underlying etiology. This article briefly elucidates the basic physiology of exosomes, their types, characteristics, and cargo that are employed for drug administration. It then delves into their therapeutic applications, such as oncology, neurological disorders, and regenerative medicine. Exosome-based therapeutic drug delivery using small molecules, nucleic acids, and proteins is also demonstrated. Finally, global organizations that are successfully creating and testing medicinal biomaterials are highlighted.

1. Introduction

Drug distribution is crucial in assuring the efficacy and safety of therapeutic molecules. Liposomes, micelles, dendrimers, polymeric nanoparticles, and inorganic nanoparticles have been adopted as means of drug delivery throughout the evolution of contemporary drug administration to improve the efficacy and therapeutic index of the Pharmacokinetic-Pharmacodynamic (PKPD) of the drug to reduce the off-target side effects and drug-related toxicity.[1] These mechanisms nevertheless face numerous challenges, including the specific organs they target, toxicity-related chemical and physical characteristics, and an unfavorable immune response.[1] To overcome these constraints, researchers have turned their attention to novel approaches. Among the different emerging approaches, exosomes are gaining momentum as a potential technique in the realm of nanomedicine. Exosomes are tiny extracellular vesicles (size 30–100 nm) whose contents are actively secreted by an array of cells and happen to exist in physiological fluids like blood, cerebrospinal fluid, urine, and saliva.[2] These nanosized vesicles possess distinctive properties that make them intriguing for drug delivery applications. They are comprised of a lipid bilayer membrane and encapsulate a diverse cargo, like proteins, nucleic acids, and lipids (Fig. 2). Exosomes engage in intercellular interactions by exchanging bioactive molecules like proteins, mRNAs, and miRNAs with recipient cells, thereby influencing cellular functions and signaling pathways.[3] Furthermore, the likelihood of exosomes traversing the blood-brain barrier (BBB) enhances neurological and motor performance in the nervous system and enables repeated intravenous injections of exosomes containing drugs without any negative side effects.[1]

The rising popularity of exosomes as drug delivery strategy because they can circumvent several challenges encountered by conventional approaches. Firstly, exosomes exhibit remarkable stability, biocompatibility, and minimal immunogenicity, making them an appealing choice for targeted delivery of therapeutic cargo.[4] Secondly, they can traverse biological barriers and selectively target specific cells or tissues, thereby enhancing the delivery efficiency and reducing off-target effects. Additionally, exosomes provide a protective environment for the encapsulated cargo, safeguarding it from degradation and enhancing its stability.[5] Exosomes can be combined with various physiologically active compounds and other readily degraded components, making them suitable carriers for the transmission of these substances.[6,7] In recent years, there has been a surge of research focused on harnessing exosomes as drug carriers. They show enormous potential as delivery systems for drugs, genetic material, and other therapeutic payloads which could be integrated into their core or incorporated into their surface by different altering approaches to optimize their intended properties. Therapeutics can be packed into exosomes utilizing a range of techniques, ranging from passive encapsulation to active loading approaches.[8] Exosomes can also be manipulated to exhibit specific ligands or antibodies on their surface, enabling targeted delivery to desired cells or tissues.[9] Beyond medication delivery, exosomes have been examined for application in a wide range of domains, including vaccine development, photodynamic immunotherapy, genome editing or gene therapy, protein therapy, regenerative therapies, and many more.[1]

The objective of this study is to present a comprehensive assessment of the most recent research and developments in utilizing exosomes as a

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https://doi.org/10.1016/j.vesic.2023.100032
Received 17 July 2023; Received in revised form 31 October 2023; Accepted 27 November 2023
Available online 9 December 2023

therapeutic distribution system. It will provide a brief insight into the types of exosomes, their properties, and their composition. It will investigate the methods used to engineer exosomes for targeted drug delivery and how exosomes are exploited as delivery systems in specific therapeutic contexts, like the treatment of cancer, neurological disorders, and regenerative medicine. This review also discusses the successful delivery of various therapeutic substances, including small biomolecules, nucleic acids, and proteins, using exosomes. Significance of exosomes in vaccine development is also highlighted. Additionally, industrial exosome preparation is discussed with commercial entities that have already developed several exosome platforms and are making exosomes for medicinal uses. The development and optimization of exosome-based drug delivery systems have also been laid out as prospects and opportunities.

This literature review attempts to illuminate the enormous scope of exosomes as drug delivery systems, by bringing together existing knowledge and highlighting recent advancements. Researchers and clinicians can optimize exosome-based drug delivery systems and maximize their therapeutic advantages by being aware of recent developments in this sector. Finally, employing exosomes as drug carriers has the potential to revolutionise the drug delivery business, which paves the way for more targeted and efficient therapeutic interventions.

2. Exosomes and types of exosomes

A subtype of EVs called exosomes was first discovered as a way for cells to get rid of waste items.[10] It is now understood that they contribute significantly to cellular communication by delivering nucleic acids, functional proteins, and metabolites to destination cells. Exosomes are tiny extracellular vesicles that develop from endosomes (Fig. 1), which are membrane-bound compartments inside the cells. They also include an array of bioactive substances, such as lipids, proteins, growth hormones, nucleic acids, and a number of other metabolites.[11] Exosomes are formed by three crucial processes, which entails: 1) endocytic vesicle formation from plasma membrane results in the formation of early endosome which eventually matures to form late endosomes. 2) The inward budding of a late endosomes to create intraluminal vesicles-containing multi-vesicular bodies (MVBs). 3) Fusion of MVBs with the parent cell's plasma membrane leads to the release of intraluminal contents in extracellular space which are referred as exosomes.[12] Every type of cell in the human body secretes exosomes, which are nanospherical structures made of bilayer lipid membranes. Exosomes are circulated throughout the body by systemic circulations.

Exosomes can be classified into three categories: natural exosomes, modified exosomes, and artificial exosomes[3] (Fig. 1). Animal- and plant-derived exosomes make up the second division of natural exosomes. Tumor and normal exosomes are two further classifications of exosomes obtained from animals.[6] Several types of cells, including epithelial, endothelial, mesenchymal stem, macrophage, dendritic, and a number of immune cells, spontaneously produce exosomes. Unlike macrophage-derived exosomes, which have an impact on the tumor microenvironment and have anti-tumor properties, MSC exosomes have the potential to be used as therapeutic agents and drug carriers. Depending on their origins, exosomes can have diverse functions, contents, and medication loading that can have a variety of therapeutic effects. The significant anticancer activity of exosomes produced from macrophages, for instance, highlighted source-specific characteristics.[6] They are kept apart from bodily fluids such as milk, serum, blood, and urine. Exosomes have been successfully tested for the delivery of the medication paclitaxel and are present in a variety of biofluids, including milk.[13] The use of miRNAs in serum exosomes for the diagnosis and prognosis of spinal cord injuries and the use of miRNA expression profiles in endometrial cancer for diagnostic biomarkers are just a few examples of the diagnostic and therapeutic potential of biofluid exosomes.[6] Modified exosomes are naturally occurring exosomes that have been altered for more effective and superior therapeutic uses, drug loading, focused delivery, biodistributions, etc. They can be altered in two separate ways: first, by altering the exosome's internal structure, and second, by altering the exosome's exterior surface. Two basic methods viz., the cell-based methodology and the lipid membrane biomembrane creation technique are used to create synthetic exosomes, which are artificial exosomes that mimic the characteristics of natural exosomes.[3] Tumor-derived exosomes, with distinct surface antigens reflecting cell nature, are vital in cancer research. They influence tumor growth, metastasis, immunity, and serve as disease markers.[6] Wu et al. in a study found that in colorectal cancer, inhibiting exosome secretion can combat metastasis.[14] Plant based exosome-like nanoparticles (ELN) from foods like ginger, grapes, carrots, and grapefruit resemble

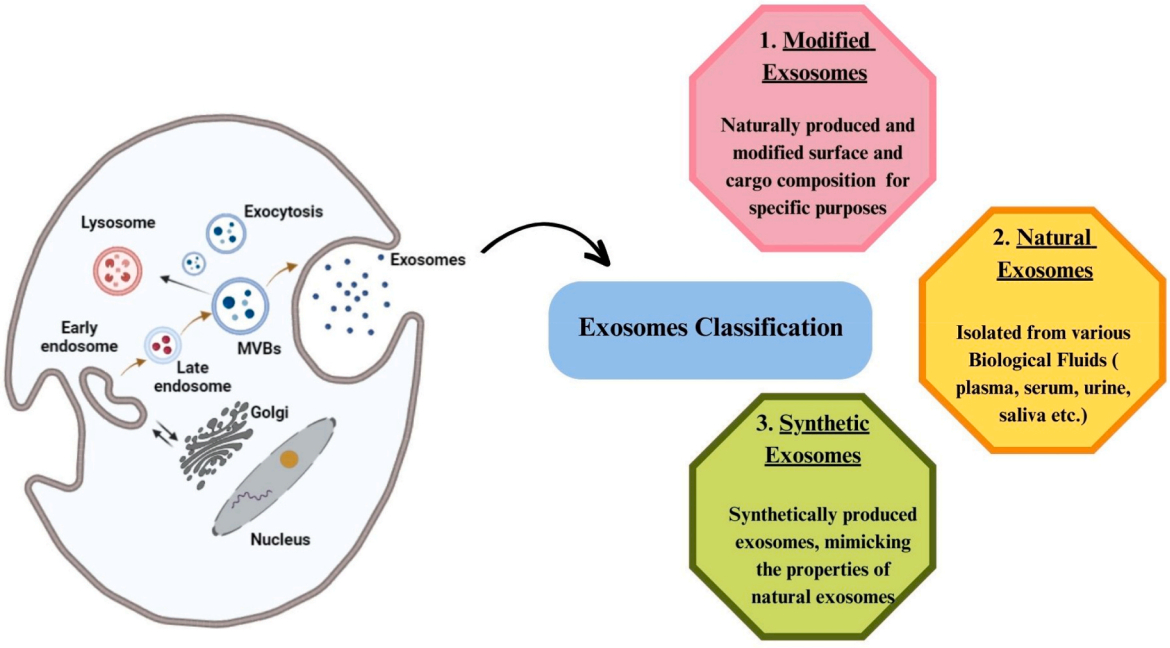

Fig. 1. Biogenesis of exosome and it's classification in accordance with origin.

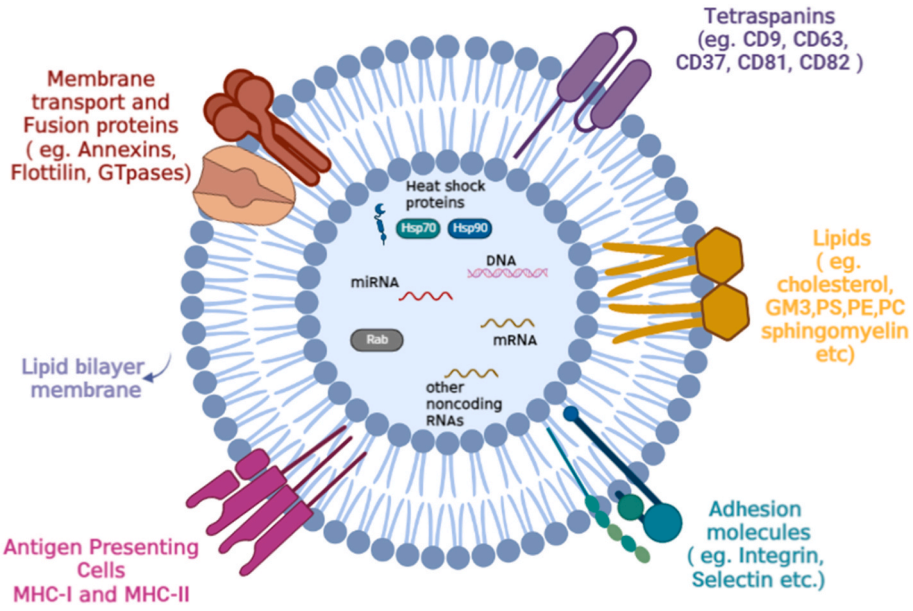

Fig. 2. Composition of exosome.

mammalian exosomes and exhibit potential for preventing liver diseases and maintaining intestinal health through anti-inflammatory effects.[6,15]

3. Characteristics, composition and isolation of exosomes

Exosomes are spherical, membrane-bound entities that are distinguished by their size, protein, and lipid composition.[16] They are lipid bilayers that produce nanoscale structures between 30 and 100 nm in size. Exosomes can traverse through the blood brain barrier (BBB) because they are small and more streamlined than other micro scaled vesicles. Exosomes originate from the parent cell's endosomes, an internal organelle from which they are secreted.

They are made up of diverse lipid and protein forms (Fig. 2). Heat shock proteins, HSP70 and HSP90, membrane transport, fusion proteins, GTPases, Annexins and flotillin, tetraspanins, CD9, CD63, CD81, and CD82 are examples of proteins.[17] The two exosome proteins that are most prevalent are integrins and tetraspanins. Exosomes also include a substantial amount of heat shock proteins and proteins from the Rab family. Integrins aid cellular adhesion by assisting the exosome in adhering to the target cell. The exosomes also contain the protein markers thrombospondin, CD55, CD59, lactadherin, ALIX, and TSG101.[18] In addition to proteins, lipids such as sphingomyelin, phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, mono-sialo-dihexosyl-ganglio side (GM3), and phosphatidylinositol are also components of exosomes.[19] Exosome stiffness and adhesion to the outside proteins are both governed by the lipid bilayer of the exosome.[18] The exosome also contains mRNA and non-coding RNAs, both of which are nucleic acids.

Exosome research is hampered by technical challenges such as simplifying extraction, increasing yield, distinguishing exosomes from other EVs, and improving analysis and identification procedures. Addressing these obstacles is critical for exosome therapy's future success, necessitating the development of efficient and quick isolation procedures.[20] Exosome isolation techniques, which are critical in biological research, have undergone significant advancements. Although more recent techniques have advantages, established ones like differential and ultracentrifugation are still frequently utilized for their dependability. While ultrafiltration enables concentration, size exclusion chromatography improves purity and efficacy.[21] Precipitation methods, including commercial kits, offer efficiency and simplicity.[22] Using exosome-specific antibodies, immunoaffinity capture is incredibly selective. For point-of-care applications, microfluidic devices provide quick, accurate isolation.[23] These developments allow for more specialized and effective exosome isolation, opening the door for innovations in diagnostics, therapy, and comprehending intercellular communication. Below is a tabular depiction of research that compares exosome separation methods, as well as the current and anticipated future developments in exosome isolation (Table 1).[20,23]

4. Application of exosomes as therapeutic agents

Exosomes are expanding their use in biomedicine on a global scale and have emerged as significant players in the creation of more effective treatment modalities for numerous diseases (Table 2). Exosomes were formerly only thought of as cellular waste, but in the last two decades, researchers have discovered a wide range of biomedical applications for them.[16] They have the inherent natural targeting potential and are stable therapeutic biomaterials. Exosomes are highly significant drug nanocarriers due to their nanoscaled size and structure, biocompatibility, non-immunogenicity, biodegradability, stability, less toxic nature, and capacity to carry target specific agents. They can be employed for immunological purposes, gene therapy, vaccine vectors, tissue regeneration, and as biomarkers in the detection and therapeutic management of a variety of diseases, including cancer, cardiovascular disease, diabetes, and neurological disease.[3] Exosome surface changes improve their capacity for cell targeting, biodistributions, and therapeutic action. Exosomes can discharge their payload by merging with the target cell's membrane or by phagocytosis.

5. Exosomes as drug delivery system for therapeutic molecules and biomolecules

Extracellular membrane vesicles known as exosomes are generated by various kinds of cells in our body and are crucial for intercellular communication and the induction of physiological responses.[3] They work well as medication delivery systems through cellular membranes as well. Exosomes and exosome mimetics are regarded as optimal delivery systems due to their distinctive properties like biocompatibility, no or minimal toxicity, increased circulating capability, specificity, etc. They are more effective and accurate transporters than the currently used delivery systems such as liposome mediated, polymeric nanomaterials, metal nanomaterials, etc. Exosomes produced by immune cells, tumor cells, and mesenchymal stem cells (MSCs) have been

Table 1
Comparison of techniques for isolating exosomes.

Isolation method	Principle	Benefit	Limitation	Recovery	Purity	Required sample volume
Ultracentrifugation (UC)/ and Density gradient Ultracentrifugation along with UC	High-speed centrifugation to pellet down the exosomes	Low reagent cost.	Time-consuming, may co-isolate contaminants, requires expensive equipment.	Low	High	Large
Differential Centrifugation	To separate the particles, use repeated centrifugation cycles at different speeds.	No other markers will be introduced and is inexpensive.	Extensive labor, sophisticated equipment, and possible exosome damage	High	Low	Large
Precipitation	Precipitates exosomes using reagents.	Ease of use, no deformation of exosome, lack of specialized equipment, and Accessible in the commercial market	Polymers may obstruct subsequent analysis, Co-precipitation of lipoproteins and other molecules with protein aggregates	High	Medium	Small
Ultrafiltration	Samples are passed through a membrane with a predetermined threshold molecular weight.	Quick and easy to execute, inexpensive, time-efficient, and simple	Potential exosome loss as a result of membrane entrapment and vesicle degradation brought on by shear stress	Medium	Medium	Medium
Immunoaffinity	Utilizes antibodies targeting exosome surface markers for capture	Demonstrates high specificity and simplicity	Compromise the integrity of the exosome, costly, non-specific binding	Low	High	Small
Microfluidics	Uses microfluidic devices to separate exosomes according to their size and other characteristics.	Quick, automated, and high-throughput capability, Economical and Enable Exosome Isolation and Characterization at the Same Time	Require significant development costs for the device, possible scaling problems, and the requirement for training, standardization, and validation in large cohorts.	High	High	Small
Size - exclusion Chromatography	Uses hydrodynamic radii to separate exosomes	Keep the biological activity and integrity intact; do not require any additional preparation.	Possibility of contamination and expensive equipment	High	High	Small
AF4	Laminar flow	Maintain Reproducibility and Integrity	Resolution is low	Low	High	Small

identified as promising, efficient nano-scaled carriers for biomolecules, drugs, and therapeutic compounds[40] (Fig. 3).

According to studies, curcumin-encapsulated exosomes (Fig. 4) improve the solubility, bioavailability, and stability of curcumin in mice with Alzheimer's disease compared to free circulating curcumin. In mouse models, it improved cognitive function and demonstrated potent anti-Alzheimer therapeutic properties.[41] When treating bacterial infections in vivo and in vitro, injection of free antibiotics has been proven to be less successful than antibiotic-incorporated exosomes. In one study, it was discovered that adding linezolid, a synthetic antibiotic against methicillin-resistant *Staphylococcus aureus,* to exosomes extracted from mouse RAW264.7 macrophages reduced the infection more efficiently than free linezolid and did not have any negative effects on macrophage cytotoxicity.[42]

Exosomes are also used to deliver biomolecules like protein, nucleic acids, growth factors, lipids, etc. to the targeted cells, causing genetic alterations in pathological as well as physiological processes.[17] Additionally, small therapeutic compounds must be delivered to the target region and transported there. These exosome properties, according to studies, have drawn a lot of interest in gene therapy, RNA interference, and other treatment modalities that affect gene expression.

6. Exosomes for delivery of proteins

Exosomes, which are natural nanovesicles, have been shown to have the inherent potential to transfer protein cargos to recipient cells for the targeted management of a variety of illnesses (Table 3). Exosomes taken out of the majority of cells are naturally present carriers of endogenous protein molecules, suggesting that using the exosome carrying mechanism as a delivery vehicle for proteins or peptides would be appropriate.[43] Exosomes are a type of naturally occurring membrane-bound protein ligands. Following their synthesis, these bioactive ligands form microdomains inside the exosomes, which provide a natural membrane habitat for the biomacromolecules. The effectiveness of membrane protein therapies is increased since this helps maintain the integrity and bioactivity of the membrane protein[44,45].

GALA-modified exosomes were created by combining modified exosomes produced from dendritic cells with pH-sensitive GALA peptides. These modified exosomes contained SAV, a protein with a high affinity for the biotin molecule, and LA, an exosome-tropic protein. Exosomes that have been created are useful for regulating the ability of tumor cells to deliver antigens and move between cells.[57] In a similar vein, exosomes containing ovalbumin or alpha-galactosyl-ceramide may be able to trigger an adaptive immune response without also inducing invariant natural killer T-cell anergy.[58] Researchers have created an exosomal delivery system to distribute the antioxidant catalase across the (BBB), which will reduce inflammatory reactions and aid in treating neurodegenerative diseases. Another example is the development of PH20 hyaluronidase-loaded exosomes for effective tumor penetration by hyaluronan degradation.[8] When compared to distribution via ferritin nanocages, exosome loading of the signal-regulating protein α (SIRPα) is more effective at inhibiting tumor growth.[44] These days, protein therapeutics are administered via protein nanocages.[1]

7. Exosomes in delivery of genetic substances and gene therapy

Exosomes serve as effective carriers of genetic materials in the context of gene therapy because of their innate ability to shield nucleic acids against destruction (Table 3). Short RNA molecules called small interfering RNA (siRNA) and non-protein nucleotides called non-coding RNAs, or miRNAs, are employed in genetic treatment to change, disrupt, or affect the expression of genes.[16] These tiny RNA-based therapies are currently recommended over DNA-based modifications, due to the likelihood of mutation in DNA-based modifications. Due to their low stability and greater susceptibility to RNase breakdown in the systemic circulation, distribution of these RNA molecules is still difficult. Additionally, in order to reach the target site and treat various illnesses, tiny RNA molecules must pass through the cellular membrane. These RNA molecules are vulnerable to lysosome trapping and acid destruction after being taken up by the cells. Exosomes are therefore regarded as the

Table 2
Various therapeutic applications of exosomes.

Type of Exosome	Therapeutic application	Mode of action	References
Exosome-loaded drugs	Anxiety disorders and refractory depression	Exosomes should be loaded with anti-inflammatory and growth substances.	24
Stem-cell derived exosomes	Cerebrovascular diseases	Stimulate innate neuronal precursors	25
Exosomes derived from neurons	Synaptic transmission regulation	Control astrocytic glutamate	26,27
Exosomes derived from cerebral cells	Tissue regeneration	Transporting regulatory components to the brain's damaged locations	28
Macrophage-derived exosomes	Parkinson's disease (PD)	Neuroprotection	29
SCAMP 5	Huntington's disease	Eliminates the aggregation of the alpha-synuclein toxin and mediates its clearance.	30,31
Natural killer (NK)-cell-derived exosomes	Glioma	Immunotherapy	32
Exosomes derived from multipotent mesenchymal stromal cells	Stroke	Facilitate angiogenesis, remodeling, and neurogenesis	33
Exosomes of human umbilical cord-derived mesenchymal stem cells	Early glioma stage	Regulate miR-10a-5p/PTEN signaling pathway	24,34
Exosomal miR-193b	Alzheimer's disease	Reduces neuronal amyloid precursor protein expression	35
Exosome-based stem cell therapy	Multiple sclerosis (MS)	Improves motor and neural function while decreasing myelin breakdown and neuroinflammation	36
Antisense molecule with exosomes	Brain glioma	Stimulate the adaptive immune system	33
Exosomes derived from stem cells	Traumatic brain injury (TBI)	Post-transcriptional gene regulation in recipient cells	37–39

Note: Some of the information collected from https://www.clinicaltrials.gov/.

finest bio-vectors for gene therapy because of their benefits as drug carriers, which were previously highlighted. They also have zero toxicity and precise delivery.

Exosomes made from stem cells are preferable to conventional delivery methods for treating cardiovascular diseases because they can prevent their nucleic acid cargo from being broken down while in circulation and deliver the medication to the cell efficiently. Recent research revealed that the tumor suppressor miRNA miR-199a-3p was present in exosomes from the omental fibroblasts of ovarian cancer patients.[59] Both in vivo and in vitro investigations demonstrated the successful inhibition of cancer cell growth and invasion by the miRNA-loaded exosome. Recently, the gene editing tool CRISPR/Cas9 has attracted a lot of interest from people all over the world and has prompted the development of numerous new treatments for diseases. Exosomes are produced and engineered in order to deliver the CRISPR/Cas9 components to the desired spot. Scientists created functionalized exosomes by conjugating Cas9 protein with the green fluorescent protein and green fluorescent protein nanobodies, as well as the exosomal membrane protein CD36, in order to increase the Cas9 protein's ability to encapsulate molecules.[60] Effective targeted gene delivery was demonstrated by the modified exosome that had been laden with CRISPR/Cas9 components. The presence of mRNA in exosomes makes them perfect bio-vectors for treating familial hypercholesterolemia because they reduce lipid accumulation in the liver and the synthesis of LDL cholesterol in the serum.

8. Exosome based drug delivery vehicles for cancer treatment

Precise means of delivering drugs to tumor cells are required to enhance the efficacy of oncotherapy, or cancer treatment. Exosome based nanocarriers are promising therapeutic vehicles in oncotherapy, propelled by notable breakthroughs, and given its innate capacity to deliver medications to the targeted tumor cells. Exosome based vehicles have intrinsic homing ability and are clinically more stable than several other cutting-edge drug delivery technologies frequently used in the treatment of cancer. The therapeutic chemicals are protected and transported specifically to the cancer cells thanks to this. The traditional methods of drug delivery, such as synthetic polymers, liposomes, micelles, viral vectors, etc., are highly immunogenic to the host, which prevents them from effectively delivering drugs to certain cells in vivo. In this regard, exosomal-based systems are produced from donor cells and have higher circulation ability and are non-immunogenic and biocompatible. Exosomes are less harmful and safe without the risk of insertion oncogenesis than recombinant viral vectors. Exosomes surface can also be altered to increase patient and cell-specific delivery. Exosomes are being used to transmit short RNA to the target region to change the gene expression of specific illnesses and enhance exosome therapy.

In a recent investigation, it was determined whether exosome-based drug carriers could deliver the chemotherapy medication doxorubicin to the target tumor cells.[61] The tumor penetrating peptide iRGD was transfected into mouse immature dendritic cells to increase the tumor cell specificity. When combined with anti-tumor medications, imaging agents, biological molecules, etc., iRGD has the ability to thoroughly infiltrate the tumor parenchyma. It binds to the Neuropilin −1 receptor and the αv integrins, which are expressed more strongly in tumor cells. The modified iRGD exosome observed a significant reduction in cancer cell proliferation after doxorubicin was loaded using an electroporation technique. The potential of iRGD modified exosome loaded with doxorubicin to inhibit the tumor growth by 3.5 folds as compared to non-modified exosome and cell free doxorubicin was reported in an in vivo study in the tumor bearing mice (MDA-MB-231). In one study, siRNA was delivered via exosomes to reduce the high rates of metastatic progression and recurrence in TNBC (Triple Negative Breast Cancer) and to manage postoperative breast cancer metastases.[62] In this study, exosomes from autologous breast cancer cells were extracted and used to make siS100A4 nanoparticles with an exosome membrane coating. After that, cationic bovine serum albumin (CBSA) was coupled to the exosomes. Drug transport, good biocompatibility, and protection against siRNA degradation were all demonstrated by the newly discovered CBSA coupled siS100A4 exosome. An effective method to suppress postoperative breast cancer was shown by in vivo testing to significantly silence genes that prevented or slowed the growth of breast cancer cells.

In addition, miRNA, whose functional mutation gain or loss is directly associated to the development of cancer, is transported by exosomes in cancer cells. In a study, a team of researchers have developed a unique exosome-based platform for miRNA replacement therapy to treat breast cancer.[63] In the study, exosomes were used to deliver the anticancer RNA let-7a miRNA, which is exogenous, to breast cancer cells that express the EGFR. In order to improve the targeted delivery of GE11, an EGFR-specific peptide was added to the surface of the exosome. Data from in vitro experiments showed that the exosome was taken up by variety of breast cancer cells, inclusive of HCC70, HCC1954, and MCF-7. When exosomes containing let-7a miRNA were administered to RAG2-/mice with tumors, it resulted in a localized reduction of tumor growth. Exosome-based drug carriers with modified surfaces have improved inherent stability, delivery effectiveness, and decreased immunogenicity. An experiment using the c(RGDyK) peptide surface demonstrated enhanced medication delivery for the treatment of cerebral ischemia.[64] Using quick bio-orthogonal chemistry, the c(RGDyK)

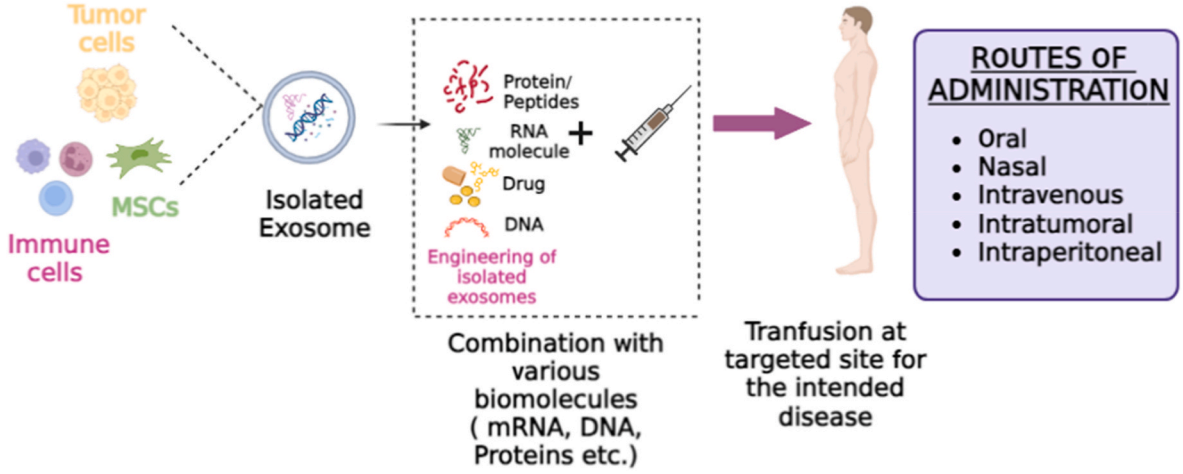

Fig. 3. An overview of drug delivery using exosomes.

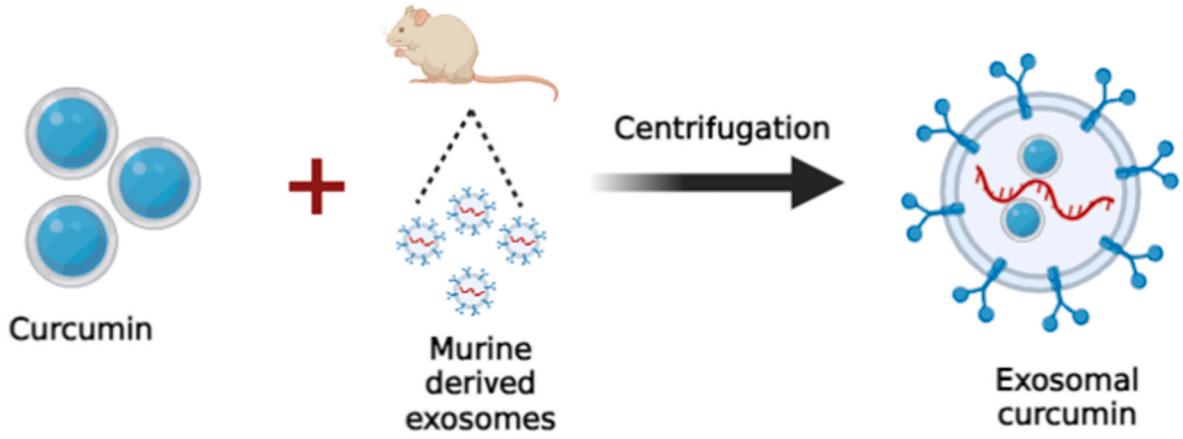

Fig. 4. Exosomal curcumin formation by incorporation into murine tumor cell derived exosomes.

Table 3
Few categories of exosome cargo as a drug delivery method.

Category of Cargo		Disease Name	Result	Exosome Source	Reference
Small molecules	Dopamine	Parkinson's disease	Increased therapeutic impact as a result of drug delivery to the brain specifically	Kunming mouse blood	46
	Curcumin	Brain tumor and autoimmune encephalitis	Reduced cerebral inflammatory response and postponed development of brain tumor	Tumor cells (GL26-Luc, BV2, 3T3L1, 4T1, CT26, A20, and EL-4)	47
	Paclitaxel	Autologous prostate cancer	Enhanced drug cytotoxicity to cancer cells	Prostate cancer cell lines (PC-3 and LNCaP)	48
	Doxorubicin	Breast cancer	Drug administration with specificity to the tumor location and tumor growth inhibition	The vector-expressing iRGD-Lamp2b fusion proteins were transfected into immature murine dendritic cells.	49
Proteins	Signal regulatory protein α	Cancer	Enhanced phagocytosis of tumor cells.	Human embryonic kidney 293T-cells	44
	20S proteasome	Mouse myocardium	Reduction in myocardial infraction	Mesenchymal stem cells (MSCs)	50
	Antiepider-mal growth factor receptor	Epidermoid carcinoma	Target specificity	Mouse neuroblastoma	51
	Survivin – T34A	Pancreatic Cancer	Apoptotic death of cells	Melanoma cell	52
Genetic Substances	siRNA	Breast cancer	Compared to non-targeted exosomes, TPD52 gene expression was downregulated by up to 70 %.	Human embryonic kidney cells (HEK293)	53
	miRNA	Ischemic kidney injury	Minimized kidney damage and safeguarded renal function	Endothelial colony-forming cells from human cord blood	54
	miRNA	Glioblastoma tumor	Providing diagnostic information	Glioblastoma cells	55
	Spherical nucleic acids	Prostate cancer	3000-fold-enhanced knockdown of miR-21	PC-3 cells	56

peptide was adhered to the exosome surface. In an in vivo study using a mouse model of middle cerebral artery blockage, it was shown that curcumin, when later loaded into exosomes, was carried precisely to the location of the lesion and that the inflammatory response was diminished.

9. Exosome based drug delivery vehicles for Parkinson's disease

Affecting roughly 65 % of the elderly population, Parkinson's disease (PD) is one of the most prevalent age-related neurodegenerative diseases.[59] Since it is difficult to get these medications into the body because of the blood-brain barrier's widely recognized impermeability, numerous treatments are now being developed to treat the disease's motor symptoms and halt its progression. The rapid identification of nanoscale delivery mechanisms within the BBB is necessary for this. The capacity to cross the blood-brain barrier makes exosomes, an ideal delivery system for the treatment of neurological diseases like PD (Fig. 5). Exosomes are ideal for crossing the BBB because of their nanoscale structure and size, which lowers the danger of embolism. Exosomes encoding dopamine that were taken from human blood were examined in vitro and in vivo. The findings stated that the exosomes traversed the BBB and supplied dopamine to the brain mediated by the interaction among transferrin and transferrin receptors.[46] When compared to freely administered dopamine, this had a more positive therapeutic effect on PD mice models. Mesenchymal stem cells (MSCs) exosomes have been found to be a potential PD treatment approach.[65,66] They are known to possess clinically advantageous miRNAs, which promote neurogenesis in PD mouse models, and they have been found to restore the dopaminergic neurons in PD mouse models. Exosomes loaded with miR-21 and miR-143 significantly modulate the immunological response and neuronal death. Exosomes from MSCs encourage neurite development at the site of the injury.

Lewy bodies, which are clumps of the peptide α-synuclein, are one of the clinical symptoms of Parkinson's disease. Antisense oligonucleotides (ASOs) of α -synuclein were delivered via exosomes, which showed enhanced cellular uptake and reduced toxicity of the neuronal culture.[67] Exosome infusion into the brain of α-syn A53T animals, a genetically-engineered rodent model of PD, resulted in reduced and attenuated expression of α-syn. After the injection of the ASOs loaded exosome, it demonstrated a considerable improvement in the mice's locomotor function. This showed that this method of treating PD worked.

10. Exosomes in vaccine development and commercial therapeutic materials

Exosomes have several therapeutic applications, among which the current application gaining worldwide attention in the realm of biomedical engineering is the use of exosomes as vaccination vectors.[3] Scientists are devoting close attention to building cell-free vaccines using exosomes for cancer and numerous other viral and non-viral infectious diseases because of its biocompatible, less toxic, and non-immunogenic characteristics.[68] Exosomes are capable of delivering antigens to the target cells, making them an effective tool for creating vaccines devoid of viruses. The MSC-derived exosomes pro-angiogenic, anti-inflammatory, and immunomodulatory properties are currently being used to create SARS-CoV-2 vaccines. Exosomes provide a novel method for antigen delivery that might be used to quickly produce multivalent protein-based vaccinations. Multivalent protein-based vaccinations are now being developed to combat the SARS-CoV-2 virus's constantly evolving strains and prevent virus transmission. Clinical testing of the STX vaccine for SARS-CoV-2 immunization has yielded

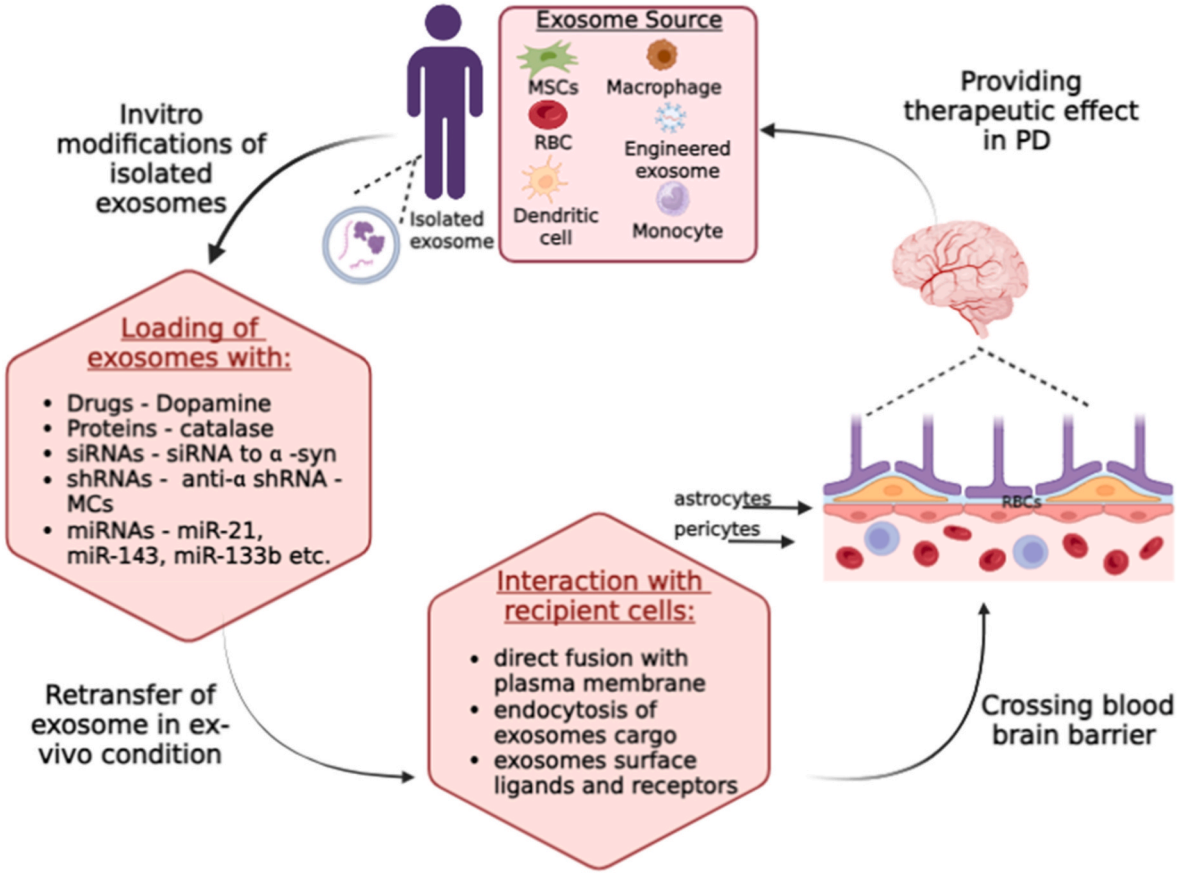

Fig. 5. PD therapy using exosomes as nano-delivery systems.

Table 4
Commercial enterprises utilizing exosome.

Name of Company	Source and incorporation of exosomes	Exosomes used in commercial or technology	Therapeutic Application
Aruna Bio	Neural stem cells	Product AB126: neuronal exosome platform.	Neurological diseases
Ciloa	Recombinant exosomes	Chikungunya (FUI granted) and Zika vaccine candidates	Antibodies, vaccines, therapeutic vectors
Evox Therapeutics	Drug-loaded exosomes	DeliverEX™ platform	Severe rare genetic disorders
Carmine Therapeutics	Red blood cells	REGENT®	Gene therapy
Direct Biologics	Human BM-MSCs	ExoFlo™	Supplying signaling proteins which regulate inflammatory response
Aethlon Medical, Inc.	-	Hemopurifier®	Cancer and infectious disease
Kimera Labs	MSCs	XoGlo®	Skin renewal/rejuvenation and wound healing
Aegle Therapeutics	Allogeneic BM-MSCs	AGLE-102	Serious dermatologic disorders
Capricor Therapeutics	-	Engineered Exosome platform	Rare and severe illnesses, such as DMD
Clara Biotech	-	ExoRelease™	Exosome Isolation Platform
Codiak Biosciences	-	engEx™ platform	cancer, neurological disorders, and development of vaccines
Anjarium Biosciences	-	Hybridosome® platform	Cancer and genetic diseases
Exocel Bio	-	EXOVEX	Regenerative medicine
ExoCoBio	Stem cells	ExoSCRT™	Technology for mass producing highly effective exosomes, including techniques for isolation and purification
		Therapeutic and cosmetic products ASCE	Diverse tissues or cells to regenerate, or to activate or deactivate
Exosome Diagnostics	-	ExoDx™ Prostate test	Diagnosis and assessment of the risk of prostate cancer
Exopharm Pty Ltd.	Stem cells	Exopharm's LEAP Technology	Purification and exosome isolation from adult stem cells
	Cargos: such as RNA, enzymes and/or small molecules	Engineering exosomes	Cancer, Neurodegeneration, and Antivirals
	Adult stem cells and platelets	Natural exosomes	Wound healing, osteoarthritis
Exogenus Therapeutics	Umbilical cord blood mononuclear cells	Exo-101	Inflammatory diseases (including chronic wounds, inflammatory skin problems, and inflammatory lung ailments) and regenerative medicine
Exosome Sciences	-	TauSome™ biomarker	Diagnosis and monitoring of neurological conditions such as Alzheimer's disease, chronic traumatic encephalopathy, and others
Exosomics Siena SpA	–	Exosome-based liquid biopsy	Cancer detection and screening using exosomes
EV Therapeutics Inc.	–	mTEV platform (EV101, EV102, EV103)	Gastrointestinal cancer, organ transplant rejection
Ilias Biologics Inc.	Therapeutic exosomes loaded with API molecule	EXPLOR™ platform technology	Controlled loading of certain proteins into exosomes
		Exo-Target®	Malignancies, metabolic disorders, and inflammatory diseases
TAVEC Pharmaceuticals	-	miRNA-loaded exosomes	Gene therapy for cancer
Stem Cell Medicine Ltd.	Adult stem cells	Exosome-based technology	Neurodegenerative and neuropsychiatric indications: autism spectrum disorder (ASD)
OmniSpirant	Bioengineered stem cells	Inhaled exosome technology platform	Currently incurable respiratory diseases, cystic fibrosis
Paracrine Therapeutics	Stem cells	Exosome Technology Platform	Regenerative medicine
XOStem Inc.	Bone marrow and umbilical cord derived MSCs	XO-Regen®	Articular damage, respiratory failure, neuroinflammation
		XO-Cutis®	Wound healing, skin renewal, and hair regeneration
ReNeuron	CTX neural stem cells	ExoPrO	cancer, neurodegenerative diseases, and vaccine development

promising outcomes for the efficient delivery of the proteins.[69]

Exosome-based therapeutic drug delivery platforms and therapeutic products are currently in pre-clinical and clinical research, thanks to the successful development of many commercial firms (Table 4).[3] Aegle Therapeutics, a biotech business in Miami, Florida, has created the exosome product AGLE-102. This medication has received approval from the FDA to treat severe dermatological diseases, including burns and wounds. Exosome mRNA vaccine and exosome VLP Display Vaccines are the two exosome-based vaccines against SARS-CoV-2 formulated by Capricor Therapeutics, and both are currently in the pre-clinical phase. A platform called engEx has been created by the pharmaceutical business Codiak Biosciences from Cambridge, UK, specifically for the creation of exosome-based medicinal agents. According to engEx, multiple promising exosomes containing a variety of therapeutic chemicals have been formed to facilitate the cure of a number of neurological illnesses. exoVACCTM is an exosomal vaccine used to treat viral infections, neurological conditions, and cancer. Plexaris, a natural exosome-based medicinal product created by Exopharm Pty Ltd with the goal of promoting wound rehabilitation, is presently undergoing a Phase 1 clinical trial. For the therapeutic management of neurological disorders and cancer, Exopharm Pty Ltd has found two modified exosomes with the names Cognevo and PlexoDOX. In order to cure cancer and neurological diseases, ReNeuron, a company located in the United Kingdom, has created a new exosome-based medication delivery system called Exo-PrO. Because of these distinguishing characteristics, exosomes are a viable and superior nanocarrier for medication delivery and other therapeutic purposes.

Numerous novelties and intellectual properties have resulted from extensive research in the field of exosomes. Innovations in exosome manufacture, isolation, transport, and therapeutic use have received patent certifications. MicroRNAs and exosomes to promote tissue regeneration. Mostly surface engineered exosomes have been generated to provide the desired therapeutic effect. Exosomes, lipid nanosized vesicles that contain specific nucleic acids that can be modified by their surface proteins, have been employed in a novel way to enhance myelination in neurological illnesses associated with demyelination, such as multiple sclerosis (MS) and others.[33] In order to target damaged tissue and encourage tissue regeneration and repair, Eduardo Marbán et al.

developed exosomes, which are loaded with proteins and produced from a specific cell type. More creative exosome-based formulations have been approved with the goal of improving biological agent distribution by bridging the blood-brain barrier and by concentrating on tumors and inflammatory areas. As a result, the therapeutic effects are enhanced while the agents' immunological reaction is diminished.

11. Conclusion and future perspective

Exosomes are promising therapeutic tools that have received a lot of interest in recent decades because of their efficiency as a delivery route for a variety of synthetic and natural medicinal substances. Exosomes have special properties that make them useful for therapeutic purposes. These characteristics include being biocompatible and biodegradable, less toxic, more stable, and are capable to pass through the BBB. They arise by several types of cells in the body, and they are the most significant drug nanocarriers for the diagnosis and treatment of a variety of illnesses, including immunological, dermatological, cardiovascular, and neurological problems. Additionally, they are employed for range of biological goals, such as vaccine development, tissue regeneration, and gene therapy. Exosomes therapeutic potential, cell-adhesion traits, and ability to successfully distribute bioactive components to target cells are all improved by surface modifications applied using a variety of techniques. Exosomes shield nucleic acids from the endosomal and lysosomal degradation pathways, in contrast to other delivery systems like liposome-mediated, polymeric and metallic nanomaterials, etc. This can improve the effectiveness of transfection of biomolecules like siRNA, DNA, and mRNA.

Despite its numerous advantages, there are still numerous challenges in correctly comprehending exosomes with reference to their therapeutic and drug delivery uses. Exosome production on a large scale for clinical studies presents a number of challenges, including the need for hybrid exosome designs for future clinical applications, high costs associated with post-drug approval, the lack of efficient methods for characterizing the safety parameters of exosomal designs, and unintended immune reactions by some exosomes. Exosome-based solutions and platforms for effective medication delivery and illness treatment, however, have been created by numerous pharmaceutical and biotechnological businesses and are currently undergoing clinical trial authorization.

Authors' contribution statement

CDM: Conceptualization, Reviewing, editing, supervision; VS: Writing original draft, editing; preparing graphical presentations.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors acknowledge all the CHST Lab members for their support and IIEST for providing the infrastructural facilities.

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