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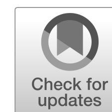

Harnessing exosomes: the future of drug delivery across biological barriers

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Abstract

A unique class of extracellular vesicles (EVs), exosomes range in size from 30 to 150 nm. They frequently travel to distant tissues inside an organism and are essential for cellular communication. Exosomes are useful in the creation of targeted therapeutics for the delivery of macromolecules and drug delivery systems because of their notable natural cell-targeting properties. Because of these features, exosomes are becoming more and more recognized as bio-derived vehicles for delivering and protecting therapeutic agents to treat various kinds of cancers (lung, pancreatic, colon, brain, and breast), viral diseases (AIDS, hepatitis B), and bacterial infections (toxoplasmosis, salmonellosis). According to research, the natural payload of exosomes can either exacerbate or lessen the severity of an illness. This calls for meticulous planning, which includes determining the exosomes' composition and source. As a result, approaches for assessing the features of intact exosomes as well as dependable and economical ways for isolating them are essential. However, several obstacles prevent them from being used in therapeutic settings. Exosome stabilization, safe and effective manufacture in large enough quantities, effective loading of therapeutic drugs into them, maximizing their removal from circulation, and scaling up production from research to clinical settings are some of these. A better comprehension of the molecular processes controlling exosome transport and activity is crucial for clinical applications. This review focuses on methods for isolating and characterizing exosomes, explores their potential as drug delivery platforms, and examines strategies to enhance therapeutic outcomes and improve their stability.

Key words exosomes, drug delivery, biogenesis, isolation, drug loading tools

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Introduction

Bonucci and Anderson discovered that chondrocytes release tiny vesicles that are smaller than 100 nm in size, which led to the discovery of tiny, secreted vesicles in the late 1960s. According to preliminary research, these vesicles, which come straight from the plasma membrane, were essential for the development of hydroxyapatite crystals [1]. Around the same time, Wolf observed that EVs, sometimes known as "platelet dust," are produced by platelets and are known to have a high clotting activity [2]. Later, it was determined that platelet dust aided in the production of thrombin, which boosted the coagulant activity in citrated plasma with longer storage times [3]. These groundbreaking findings laid the foundation for further research, demonstrating that osteogenic exosomes not only participate in normal bone and tooth deposition but are also implicated in pathological calcification of arterial and cardiac tissues associated with cardiovascular diseases [4].

EVs were mainly unknown until the 1980s, when their secretion was recognized as a common cellular function, notwithstanding these early findings. In the 1980s, exosome research became popular, especially as a result of Trams et al.'s ectoenzyme studies [5]. Exosomes were also discovered in the seminal fluid around this time, and it was hypothesized that they came from the prostate and facilitated the transfer of proteins and lipids that were necessary for sperm maturation [6]. All prokaryotic and eukaryotic cell types secrete EVs, which are now characterized as membrane-bound vesicles with micro- or nano-scale dimensions [7]. EVs are classified as exosomes (30–150 nm), microvesicles (MVs) (100–1000 nm), and apoptotic bodies (>1000 nm) according to their shape and generation methods (**Figure 1**) [8]. Numerous components found in these vesicles mediate intercellular communication and impact a variety of cellular functions [9].

The processes of biogenesis distinguish apoptotic bodies, MVs, and exosomes [10]. MVs are created by the budding and blebbing of the cell membrane, exosomes come from endocytic pathways, and cells going through programmed cell death produce apoptotic bodies, which act as cues for phagocytic clearance. Exosomes are sometimes referred to as nanosomes and these vesicles are a promising platform for the development of targeted drug delivery systems to deliver therapeutic payloads to particular cells, tissues, or organs since they can transfer intracellular components and functional molecules to destination cells [11].

Exosomes are commonly found in a variety of biological fluids, such as urine, saliva, serum, and cerebrospinal fluid (CSF), as well as a broad spectrum of cell types, such as platelets and immune cells. According to preliminary studies, exosomes have been linked to several biological activities, including apoptosis, coagulation, inflammation, and maintaining cellular homeostasis [12]. Exosomes are lipid bilayer-encased, nanoscale vesicles that contain proteins, RNA, and other bioactive materials that facilitate intercellular communication. Their potential uses include serving as therapeutic tools and biomarkers for the identification and management of different illnesses [13].

The endosomal process, which starts with the bio-membrane budding inward and creates endosomes-membrane-bound vesicles inside cells-is how exosomes are produced [14]. These EVs transport biological signals, including transferrin receptors and transmembrane proteins, from their host cells to the outside world [15]. Exosomes engage with destination cells and transfer their molecular payload via this process [16]. Exosomes are important for intercellular communication because they carry proteins and RNA, which allows signals to be sent between cells and even distant organs [17]. Through receptor-ligand interactions, they interact with cell membranes and take part in several events, including the presentation of antigens and the advancement of cancer. Exosomes discharge their internal contents and surface proteins into the destination cell after attaching themselves to the target cell membrane. Exosomes have been shown to enter cells, carry their cargo, and alter different physiological and pathological functions [18].

Exosomes and their method to release

Exosomes are known to participate in both local and systemic signaling and regulatory processes and act as carriers of different proteins and nucleic acids [19]. The endosomal system is the source of exosomes. Early endosomes, which are produced when the membrane buds inward, are the first to form. The final fate of these early endosomes is determined by sorting. Early endosomes can combine with endocytic vesicles, guiding their contents toward secretion, destruction, or recycling pathways. If the cargo is intended for reuse, recycling endosomes will form [20]. On the other hand, certain early endosomes develop into late endosomes. Multivesicular bodies (MVBs), which contain a large number of intraluminal vesicles (ILVs), are formed during the mechanism of inward budding of the vesicle membrane during this maturation phase [21]. At this point, the MVBs can either fuse with the plasma membrane, which releases ILVs into the extracellular environment, or they can fuse with lysosomes, which causes ILV destruction (**Figure 2**). These liberated ILVs, also known as exosomes, transport lipids, proteins, polysaccharides, and nucleic acids that come from the inside of the cell [22].

Exosome isolation and separation methods

Numerous biological fluids, such as CSF, saliva, urine, amniotic fluid, semen, breast milk, and tears, have been found to contain exosomes [23]. Additionally, conditioned media made from tissues, cultured organs, and cell lines can yield them [24]. Usually, different laboratory methods are employed to isolate exosomes from biological fluids and conditioned media. These methods consist of size-exclusion chromatography (SEC), polymer-based precipitation, magnetic separation, acoustic fluidic separation, dielectrophoretic (DEP) separation, deterministic lateral displacement (DLD) separation, immunological separation, ultrafiltration, ultracentrifugation, and, more recently, microfluidic devices [25].

Ultrafiltration

The method of exosome separation is simple, size-dependent, and useful [26]. The benefit of ultracentrifugation is that it only needs a small sample volume to provide the required yield while being slower, more complicated, and less effective. The remarkable effectiveness of ultrafiltration was established by Cheruvanky et al., who presented that exosomes could be successfully separated using only 0.5 mL of a urine sample [27]. Techniques like tangential flow filtration and direct flow filtration are used in ultrafiltration. A common method for filtering tiny sample amounts (up to 30 mL) is direct flow filtration. However, issues including membrane fouling and insufficient particle separation limit this approach. Tangential flow filtration is a more potent and useful substitute for large-scale exosome isolation. Tangential flow filtration reduces clogging and stops membrane fouling layers from forming by guiding the sample stream across the ultrafilter membrane [28].

Immunological separation

Another technique for purifying exosomes is immunoaffinity. This method allows for more precise exosome isolation by using different antibodies. It is possible to apply these antibodies

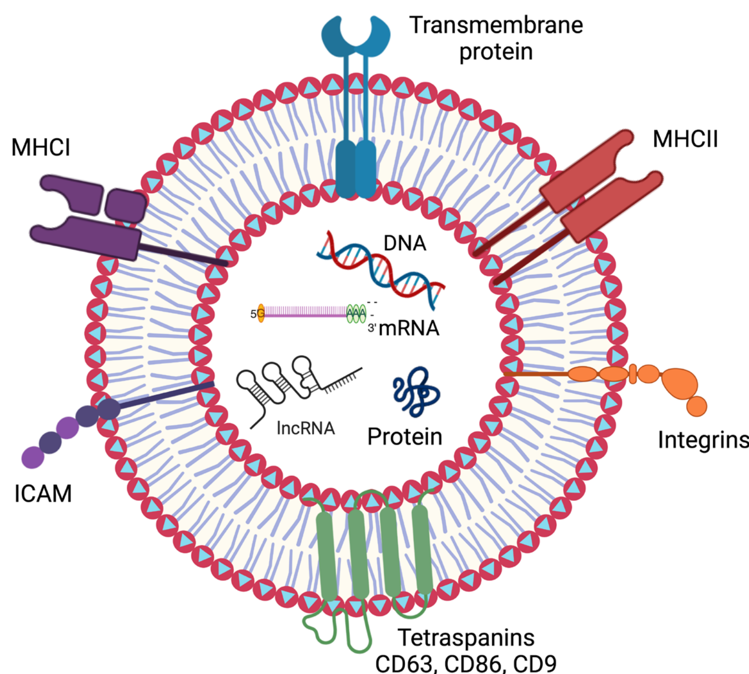

Figure 1. The figure depicts the structural elements of an exosome. A lipid bilayer membrane enclosing the exosome is embedded with a variety of surface proteins, including integrins, transmembrane proteins, major histocompatibility complexes (MHCI and MHCII), and tetraspanins (e.g., CD63, CD86, and CD9). Furthermore, proteins and nucleic acids like DNA, mRNA, and lncRNA are found within cells, which reflects the function of exosomes in molecular transport and intercellular communication.

separately or in combination. Numerous substrates, including magnetic beads, chromatography matrices, plates, and microfluidic devices, can be used to immobilize the antibodies [29]. Although this approach is scalable, many of the extracellular antigens linked to exosomes are not unique to them. Therefore, protein complexes and other particles, including antigen-carrying vesicles unrelated to exosomes, such as cell debris, may also be purified using immunoaffinity-based isolation approaches. Additionally, this method may limit exosome isolation to those associated with a specific biomarker [30].

Ultracentrifugation

Ultracentrifugation is widely regarded as the conventional and benchmark technique for isolating exosomes. This technique relies on centrifugal force to separate cells and major cellular debris from biological fluids based on density, size, and shape [31]. Théry et al. outlined a detailed experimental protocol for exosome extraction and separation using ultracentrifugation. As centrifugation technologies continue to advance, the use of density gradient separation in combination with ultracentrifugation can further enhance exosome isolation [32]. However, despite its established effectiveness, prolonged application of ultracentrifugation may lead to the rupture of exosome membranes due to the sustained effects of centrifugal force [33].

Size exclusion chromatography

By trapping smaller particles in a column full of porous beads, size exclusion chromatography (SEC) is a chromatographic method that filters out particles according to their size. Larger particles, on the other hand, elute more rapidly. Exosomes are larger than most proteins, which usually have diameters of less than 10 nm. They can range in size from 40 to 100 nm. Using beads of the right size, centrifugation can be used to separate exosomes from proteins, leaving behind bigger cellular detritus. With pore diameters of 42, 31, and 75 nm, respectively, Sepharose CL-4B, Sephacryl S-400, and Sepharose CL-2B beads have been used to successfully separate exosomes from proteins [34]. SEC offers several benefits. It preserves the vesicular structure of exosomes, as they are not subjected to harsh conditions like centrifugal forces [35]. Due to its relatively low cost and rapid separation process, SEC is regarded as a practical method for large-scale exosome development.

Polymer-based precipitation separation

The current technique uses hydrophilic polymers, such as polyethylene glycol (PEG), to precipitate exosomes based on charge. By attaching themselves to water molecules, PEG decreases the solubility of exosomes, resulting in their precipitation at comparatively low centrifugal forces. Following an overnight incubation period at 4°C, exosome-containing samples that are treated with a PEG solution precipitate. Filtration or centrifugation can then be used to recover or isolate the precipitated exosomes. This method is comparatively easy to use, requires little downtime, and doesn't require sophisticated equipment [36].

Magnetic separation

Clayton et al. used immuno-magnetic isolation of exosomes expressing human primary histocompatibility complex class II molecules to suggest a quick and effective technique for routine exosome separation and exploration [30]. This technique makes use of magnetic beads coated with antibodies that are particular to the proteins found on the surface of exosomes. There are numerous benefits linked to this strategy. For example, the bead-exosome complexes can be analyzed using flow cytometry in conjunction with fluorophore-conjugated antibodies, allowing for a rapid and quantitative evaluation of the exosome surface composition [37].

Dielectrophoretic separation

The dielectric force acting on polarized particles in a non-uniform electric field is the basis for DEP separation. Larger objects like cells are drawn to the low-field zones between the electrodes, whereas nanoscale particles are drawn to the high-field zones close to the edges of circular microelectrodes. However, the DEP field has little influence on cations, anions, and small biomolecules. The dielectric characteristics of the surrounding fluid and the nanoparticles differ, which results in the DEP force [38]. The rate at which charges flow through a material in response to variations in the external electric field is determined by the dielectric constant. Transient dipoles are created across the nanoparticles as the fluid's charges and the nanoparticles' realignment rates diverge. The forces produced by these dipoles draw the nanoparticles into the non-uniform electric field's DEP high-field zones [39]. Although the technique's use may be restricted by electrothermal heating, its efficiency and speed are significant benefits.

Microfluidic devices

Even with small sample volumes, exosome separation and purification can be automated thanks to microfluidic devices [40]. EVs and their subtypes can be quickly and precisely purified with these technologies. These devices combine features including sieving barriers, mixing chambers, and antibody-coated functionalized layers to make it easier to separate various EV subclasses. The majority of these separation techniques rely on affinity [41]. Because microfluidic devices can handle small volumes (μL), they are very helpful as diagnostic instruments. They can, for example, enhance cell-specific EVs associated with particular illnesses. While they show great promise in diagnostics, these devices face significant challenges for large-scale applications due to issues such as the lack of standardization and the absence of consistent benchmarking, limiting their scalability in broader diagnostic contexts [42]. The principles, advantages, and disadvantages of different exosome isolation methods are summarized in the accompanying **Table 1**.

Drug loading methods of exosomes

The lipid bilayer membrane of exosome vesicles acts as a natural

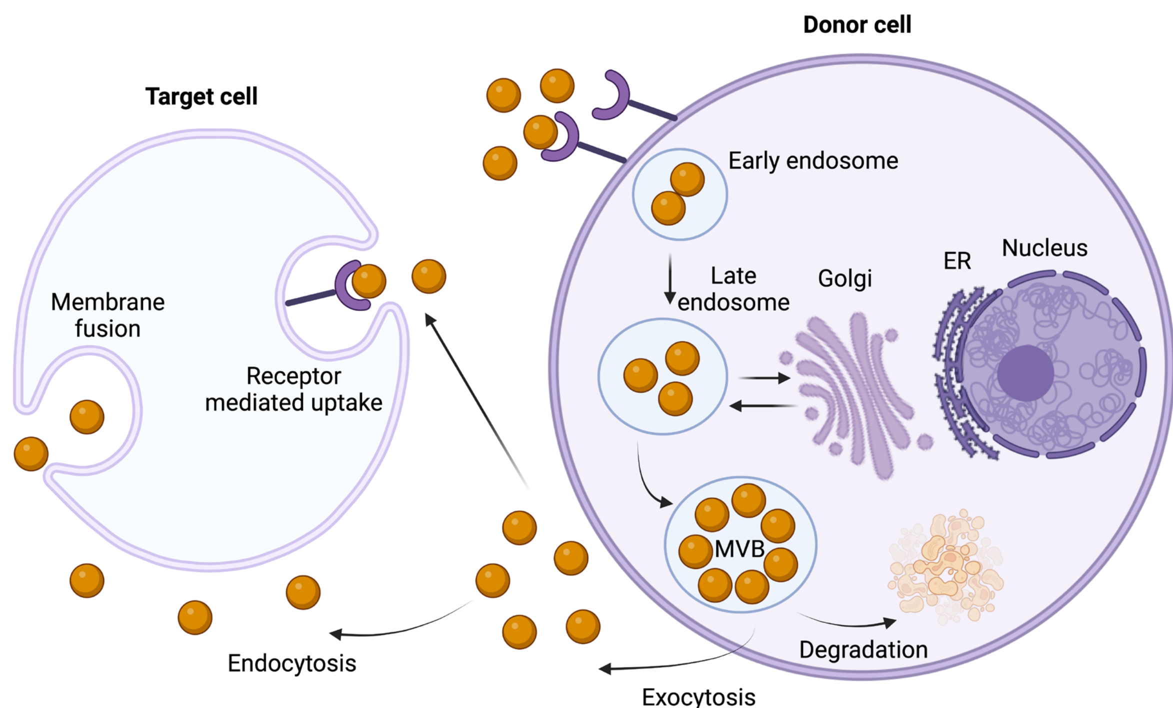

Figure 2. The synthesis, release, and absorption of exosomes are depicted in this figure. When early endosomes develop into multivesicular bodies (MVBs) with intraluminal vesicles holding RNA, DNA, proteins, lipids, and other macromolecules, they give rise to exosomes. Exosomes are released into the extracellular environment by exocytosis when MVBs fuse with the plasma membrane. Target cells then absorb these exosomes through endocytosis, membrane fusion, or receptor-mediated uptake, which promotes molecular exchange and intercellular communication.

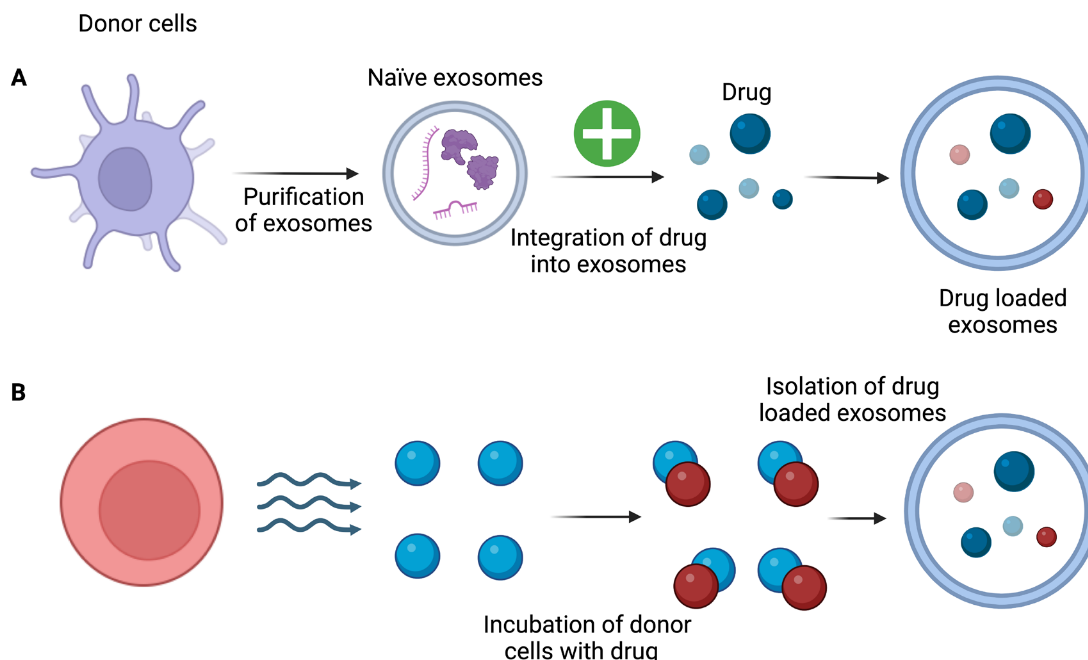

Figure 3. The figure shows the two methods for loading drugs into exosomes. (A) Post-loading strategy: Drugs are added to purified exosomes after they have been separated from donor cells. (B) Pre-loading strategy: Drugs are added to donor cells during incubation, which permits drug uptake and packing into exosomes during exosome biogenesis. Drug-loaded exosomes are then isolated.

barrier, protecting their contents from degradation during circulation in the bloodstream. However, this membrane, along with the internal components of exosomes, complicates the process of loading drugs into these vesicles. Typically, drugs can be incorporated into exosomes using either active or passive loading techniques [43]. Active loading, also referred to as remote or postdrug loading, involves incubating the drug with isolated exosomes. Passive loading, or preloading, entails the secretion of drug-loaded exosomes from cells that have been pretreated to sort the drug into the vesicles. Drugs do not need to be added directly to the exosome vesicles to use this strategy. Because of the active pumping mechanism, active loading is typically more functional in reaching a greater drug-to-vesicle ratio. For hydrophobic medications, the postloading method is generally more successful than for hydrophilic ones [44]. **Figure 3** shows a variety of drug-loading techniques for exosomes, and **Table 2** lists the benefits and drawbacks of each technique.

Incubation of drugs with exosomes

By incubating the drug with exosomes, this method often called passive drug loading allows the medication to diffuse into the exosomes following the concentration gradient. Given that hydrophobic medications are more likely to engage with the vesicle's lipid bilayer, the effectiveness of drug loading with this technique is directly correlated with the hydrophobicity of the drug molecules [44]. In one investigation, Dongmel et al. treated mouse lymphoma exosomes with curcumin in PBS for five minutes at 22°C. A sucrose gradient was then used to centrifuge the mixture. When compared to its free form, curcumin's solubility, stability, and bioavailability were all increased by this encapsulating technique [45]. Similarly, when curcumin was incubated with exosomes, Vashisht et al. [46] showed a 70.46% drug loading efficiency. Catalase was also encapsulated into exosomes by an 18-hour incubation period in PBS at room temperature. Nonetheless, this approach's comparatively poor drug-loading capacity is a major drawback [47].

Incubation of drugs with donor cells

This technique involves administering a medication to the target donor cells, which enables the cells to accumulate therapeutic or bioactive substances. The therapeutic chemicals can then be transported by the exosomes secreted by these pretreated cells. This method aims to deliver the therapeutic compounds into the exosomes after they have been absorbed and packaged by the donor cells. However, this strategy can result in a reduced exosome yield because it is untargeted [44]. A modest dose of paclitaxel was administered to SR4987 mesenchymal stromal cells for 24 hours in a study conducted by Pascucci et al. [48]. Following a wash, the cells were moved to a fresh culture flask containing new media. The paclitaxel-loaded exosomes were separated from the conditioned media after 48 hours of culture. To promote the release of drug-loaded exosomes, donor cells can also undergo mechanical or biological treatments, such as exposure to heat, UV light, or a combination of these [40, 49].

Table 1. Principles, advantages and disadvantages of exosome separation methods.

Method	Principle	Advantages	Disadvantages	Reference
Ultrafiltration	Separation is based on molecular weight and size	Faster, requires no special equipment	Exosome clogging, deformation, and damage to large EVs	[66]
Immunological Separation	Exosomes are captured via an antigen-antibody response	Saves time, isolates of high purity, and a simple process	poor capacity, and yield, non-physiological salt and pH conditions are required	[67]
Ultracentrifugation	Sedimentation coefficient of exosomes and other substances in the sample	Exosomes may be produced in enormous quantities, with great separation purity	Time required (>4 h), low recovery rate (5–25%), and poor reproducibility	[66]
Size-Exclusion Chromatography	Utilizes a column of porous polymeric beads to separate exosomes based on size	High yield and purity	Expensive, time-consuming post-isolation analysis and column contamination	[68]
Polymer-based precipitation separation	Hydrophobicity	Simple procedure with a small sample volume	Long run periods and post-separation cleaning are required	[69]
Magnetic separation	Magnetic Force	The contactless separation, high specificity, and high throughput	Magnetic labeling	[70]
Dielectrophoretic separation	Polarized particle's size and electric properties	Label-free, contactless, fast, and high-throughput	Low resolution, low purity, Joule and electrothermal heating problems	[71]
Microfluidic devices	Separation based on size, charge, surface properties and interactions	Fast, High precision	Non-scalability on large-scale diagnostics	[72]

Active drug loading approaches

By momentarily rupturing the exosome membrane, active drug loading facilitates the drug's easier diffusion into the vesicles. The exosome membrane is repaired to preserve its integrity after the intended payload has been loaded. The exosome membrane can be broken by a variety of techniques, such as sonication, extrusion, and freeze-thaw cycles [43]. The active approach can boost the drug-loading capacity of exosome vesicles by up to 11 times compared to passive drug loading [50]. The possibility of harming the exosome's original structure and targeting capabilities during the disruption process is a significant obstacle to this strategy, but [43].

Sonication

After combining the medication or protein of interest with exosomes generated from donor or target cells, a homogenizer probe is employed to sonicate the mixture. The exosome membrane is broken by the mechanical shear force which also deforms the membrane and permits the diffusion of bioactive substances into the vesicle [51]. After sonication, Kim et al. [52] found that the exosome membrane's microviscosity significantly decreased. However, the exosome's lipid contents and membrane-bound proteins are not significantly impacted by this deformation procedure. They discovered that an hour of incubation at 37°C might restore the integrity of the exosome membrane. Furthermore, when medications are both contained inside and connected to the outside membrane of exosomes, biphasic drug release may occasionally happen. A burst release occurs when the medication attaches to the outside membrane of the exosome, whereas a gradual release occurs when the drug is encapsulated inside [52].

Extrusion

Extrusion is a post loading technique that loads medications into exosomes using a lipid extruder based on a syringe. Donor cell exosomes are combined with a target medication and put into a syringe-based lipid extruder, which is kept at a regulated temperature and contains a membrane with pores that range in size from 100 to 400 nm. The medication and the damaged exosome membrane are well combined during extrusion [53]. The benefits of using the extrusion technique to load medications into exosomes were emphasized by Fuhrmann et al. [50]. Porphyrin was extruded into exosomes made from MDA-MB231 breast cancer cells for their investigation. When compared to the incubation procedure, this method produced a higher cytotoxic effect. Furthermore, the extrusion process modifies the exosomes' zeta potential, and by changing the makeup of the vesicles, increasing the number of extrusions during an intense extrusion procedure can improve drug loading even more.

Freeze-thaw cycles

Exosomes are first incubated with the target drug at ambient temperature for a certain amount of time, and then they are quickly frozen at -80 °C or in liquid nitrogen as part of the freeze-thaw process for drug loading. After that, the mixture is allowed to defrost at room temperature. Freeze-thaw cycles are usually performed at least three times to improve medication encapsulation. However, in comparison to sonication or extrusion procedures, this technique often has a lesser drug-loading

Table 2. Advantages and disadvantages of different exosome drug loading approaches.

Drug Loading Approach	Principle	Advantages	Disadvantages
Incubation of exosomes and free drugs/ Incubation of the donor cells with free drugs	Diffusion of cargo into a cell or exosomal membrane	Simple operation	Loading efficiency; Drugs may cause cytotoxicity to the donor cells
Sonication	Creation of micropores for diffusion by mechanical shear force	Higher loading capacity than the simple incubation method	Sonication-induced membrane damage is a roadblock for large-scale application
Extrusion	Membrane recombination	High cargo loading efficiency. Repeated extrusion provides a homogeneous blend of exosomes with cargoe	Recombination of exosomal surface structure may compromise the immune-privileged status of exosomes, making exosomes visible to immune cells such as mononuclear phagocytes
Freeze-thaw cycles	Membrane fusion	A simple and effective strategy to load various cargoes into exosomes directly	Repeated freeze-thaw may cause protein degeneration and exosome aggregation
Electroporation	Creation of micropores for diffusion by the electric field	High loading efficiency	The loading efficiency and aggregation of cargo are major limitations
Incubation with membrane permeabilizes	Dissolves membrane molecules, creates pores on the exosomal surface	Higher loading capacity as compared with the simple incubation method	An extra purification process may be required to remove saponin

Note: Reference (47, 56).

potential. Furthermore, it might result in exosome aggregation, which would cause the drug-loaded exosomes to have a larger size distribution [54].

Electroporation

Through the disruption of the phospholipid bilayer and the creation of pores in it, electroporation uses an electromagnetic field to encourage the passage of medicinal molecules into the exosome lumen [55]. Drug molecules permeate the exosome membrane's pores during this process, and following loading, the membrane's integrity is restored. This method is frequently used to load big molecules into exosomes, like nucleotides (like siRNA or miRNA) [50]. However, because of problems such as RNA aggregation and exosome instability, electroporation often has a reduced loading capacity. Despite this, the technique can increase the incorporation of hydrophilic small molecules into exosomes and substantially raise RNA loading [50].

Incubation with membrane permeabilizers

The cholesterol in the cell membrane interacts with membrane permeabilizers and surfactants, including saponin, to create pores that make the exosomal membrane more permeable. The membrane permeability technique can increase the effectiveness of catalase loading into exosomes in comparison to the incubation approach [56]. In comparison to the passive loading technique without saponin, a previous study showed that the use of saponin increased the loading of hydrophilic medicines into exosomes by 11-fold [50]. Using the right amount of saponin for drug loading is crucial in this method, and purification of the exosomes is necessary following the saponin incubation.

Exosomes current state and potential future

Exosomes and their possible uses are currently the subject of a large number of scholarly papers, as well as an increase in clinical trials investigating their potential as diagnostic and therapeutic tools. Exosomes may be the basis for creating novel drug delivery systems if the findings of these investigations are encouraging. To fulfill future market demand, Aruna Biocompany has revealed its plan to commercialize pharmaceutical exosomes for CNS-specific drug delivery. The business is currently searching for options for effective, large-scale exosome production. AB126, a novel neural-derived exosome from ArunaBio, has been manifested to cross the blood-brain barrier (BBB). Both intrinsic therapeutic qualities like neuroprotection, anti-neuroinflammation, and neuro-regeneration, as well as functional qualities like neural cell targeting, are advantages of this exosome. It can pass the BBB to deliver proteins, siRNAs, allele-specific oligonucleotides, and monoclonal antibodies [57].

ExoSTING, an antigen-presenting cell-targeting agent created by Codiak, distributes interferon gene stimulators within the lumen of exosomes. It has been demonstrated that this drug maintains memory responses against tumor cells. Furthermore, Codiak has created an exosome product called exoASOTM-STAT6 that specifically targets tumor macrophages [58]. Exosomal topical solution for diabetic ulcers and chronic wounds, as well as exosomal eye serum for glaucoma and age-related macular degeneration, are developments for Vittilabs. Exosomes have been shown in numerous studies to be effective ocular pharmacotherapeutics for a variety of diseases [59].

To enable tailored delivery to particular organs, the Evox Therapeutics scientific team is working on creating protein engineering methods that load proteins and nucleic acids into exosomes. Along with implementing trade secrets, copyrights, and trademarks to safeguard its assets, Evox has also developed a portfolio of intellectual property that includes its exosome drug

delivery systems (DDS) for mRNA, siRNA, antibodies, receptors, antisense oligonucleotides, enzyme replacement therapies, and CRISPR technologies. Evox also possesses proprietary technology for downstream processing, upstream process optimization, and cellular engineering. Other top businesses, like XOSTEM, TEVAC Pharmaceuticals, OmniSpirant, and ILIAS, are also attempting to resolve the manufacturing and engineering issues required for the repeatable production of transformational exosome medications. Bone diseases, fibrotic conditions, alopecia, heart diseases, skin problems, gastrointestinal disorders, kidney diseases, rare genetic disorders, autism spectrum disorder, neurodegenerative diseases, cancer, diabetes, wound healing, and transplant rejection are just a few of the many illnesses for which exosomes are being studied [57]. The thorough analysis of exosomes highlights that to better understand the diverse ways that exosomes respond to communication between normal and malignant cells, future research should concentrate on filling in the gaps that currently exist, especially in vivo studies. Deciphering exosomal transport pathways and target cell physiological regulation are part of this. Furthermore, to fully evaluate toxicity and direct the therapeutic potential of exosomes, more methodical in vivo research is required. These new nanoplatforms may become closer to clinical use as a result of these investigations. Even at the preclinical level, it is critical to choose and create suitable animal models that also satisfy economic sustainability, even if exosome clinical trials are still in their infancy and have not yet addressed numerous clinical transition issues. In this regard, organ-on-a-chip systems may be a strong and trustworthy instrument for investigating the efficacy, drug delivery potential, and mechanisms of action of exosomes [60].

Animals are not the only things that have exosomes; plants and plant-derived products, such as fruit juices, have also been found to contain them. Grape exosomes, for instance, have demonstrated potential in the treatment of radiation or chemotherapy-induced oral mucositis [61]. Exosomes generated from grapes have been shown in preclinical research to contribute to tissue remodeling following pathological injury and support intestinal tissue regeneration [62]. To treat patients with polycystic ovarian syndrome and lessen insulin resistance and chronic inflammation, another clinical trial is examining the effects of exosomes made from ginger or aloe (NCT03493984). Furthermore, because curcumin is hydrophobic, Donar Millar et al. investigated the utilization of plant-derived exosomes as a hydrophobic drug delivery method to encapsulate the molecule for the treatment of intestinal diseases [63]. To make the exosome isolation procedure simpler, more studies should concentrate on sources that don't include cells or animals.

It has been noted that mesenchymal stem cells or other stem cell types are the source of the majority of exosomes that make it to human clinical trials. To address the increasing demands of cutting-edge research, biobanks devoted to the preservation of stem cells have grown significantly on a global scale. In light of this growing demand, the discipline of biobanking emphasizes the exosome's collection, storage, and dissemination, in addition to the exosome's place of origin. Addressing information gaps about specific processes, transport variables, isolation yields, preclinical parameters, and other elements that are frequently underreported in the literature requires an understanding of the exosome lifecycle. To overcome the present obstacles in exosome research, biobank operations are crucial in bridging the gap between bench research and clinical applications [64]. To guarantee the quality of exosomes in biobanks, however, additional standardized techniques, regulated processes, and accurate data archiving are urgently needed. Monitoring negative reactions when exosomes are released onto the market is equally crucial [65].

Conclusion

Exosomes are increasingly recognized as promising drug delivery systems, offering unique strategies for managing a variety of diseases. Meanwhile, our current understanding of exosomes remains incomplete and, at times, inconsistent, posing challenges to their development for diverse applications. To advance the use of exosomes in research and therapy, there is a dire need to prioritize their large-scale production while maintaining high quality and purity standards to maximize their therapeutic potential. Exosomes, whether naturally derived or modified, hold immense promise as efficient nanocarriers for drug delivery. Their inherent role in cell-to-cell communication equips them with valuable natural delivery properties, making them effective even without modification. At the same time, the creation of engineered exosomes presents exciting opportunities for advancing drug delivery research. Nevertheless, future research is needed to refine the mechanism for characterizing and standardizing the production of both natural and modified exosomes for therapeutic applications. Addressing current limitations will require innovative approaches and new principles to enhance the success rate of translating exosomes into viable medical treatments.

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Ethics approval

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Data availability

The data will be available upon request.

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Authors' contribution

Rahul Choudhury contributed to draft, critical revision of the article, table making, figure production and submitted the final manuscript.

Competing interests

None.

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