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A Review of Exosomes: Isolation Methods and Applications

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Abstract:

Exosomes are becoming recognized as crucial facilitators of interaction between cells. They deliver biological agents to target cells, play essential roles throughout multiple biological and pathological events, and have significant potential as innovative alternative therapies for illnesses. Exosomal communication between cells appears to have a role in the development of several illnesses, involving cancer, neurodegenerative conditions, and inflammatory disorders. Exosomes are diminutive (20–150 nm) entities characterized by a unique bilipid protein architecture. They transport and switch diverse cargos across cells and serve as noninvasive indicators for multiple disorders. Exosomes are regarded as the most effective indicators for cancer detection due to their distinctive properties. This document will examine the current uses of exosomes, their origins, and diverse isolation techniques. Furthermore, the function of exosomes and their use in biomedical studies and preclinical experiments are succinctly addressed.

Summary of extracellular vesicles (EVs)

Synthetic delivery of drug technologies, such as polymeric nanoparticles, dendrimers, micelles, and liposomes, have been utilized to improve pharmaceutical efficacy and curative usefulness in clinical contexts (1). Notwithstanding the considerable benefits of liposomes, the most established and extensively researched drug delivery system, their utilization is constrained by their low stability, long-term safety concerns, and the potential to trigger severe hypersensitivity responses (2). EVs, considered a natural transport system, may surmount the limitations associated with liposomes as well as additional artificial drug transport methods. EVs are categorized based on their size, source, appearance, and operations, including numerous kinds of microvesicles generated from the plasma membrane (3). Exosomes, as a subgroup of EVs, are now garnering significant interest from the scientific community. Exosome creation transpires in three phases: budding, multivesicular body (MVB) development, fusion of the plasma membrane with MVBs, and the dissolution of vesicular components as exosomes (4). In contrast to apoptotic bodies (1000–5000 nm) and microvesicles (50–1000 nm), which are produced by dead cells and the external budding of the plasma membrane respectively, exosome biosynthesis commences with the inward budding of the plasma membrane, typically initiating the formation of intraluminal vesicles (ILVs) within early endosomes (5). Endocytosis results in the formation of early endosomes that sequester cellular proteins and genetic materials from the cytoplasm, subsequently transforming to late endosomes, which give rise to multivesicular bodies (MVBs). MVBs are subsequently destroyed by lysosomes or merged with the plasma membrane to release ILVs as exosomes (6).

The therapeutic uses of exosomes are many and diverse. A very interesting study domain is reconstructive medicine, whereby exosomes produced from stem cells have demonstrated the capacity to facilitate regeneration and repair of tissues (7). Exosomes have been employed for the treatment of heart disorders, including myocardial infarction,

by facilitating angiogenesis and enhancing cardiac function. Exosomes have also been investigated as a therapeutic modality for neurological illnesses, such as Parkinson's disease and Alzheimer's disorder (8).

Besides the possibility of treatment, exosomes have been examined for their monitoring qualities. Exosomal indicators have been discovered for several illnesses, such as cancer, facilitating the creation of non-invasive diagnostic assays (9). Moreover, exosomes have been investigated as a strategy for delivering tailored medicines to certain cells or tissues, overcoming the constraints of conventional delivery techniques. Notwithstanding the considerable advancements in exosome research, several obstacles and ambiguities remain to be resolved (10). The best techniques for the separation and purification of exosomes are still under discussion, and both the stability and the bioavailability of exosomal cargo continue to be problematic. The regulatory framework governing the application of exosomes as medicinal products is in flux (10).

This critical study seeks to offer a thorough summary of the existing information about the therapeutic uses of exosomes. We will conduct a critical study of the current literature to examine the advantages and drawbacks of exosomes as a means of treatment, discuss the obstacles and difficulties that require resolution, and provide suggestions for additional study avenues.

Composition of exosomes

Exosomes are endowed with lipids, proteins, and DNA or RNA throughout biosynthesis. Lipids such as sphingomyelin, cholesterol, ganglioside GM3, desaturated lipids, phosphatidylserine, and ceramide constitute the bilayer framework of the exosomal membrane and enable exosomes to serve as cargo transporters (11). Proteins found in the outer layer or cytoplasm of exosomes involve fusion proteins, enzymes, chaperones, and proteins associated with MVB production (e.g., CD9, Annexin A2, Meosin, ICAM1, MHC) (12).

Nucleic acids present in exosomes including mRNAs, miRNAs, other non-coding RNAs, and DNAs. Our high-through investigations identified around 6000 recognized proteins, with over 85% of these recognized miRNAs residing in brain cell-derived exosomes, indicating the exceptional compatibility of exosomes in encapsulating molecules with diverse physical and biological characteristics (13).

The chemical compositions of exosomes are significantly affected by the kinds and conditions of their parent cells. We noted that stimulated microglia, the resident immune cell population in the Central Nervous System (CNS), emit exosomes full of cytokines, while neural stem/progenitor cells (NPCs) generate exosomes plentiful in growth factors (14). These exosomes may permeate the blood-brain barrier into the peripheral circulation. Consequently, the unique profiles of illness-associated chemicals in exosomes separated from bodily fluids are regarded as prospective markers for diagnosing illnesses (15).

Exosomal cargos may be passively encapsulated; however, new research has also found several sorting mechanisms that actively incorporate molecules into exosomes. The endosomal selecting complex needed for transport (ESCRT) is identified as an essential carrier for the translocation of proteins into exosomes (16). Ubiquitylated proteins are identified by ESCRT-0 and directed to the endosomal membrane, while ESCRT-1 and ESCRT-2 facilitate bud development with the chosen proteins, and ESCRT-3 finalizes vesicle scission (17). Additionally, an ESCRT-independent process contributes to protein sorting into exosomes, primarily facilitated by ceramides, tetraspanins, or heat shock proteins. Nucleic acids are compartmentalized into exosomes with the aid of RNA-binding proteins (18). The ESCRT-2 complex, in conjunction with tetraspanin-mediated microdomains, the miRNA-induced silencing complex (miRISC), and phosphorylated argonaute 2 (AGO2), identifies certain RNA patterns and aids in their entrapment by exosomes. Consequently, although the mechanisms behind cargo sorting into exosomes are mostly unclear, it is apparent that cargos may be incorporated into exosomes by both random and selective processes (18).

Application of exosomes

Extracellular vesicles execute several roles in biological circuits. Microvesicles are involved in cell signaling, apoptotic bodies convey the contents of dying cells to healthy cells, and exosomes facilitate interactions between cells in many physiological and pathological processes. This text presents instances of exosome function in its native form (19).

Proteins on exosome surfaces

Exosomes are pivotal in the induction and inhibition of the immune system, capable of modulating immune system reactions through antigen presentation on their outermost layer (20). Exosome membranes merge with MHC–antigen complexes, eliciting antigen-specific T-cell responses that contribute to the beginning and development of inflammation. The exosomal receptors on the surface CD86 and lymphocyte function-associated antigen 1 (LFA-1) initiate inflammatory processes that stimulate immune system cells (21). Exosome surface proteins contribute to immune repression; exosomes from tumor cells expressing programmed death-ligand 1 (PD-L1), a regulatory checkpoint molecule, may block cytotoxic T-cell activity and enable the immunological evasion of tumor cells. In addition to surface proteins, exosomes

have been shown to transport protein, DNA, and RNA cargos that may elicit immune system reactions and other biological processes (22).

Transportation of cargo

The exosomal transfer of proteins, nucleic acids, and lipids from donor to recipient cells signifies a recently recognized mechanism of communication between cells. The delivery of exosomal cargo, particularly nucleic acids, might influence the behavior of the destination cell (23). The miRNA content of mesenchymal stem cell (MSC)-derived exosomes facilitates myocardial healing, acting as a substitute for MSCs (24). Exosomes produced from MSCs have a greater capacity to avoid hypertrophy or destruction compared to the MSCs themselves in vitro, attributable to variations in miRNA content among the exosomes and their original cells (25). The exosomal transport of water-soluble cytokines, growth factors, and hormones serves as an essential system for intercellular interaction, facilitating interaction over extensive distances to influence systemic immune responses. The lipid content of exosomes seems to possess many activities, including the regulation of metabolism and immunological responses in recipient cells (26).

The onset of metastasis in cancer development is facilitated by the epithelial-mesenchymal transition, which involves the conversion of sticky epithelial cells into migratory, aggressive mesenchymal cells (27). Exosomes originating from mesenchymal cells possess a unique payload compared to those from epithelial cells, and these mesenchymal-derived exosomes may facilitate migration. Exosomes inside the tumor environment may facilitate tumor proliferation by transporting proteins, lipids, and nucleic acids that induce immune suppression. Fatty acid cargo from tumor-derived exosomes may inhibit the immune system reactions of recipient dendritic cells (28).

Application of biomarkers

Exosomes are found in several biofluids, potentially offering a novel means to assess cellular state in both normal physiology and the emergence of disease disorders (29). Exosomes and other EVs may be profiled for their cargo by an easy liquid biopsy of bloodstream fluids, urine, or saliva, facilitating applications in evaluation, prediction of disease progression, and chemoresistance biomarkers (30). Although various cell types secrete exosomes, data indicates that exosome production elevates in certain pathological situations, including cancer. Exosomes exhibit stability in many bodily fluids and protect biomolecules from destruction. Pathological situations may manifest cellular alterations in the physiological constituents of exosomes produced by cells (31). The modified cargo of exosomes may be detected and evaluated using transcriptomics, proteomics, and lipidomics studies; hence, variations in the quantities of certain compounds might be advantageous for biomarker usage (32). Various laboratories have demonstrated variable expression of miRNAs in cancer cells and their exosomes in vitro; also, some research done on ex vivo and animal cancer models have corroborated alterations in miRNA expression levels. Various miRNAs are under development, involving miRNA-200-5p, miRNA-378a, miRNA-139-5p, and miRNA-379 for lung cancer, as well as miRNA-21 for oesophageal squamous cell carcinoma, which has been proposed as non-invasive detection biomarkers (33). Furthermore, exosomal proteins have biomarker potential for several malignancies. In oncology, the prompt identification of a neoplasm is crucial for successful treatment (34). A comprehensive understanding of the mechanisms governing exosomal cargo organizing and production, along with their transport via specific bodily fluids and stable levels throughout physiological conditions and various disease situations, would yield more robust biomarkers for tracking the start of disease and progression. Moreover, additional research is necessary to confirm the specificity and sensitivity of exosomal biomarkers in comparison to conventional cancer markers (35, 36).

Principal methods for the isolation of exosomes

Various exosome separation techniques have been established depending on their size, morphology, density, and surface protein composition (37). The significant similarity in physical and chemical characteristics among exosomes and non-exosome vesicles resulted in the presence of many non-exosome vesicles, including microvesicles and apoptotic bodies, in “exosome specimens” generated using established methods. Consequently, unless stated contrary, the word “exosome” in this article denotes a composite of tiny extracellular vesicles, including exosomes, apoptotic bodies, microparticles, and microvesicles (38).

Ultracentrifugation

Ultracentrifugation is the benchmark method for exosome separation, employed in 80 percent of exosome processing procedures. Ultracentrifugation requires little preparation of the sample and is cost-effective, with the exception of the initial expense of the equipment. Nonetheless, it is labor-intensive and attains only modest exosome purity (39). Ultracentrifugation segregates specimen constituents according to density; yet, there exists considerable overlap in the density areas of several EV kinds. Centrifugal forces segregate samples into distinct layers; high-density particles descend to the bottom of the tube, whilst low-density fragments ascend to the

top. The ultracentrifugation rates utilized for exosome separation vary from 100,000 × g to 210,000 × g. Enhancing the velocity may facilitate extractions but poses a risk of compromising the exosomes (40, 41).

Differential centrifugation is a type of ultracentrifugation method that uses several sedimentation cycles to isolate target exosomes from cellular detritus, bigger vesicles, and proteins (42). The technique requires regular user interaction to eliminate supernatants and pellets and to initiate spin cycles. Exosomes may be lost throughout the multiple extractions of supernatant and the movement of samples between tubes; hence, exosome attrition is anticipated, prompting the application of larger sample amounts at the outset of processing to achieve the required yield. Notwithstanding these limitations, differential centrifugation is a reliable method that routinely yields exosomes of intermediate both purity and quality (43).

Ultrafiltration

Ultrafiltration is characterized by the application of minuscule pores (about 100 nm in diameter) and may be employed to segregate exosomes according to size. Ultrafiltration techniques are expeditious, with a single filtration cycle lasting from seconds to 30 minutes, facilitating high efficiency (41). Ultrafiltration separates vesicles by exerting force to propel sample fluid across membranes with about 100 nm holes. Membranes with varying pore diameters might be utilized in subsequent stages to eliminate further undesirable particles (44). The procedure is more rapid than ultracentrifugation; nevertheless, the applied pressure may harm exosomes owing to shear stress and might lead to exosome depletion from membrane adherence and obstruction caused by particle buildup, hence diminishing exosome yield and extending processing time. Membrane cleaning methods have been established to address these challenges (45).

Exosome ultrafiltration methods encompass arranged filtration, conjunction filtration, centrifugal ultrafiltration, and tangential flow filtration. Sequential and tandem filtration are dead-end techniques that are executed with a syringe (46). Sequential filtration involves numerous filtration phases, everyone utilizing a distinct molecular weight threshold, while tandem filtration integrates several filters within a single needle. The size-exclusion thresholds for exosomes generally range from twenty to two hundred nm; in tandem filtration, exosomes are retained in an intermediary membrane (47).

Precipitation

Precipitation techniques are extensively used in the characterisation of electric vehicles. A global study indicated that precipitation is the favored method for EV RNA investigation. Precipitation techniques employ volume-excluding polymers to sequester water molecules and precipitate less soluble constituents from the solution (48). Biological substances are removed from the solvent-containing areas inhabited by these polymers and become concentrated when their solubility limit is surpassed, resulting in precipitation. This strategy is frequently employed with other isolation techniques. This approach yields more output; nevertheless, it is constrained by the reduced quality of the goods (49).

Size-exclusion chromatography

Size exclusion chromatography (SEC) is the most delicate chromatography technology, extensively utilized in the separation as well as purification of biopolymers, including proteins and polysaccharides (50). The isolation of exosomes by SEC maintains vesicle integrity and biological activity while achieving a high yield. The SEC technique segregates biomolecules according to variations in hydrodynamic radius as they traverse an inert, low-adsorption resin composed of a hollow bead matrix inside a column (51). The particles exceeding pore size elute initially, however, smaller fragments and molecules infiltrate the pores to differing extents according to their size, resulting in prolonged elution times as particle or molecule size diminishes (52). To achieve high-resolution particle size, it is essential to examine processing parameters like column measurements, bead wrapping, resin type, velocity, and system capacity. This technique may be used to differentiate samples over a spectrum of viscosities, ranging from low-viscosity urine and cells grown in medium to high-fluidity plasma. Nonetheless, pretreatment of materials using ultracentrifugation or ultrafiltration is essential to get extracellular vesicle preparations devoid of protein and lipoprotein contaminants (53).

Exosomes and oncological biology

Exosomes stimulate tumor cell growth

Recent research indicates that cell-derived exosomes significantly contribute to tumor development and advancement (54). Recombinant epidermal growth factor (EGF) administration may enhance the absorption of exosomes generated from oral squamous cell carcinoma (OSCC) cells into OSCC cells (54). Conversely, the suppression of the EGF receptor (EGFR) or the use of EGFR blockers, such as erlotinib and cetuximab, eliminates exosome absorption by OSCC cells. Exosomes generated from cells have been demonstrated to promote growth, movement, attack, stemness, and chemoresistance in OSCC cells. These chemicals may initiate signaling cascades and enhance tumor cell growth by attaching to receptors on the tumor cell membrane (55).

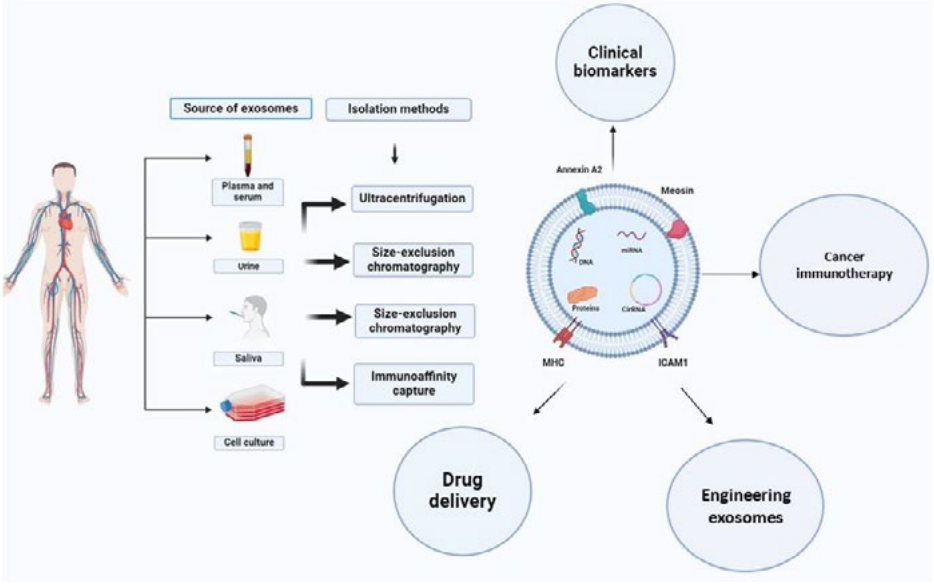

Fig 1. Schematic representation of exosome sources, isolation methods, and their uses. Exosomes include various proteins and nucleic acids that are characteristic of the kind and condition of the originating cells. The distinctive double-layer membrane of exosomes safeguards the biomolecules they transport, allowing these exosomes, which contain specific chemicals, to persist in diverse bodily fluids for extended periods, facilitating their detection and extraction. These exosomes may be modified for several therapeutic uses.

Exosomes facilitate the spread of malignant cells.

Comprehensive studies on the tumor environment indicate that exosomes generated by cancer cells play a significant role in essential aspects of cancer development, such as blood vessel formation, premetastatic niche formation, extracellular matrix development, epithelial-mesenchymal transition (EMT), cancer stem cell proliferation, and persistence against treatment (56). Research established that exosomes produced from cancer-associated fibroblasts (CAFs) are a significant factor in the advancement of ovarian cancer (OVCA). This work demonstrated that the circRNA molecule hsa_circIFNGR2 may clarify the new role of CAF-derived exosome circIFNGR2 in ovarian cancer cell development and metastasis (57). Metastatic organ tropism continues to be one of the most significant enigmas in cancer genesis since the formulation of the "seed and soil" concept. Researchers discovered that EGFR present in exosomes released by gastric cancer cells may be transported to the liver and incorporated into the plasma membrane of liver stromal cells (58). Translocated EGFR has been demonstrated to efficiently stimulate hepatocyte growth factor (HGF) by suppressing the expression of miR-26a/b. Furthermore, the increased paracrine HGF interacts with the c-MET receptor on migratory cancer cells, creating a conducive environment for the establishment and multiplication of metastatic tumor cells (58).

Exosomes and the immunological response

Exosome components secreted by tumor cells during development may suppress the normal functioning of the immune system, therefore evading assault and elimination by the host immunological response (59). This inhibitory action is primarily accomplished by modulating immune cell activity, facilitating immune cell death, and diminishing the population of immune cells (60). The exact mechanism by which exosomes contribute to tumor growth is unclear, complex, and dual-natured, necessitating more elucidation. Exosomes may mediate the interaction between innate immune cells and tumor cells, influencing tumor growth either positively or negatively. A previous study concentrated on exosome-mediated interaction among tumor cells and macrophages, neutrophils, mast cells, monocytes, dendritic cells, and natural killer cells (61).

Novel uses of exosomes in cancer detection and treatment

Exosomes used as diagnostic instruments

As previously stated, exosomes are found in many biofluids, providing a chance to assess cellular health in both normal and diseased settings. Exosomes in biopsies (liquid, plasma, serum, urine, saliva) have been utilized to assess and ascertain a patient's diagnosis, outlook, progression, and chemoresistance condition (62). Moreover, increased exosome production has been seen in other complicated diseases, notably cancer. Pathological states may be assessed by the analysis of exosomes generated by cells. Transcriptomics, proteomics, and lipidomic analyses may identify and examine discrepancies in the quantities of certain molecules. Exosomes have been explored for their potential in the early detection and prediction of cancer and cardiovascular illnesses (63, 64).

The use of exosomes in blood samples for cancer diagnosis has recently garnered significant interest.

In a retrospective investigation, scientists integrated artificial intelligence with surface-enhanced Raman spectroscopy to effectively identify exosomes in the initial stages of malignancies, including lung, breast, colon, liver, pancreatic, and gastric cancer (65). The work incorporates a classification algorithm to discern plasma exosome signaling patterns for determining their existence and tissue of origin. The conclusive complete decision model demonstrated an accuracy of 90.2% and a sensitivity of 94.4%, accurately identifying tumor organelles in 72% of positive individuals (66). Exosomes are becoming known as prospective biomarkers for diagnosing colorectal cancer owing to their extensive biological signatures and remarkable durability. A new study emphasizes the development of an exosome enriching platform using a 3D porous sponge microfluidic chip, which achieves an exosome capture rate of over 90% (66).

In comparison to conventional biomarker detection techniques, using exosomes for illness diagnosis has the following advantages: Exosomes possess excellent sensitivity due to their capacity to transport several biomarkers. High precision: Exosomes may transport cell-specific indicators, resulting in enhanced specificity (67). Non-invasive: Exosome extraction may be accomplished using standard bodily fluid techniques, eliminating the need for invasive procedures like tissue sampling or puncture. The harvested exosomes may be preserved for extended periods using cryopreservation and other techniques to enable future investigation. Despite the many benefits of using exosomes for illness detection, some problems persist Standardization concerns: The absence of standardized protocols for exosome gathering, processing, and identification may lead to variability in findings across various labs. (2) Technical challenges: The existing detection technology for exosomes is insufficiently advanced, necessitating the development of novel detection methodologies. (3) Sample volume concern: Due to the insufficient comprehension of exosomes, a substantial number of samples are required for validation and corroboration (68, 69).

Therapeutic application of exosomes

The employing of exosomes in cancer therapy primarily relies on two approaches: (1) their function as drug carriers for encapsulating pharmaceuticals; (2) their role as bioactive compounds (e.g., miRs) to modulate the tumor environment and impede tumor development and spread (70). Experimental investigations indicate that employing exosomes as drug carriers enhances medication inhibition and bioavailability while minimizing negative effects. RNA nanoparticles of this kind may enhance targeting efficacy and facilitate the delivery of therapeutic agents to targeted cancerous cells (71). Reports indicate that RNA nanoparticles may be utilized to provide chemical ligands (such as folic acid), pharmaceutical agents, or RNA aptamers on the exosomal surface. The above combinations may transport small-molecule chemical medicines to a target using chemical ligands or RNA aptamers (72). Binding RNA particles to particular tumor cells may minimize medication dosage and adverse reactions, facilitating targeted cancer therapy. Moreover, RNA nanoparticles altered on exosomes may associate with siRNA, miR, RNA aptamers, or ligands to selectively suppress cancerous cells (73).

Ongoing clinical studies using exosomes

Exosomes have been examined in therapeutic studies across several settings and uses in recent years. An assessment of the contemporary research landscape from the GlobalData database indicates that the primary domains in which exosomes are being investigated for therapeutic treatments are cancer (54%), central nervous system (13%), infectious illness (13%), and immunology (8%) (29). Among the 420 exosomal drugs currently in clinical development, over 65% are in the initial stages of development and have not yet been submitted for independent new drug review by the U.S. Food and Drug Administration (74). Only 6.4 percent, 7.3 percent, and 2.3 percent are in Stage 1, 2, and 3 clinical trials, respectively. As of now, only two items have been introduced for therapeutic use. Patisiran is intended for the treatment of polyneuropathy associated with hereditary transthyretin-mediated amyloidosis (hATTR) in adults. Patisiran is an innovative RNA interference (RNAi) medication aimed at decreasing levels of both wild-type and mutant transthyretin (TTR) for the treatment of individuals with hereditary transthyretin-mediated amyloidosis, a genetic neurological and cardiac condition (75). Ibudilast is a further readily available item associated with exosomes. It is a somewhat nonselective phosphodiesterase inhibitor that has been available in Japan for approximately twenty years for the treatment of asthma. Recent findings indicate its anti-inflammatory effect in both the peripheral immune system and the central nervous system through glial cell modulation in relapsing-remitting and/or secondary advanced multiple sclerosis (76).

The use of exosomes for the treatment of neurological and cardiovascular illnesses has garnered significant interest. Although there is now no efficacious therapy for neurological disorders like Alzheimer's and Parkinson's diseases, certain bioactive chemicals found in exosomes may facilitate neuronal development and repair, potentially offering a novel therapeutic approach for these conditions (77). Scientists have discovered that neurotrophic substances inside exosomes may facilitate neuronal development and repair. Exosomes were injected into the brains of mice, resulting in the

promotion of neuronal development and repair, as well as enhancement of cognitive performance (77). In cardiovascular research, diseases such as coronary heart disease and myocardial infarction are among the primary causes of mortality globally. Bioactive substances, including microRNA-132, transported by exosomes produced from mesenchymal stem cells, facilitate angiogenesis in myocardial infarction (78). Researchers have discovered that miR-126 present in exosomes may enhance endothelial cell proliferation and angiogenesis while diminishing vascular permeability. They encapsulated miR-126 into exosomes and administered it to the myocardial infarction location in mice. Exosomes were discovered to enhance the development and regeneration of cardiomyocytes, hence improving cardiac function in mice (79).

Exosomes and their applications must adhere to GMP, irrespective of their therapeutic reason. A GMP-grade exosome production method entails the use of superior quality ingredients, cells, culture environments, manufacturing technology, and proficient staff, all under carefully regulated and meticulously overseen settings (80). Moreover, separation and quality control release assessments are needed post-production to guarantee that the finished product meets the highest requirements before administration to patients for its intended application (81). The European Medicines Agency and the US FDA have organized seminars and recognize that recent advancements in cellular and molecular biotechnology have resulted in the creation of innovative medicines. Both authorities have issued suggestions and classifications for the manufacture of advanced therapies. As the emerging area of exosomes expands in treatment and diagnostics, further suggestions, standards, and requirements for investigators and producers are necessary to ensure the safety of modern therapies for patient usage (82, 83).

Prospective advancements of exosomes in medicinal applications

Advancements in accuracy gene editing technology will enable the accurate alteration of exosomes by genetic engineering, facilitating more exact control in the future. A prior work presents the application of precision gene editing technology to accurately change exosomes for therapeutic impacts on Parkinson's disease. Genetic engineering enables the incorporation of therapeutic carriers into exosomes, which are then introduced into cells. The essay examines the potential applications and obstacles of exosomes in neuroscience (84, 85).

Cell engineering technology may regulate the cells that produce exosomes, hence influencing their release volume and quality. Separate research presents cell engineering techniques to regulate the cells from which exosomes originate, therefore influencing the amount and quality of exosome release (86). A scientist investigates the use of human umbilical cord mesenchymal stem cell exosomes to enhance angiogenesis via the Wnt4/β-catenin pathway and presents the potential applications of cell genetic engineering in regulating exosome release (87).

Nanotechnology has significant potential to enhance the understanding and influence of cell physiology and disease. Recent studies highlight the application of nanotechnology to manipulate the dimensions, form, and exterior features of exosomes to affect their physiological and pathological impacts (88). The paper examines the prospect of employing nanotechnology to modify the features of exosomes for use in cancer therapy. In summary, exosomes are extracellular vesicles with significant potential and are crucial in both physiology and disease. Altering it in various manners allows for the regulation of its biological functions and stability, allowing comprehensive investigation and application of its purpose and mechanism of action. As technology advances, exosome alteration technologies will see greater application.

CONCLUSION

Exosomes provide significant advancements in drug transport, noninvasive illness detection, therapy, and several other domains due to their therapeutic potential and distinctive biological roles. In comparison to liposomes, nanoparticles, microspheres, microemulsions, and other artificial pharmaceutical delivery systems, exosomes have distinct natural benefits as possible indicators for prognosis and disease diagnosis, drug delivery vehicles, cell-free therapies, and cancer vaccines. Despite the potential of exosomes in the diagnosis and treatment of numerous illnesses, obstacles persist in their transition from laboratory research to clinical use. Initially, there are constraints regarding vesicle extraction and the assessment of whether the separated exosomes are suitable candidates for therapeutic use. This may result from several variables, including discrepancies in separated particle quantity, morphology, methods, circumstances, and the source of separated exosomes. A significant restriction is the heterogeneity in exosomal quantity and purity associated with isolation methods. Although some isolation procedures may offer superior purity and yield compared to others, variations in technique, machinery, and human error complicate the determination of the best suitable approach. Additionally, there are discrepancies in cargo loading efficiency, including electroporation sonication, and extrusion. Although exosome research is still in its early developmental phase, a more comprehensive knowledge of subcellular components and processes related to exosome production and targeted cellular

interactions will illuminate their physiological roles and therapeutic applications. Exosomes undoubtedly constitute a potential instrument in medicine and may provide solutions to several contemporary medical difficulties.

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Authors' contributions

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The author who supplied the data may provide the raw data used in this work upon an appropriate request.

Ethics approval and consent to participate

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Consent to publication

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