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Exosomes as drug delivery systems: A brief overview and progress update

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ABSTRACT

Exosomes are intracellular membrane-based vesicles with diverse compositions that are involved in biological and pathological processes. Since the discovery of exosomes, they have been used as diagnostic biomarkers and as potential drug delivery vehicles based on their size and competence to transfer biological materials to recipient cells. The properties of exosomes such as biocompatibility, preferred tumor homing, adjustable targeting efficiency, and stability make them striking and excellent drug delivery vehicles for use in various diseases and cancer therapy. In this article, we provide a brief overview of the biogenesis, functions, and contents of exosomes along with the separation and characterization techniques. Our major focus is on the recent progress made in application of exosomes as drug delivery systems involving delivery of small molecules, macromolecules, and nucleotides. Further, we discuss the challenges faced when using exosomes as a drug delivery vehicle.

1. Introduction

"Communication is an art which leads to community." The cells in our body communicate, function in unison and are in complete synchrony with each other leading to a cellular community. Cellular communication plays a pivotal role for the cells to work in coordination and to effectively carryout their functions.

Intercellular communication occurs due to the presence of connections such as the gap junctions or by the process of cell signaling. Cell signaling involves the release of chemical mediators or bioactive molecules that alter or regulate other cells in the vicinity [1]. For instance, the hormones and enzymes secreted by a cell produces an effect onto another cell at a distant location. A peculiar mode of intercellular communication with distant cells occurs *via* the extracellular vesicles. These membrane vesicles serve as cargo transporters and are responsible for the transport of lipids, proteins, and genetic material to the recipient cells [2]. Exosomes are a type of extracellular vesicle with size ranging from 30 to 150 nm. The exosomes can enter the cells, release its cargo and mediate physiological and pathological processes. For example, they are capable of regulating the gene expression of the recipient cell *via* the delivery of specific mRNA [3], mediate immune stimulation or repression [4], promote excretion from organs, and the elimination of waste from the brain [5].

The ability of exosomes to mediate certain physiological and pathological processes is exploited to design the delivery of therapeutic agents to a specific target with the aim of mitigating the systemic toxicity. In view of their natural origin, exosomes show little long-term accumulation in any organ or tissue thus, resulting in minimal or no systemic toxicity. Owing to their compatibility with the biological system and their organotropic behavior [6], exosomes are employed as natural drug delivery vehicles to deliver a wide range of therapeutics including genetic material for their inherent ability to transport the therapeutic cargo into the cells [7]. In addition, the low immunogenicity and toxicity make exosomes an ideal candidate for designing novel drug delivery systems.

Currently, liposomes and polymeric nanoparticles are the most preferred drug delivery systems. Liposome, a synthetic vesicle with a phospholipid membrane that self assembles into various shapes and sizes in an aqueous environment. Polymeric nanoparticles, as name suggests uses polymers to help in the entrapment, encapsulation, or adsorption of drug molecules. Both these drug delivery systems have been used to deliver different drugs including proteins and peptides, anti-cancer drugs and analgesics. Still, the ability of liposomal system to invade host immune system along with circulating capability and stability, without toxicity remains incomprehensible. Though polymeric nanoparticles solve the stability issue, their toxicity and biocompatibility remain a major concern, especially when using nonbiodegradable polymers. Exosomal drug delivery system with minimal toxicity, biocompatibility, tissue and tumor targeting, and long circulating half-life emerges to be a superior choice, overcoming the shortcomings of liposomes or polymeric nanoparticles.

In the last decade, there have been numerous publications exploring the potential of exosomes as drug carriers. Fig. 1 below shows the number of publications published per year, as per Pubmed, containing

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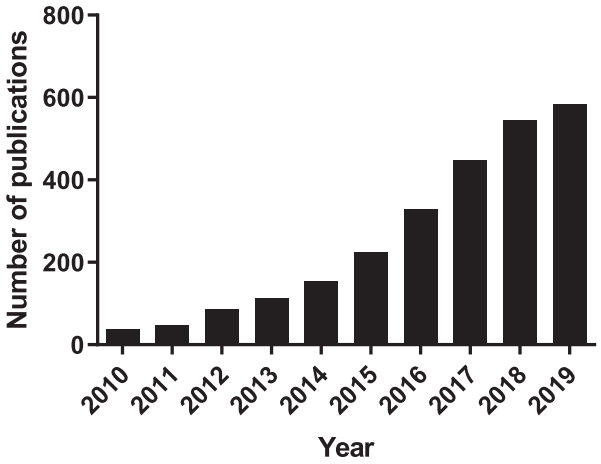

Fig. 1. Number of publications containing the search terms 'exosome' and 'drug delivery', obtained from Pubmed.

the term 'exosomes' and 'drug delivery'. In this review, we present a brief overview of exosomes, source, isolation, purification, and characterization. Further, we will focus on the recent progress made in exosome-based drug delivery.

2. Source/biogenesis of exosomes

The exosomes originate from the endosomal system. The early endosomes mature to form late endosomes, also known as multivesicular bodies (MVBs). The inward budding of the MVBs produces intraluminal vesicles (ILVs) in the lumen of organelles. The ILVs are also known as exosomes. The MVBs fuse with the plasma membrane and release exosomes into the extracellular environment (Fig. 2) [2,8,9].

The extravascular vesicles released may be classified into three types based on their origin and size: (a) Exosome (diameter in the range 30–150 nm), (b) Microvesicles (50 nm–1 μm), and (c) Apoptotic body (50 nm–5 μm). The exosome subpopulation further has large exosomes (Exo-L; 90–120 nm), small exosomes (Exo-S; 60–80 nm), and exomeres

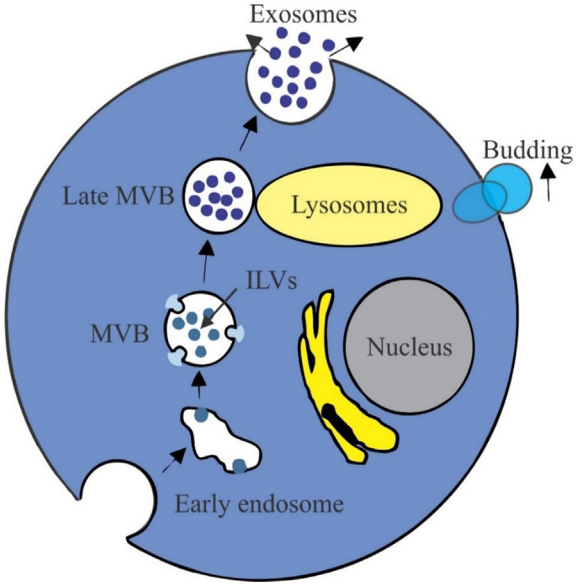

Fig. 2. Formation of exosomes. Exosomes are derived from the endosome formed from plasma membrane. As early endosome matures into the late endosomes, inward budding occurs and forms multivesicular bodies (MVB) containing numerous intraluminal vesicles (ILV). The MVBs either get degraded by lysosomes or fuse with the plasma membrane to release ILVs called exosomes. Adapted from [2]

(~35 nm) [9].

Exosomes are membrane derived, homogenous vesicles having multiple origins and can be classified as ectosomes (exosomes secreted by neutrophils or monocytes) or cardiosomes (exosomes produced by cardiomyocytes) [1]. The released exosomes generally loaded with genetic and proteomic information acts as a cargo transporter by three mechanisms. Firstly, ligand receptor binding – the exosomes activate the target cells by binding with specific ligands on their receptors. Secondly, the exosomes may transfer surface receptors from one cell to another target cell by budding followed by fusion with the plasma membrane. The third mechanism involves horizontal transfer of cytosolic contents from donor cell to the target cell by fusion of the membrane or internalization [10]. The exosome uptake by a recipient cell is highly cell-specific and the specificity depends on the interaction between the surface molecules of the recipient cell and the exosomes. This attributes to the cell targeting and adhesion property of the exosomes.

3. Contents of exosomes

Since exosomes are derived from endosomes, they have intercellular material derived from the parent cells. Exosomes have wide array of constituents such as surface proteins, heat shock proteins, lysosomal proteins, tumor sensitive gene, fusion proteins and nucleic acids, each exhibiting certain functions (Table 1). The lipid bilayer of the exosome is rich in cholesterol and diacylglycerol [6]. Lipids such as sphingomyelin and monosialo tetrahexosylganglioside (GM3) determine the rigidity of the exosomes. In addition, the different types of phospholipid transportation enzymes in exosomes are expressed by phosphatidylserine [11]. Other functions of lipids include docking outer proteins and fusion of exosome to plasma membrane and signaling [12]. Prostaglandins are reported to be involved in the biological responses and inflammatory processes [13].

Proteins in exosomes exhibit a distinct function in biogenesis and transport mechanisms. The intracellular assembly and trafficking in

Table 1
Composition of exosomes, functions of components and examples [14].

Composition	Function	Example
Lipids and Metabolites	Vesicle formation	Glycosphingolipids
	Biogenesis	Monosialotetrahexosylganglioside (GM3)
	Release & Interaction with target cells	Sphingomyelin
	Pathophysiological conditions	Cholesterol
	Inflammatory processes	Phosphatidylserine
	Rigidity	Prostaglandins
		Glycerophospholipids
		Organic acids, alcohols, steroids, phenols and amino acids conjugates, sugar and conjugates
Proteins	Transporters	ATP7A, ATP7B, MRP2, SLC1A4, SLC16A1, CLIC1
	Receptors	CD46, CD55
	Heat Shock Proteins	Hsc70, Hsp70, Hsp90
	Tetraspanins	CD9, CD81, CD82
	Metabolic enzymes	GAPDH, Pyruvate
	Antigen Presentation Proteins	HLA Class I & II, Peptide complexes
	Lysosomal markers	CD63, Lysosome membrane protein 2
	Membrane Adhesion Proteins	Integrins
Nucleic Acids	Mediator of horizontal transfer of genetic information	mRNA
	Gene regulation	Non-Coding RNA
	Target Cells	miRNA
	Gene Silencing	
	Carcinogenesis and cancer progression	Long Non-Coding RNAs

exosomes are carried out by heat shock proteins (Hsc70, Hsp70, Hsp90). Tetraspanins (CD9, CD81, CD82) mediate signaling, cell fusion, and migration. Membrane adhesion proteins and integrins adhere the vesicles to their target cells. Transporters (ATP7A, ATP7B, MRP2, SLC1A4, SLC16A1, CLIC1) and receptor (CD46, CD55) proteins carry out their function of communication and cargo transport [14–16].

Exosomes contains various nucleic acids to carry out a definite purpose. Messenger RNA (mRNA) is the mediator of horizontal transfer of genetic information in exosomes [17]; whereas micro RNA (miRNA) serves the function of cell targeting and gene silencing [18]. Exosomes also have noncoding RNA out of which shorter ones regulate the gene [19] and long non-coding RNAs are involved in carcinogenesis and cancer progression [20]. Circulating DNA (cDNA), a heterogenous population of genomic and mitochondrial DNA, contains genetic alterations and reflects mutations, rearrangements and amplifications in tumor tissues [21]. Table 1 lists the different components of exosomes and their functions.

The natural exosomal cargo such as lipids, metabolites, functional proteins, and nucleic acids (mRNAs, miRNAs, DNA) play an important role in inter and intracellular communication and shows the possibility for using exosomes as drug delivery systems to deliver therapeutic drugs. However, in order to use exosomes as biomarkers and drug delivery systems, their isolation, purification, and characterization is extremely important and can be improved by innovative technologies.

4. Isolation & separation

Exosomes are always associated with cellular components and hence, isolation of exosomes becomes vital to understand their mechanism and application in biologics. Many methods have been developed to facilitate the isolation of exosomes from body fluids. However, similar morphology to that of other extracellular vesicles, overlapping size range, and exceedingly smaller size makes it difficult to isolate exosomes. To achieve high recovery rate, high purity and high throughput separation, researchers have implemented various methods and each method has its advantages and disadvantages that are discussed below (Table 2).

4.1. Ultracentrifugation-based separation

The most common isolation technique is centrifugation. Ultracentrifugation is based on the sedimentation coefficient difference between exosomes and other extracellular content. Ultracentrifugation can be further classified as differential ultracentrifugation and gradient density ultracentrifugation. Differential ultracentrifugation involves sequential differential velocity centrifugation with low-speed, high-speed, followed by ultracentrifugation. At low-speed centrifugation, the cell debris is removed and the cells are separated. At high-speed centrifugation, large extracellular vesicles, apoptotic bodies, and microvesicles are separated and removed. Finally using the ultracentrifugation, exosomes are sedimented and collected. However, this method has low purity of separation that can be overcome by gradient ultracentrifugation. This method uses two or more different gels or solutions of different densities. The EVs are separated according to their densities and hence, achieve high separation rate. However, this method is time consuming since it takes a while to attain equilibrium of solutions [22,23].

4.2. Immunological separation

The principle of immunological separation is based on the antigen-antibody reaction to capture exosomes. This method exploits the presence of various proteins on exosomes membrane to capture them. Recent studies involve antibody-coated plates, chromatography matrices and beads for immunological separation with high purity and less time consumption. It is an expensive method as it involves special reagents and cell free samples and limits its use in large scale samples [22,24].

4.3. Ultrafiltration

Ultrafiltration is a membrane separation technique that works on the principle of separation based on size and molecular weight of exosomes and other contents. Exosomes can be separated from macromolecules using membranes containing pores equivalent to exosomes size of 100 nm so that it passes through and other contents are retained on the membrane. Ultrafiltration is often applied in combination with ultracentrifugation to decrease the process time and ease of operation.

Table 2
Advantages and disadvantages of different isolation methods for exosomes.

Method	Principle	Advantages	Disadvantages
Ultracentrifugation	Sedimentation coefficient of exosomes and other substances in sample	Can produce large number of exosomes, high separation purity	Unsuitable for clinical diagnosis due to time required (> 4h), low recovery rate (5–25%), and poor repeatability
Immunological Separation	Antigen-antibody reaction to capture exosomes	Time-saving method, can directly separate from body fluids, high-purity isolation, simple procedure	The requirements of nonphysiological salt and pH conditions, costly reagents, cannot be used for large scale exosome separation
Ultrafiltration	Size and molecular weight	Simpler and faster procedure, lack of special equipment	Clogging and trapping of exosomes in filters results in low recovery rate, deformation and damage of larger EVs due to force to drive through filters
Size-Exclusion Chromatography	Pore size of gel and size of exosome	High purity and yield	Expensive, column contamination, time consuming post-isolation analysis
Polymer-based Precipitation Separation	Hydrophobicity	Small sample volume and simplicity of operation	Low specificity and high cost
Magnetic Separation	Magnetic Force	Contactless, high specificity and high-throughput separation	Magnetic labelling
Acoustic Fluid Separation	Size-based separation	Label-free, contactless and fast technique	Not widely applied for separation yet
Dielectrophoretic (DEP) Separation	Size and electric properties of polarized particle	Label-free, contactless, rapid and high-throughput characteristics	Low resolution, low purity, drawbacks of Joule and electrothermal heating
Deterministic Lateral Displacement (DLD) Separation	Critical size for particle separation	Label-free, easy to use	Low separation purity, clogging

But, multiple membrane washing steps will significantly increase the process time, and the possibility of clogging of membrane pore limits the use of this process [25].

4.4. Size exclusion chromatography

Size exclusion chromatography separation is based on the relative pore size of gel and that of the sample to separate under gravitational force. It preserves the biological activity and integrity of exosomes as no external driving force is used for separation. It is an expensive method to implement but has high purity and yield [25].

4.5. Polymer-based precipitation separation

The polymer-based precipitation separation method employs the hydrophobicity of exosomal membrane and other water-soluble compounds to bind water molecules and precipitate exosomes. Polyethylene Glycol (PEG) method is widely used for exosome separation. Separation kits are available commercially which requires small sample volume but possess low specificity [26].

4.6. Magnetic separation

Magnetic separation requires antibody-modified magnetic beads (magnetic labelling) to capture and separate exosomes. This method is mostly used owing to its contactless, high-throughput and specific separation. The exosomes are retained by immunomagnetic beads while other contents are washed away by PBS. The antibody-labelled magnetic beads are lysed, captured and analyzed in the chamber [27].

4.7. Acoustic fluid separation

Acoustic fluid separation is another method that is based on the principle of size-based separation. In acoustics, particles are subjected to different acoustic force depending on their size and can be separated accordingly. It is a contactless and label-free technique, but needs to be validated and exploited for different sample separation [28].

4.8. Dielectrophoretic (DEP) separation

Dielectrophoretic separation works on the principle of polarized particles experiencing dielectric force in a nonuniform electric field. The magnitude of the forces exerted on particles *via* DEP is dependent on the cells' intrinsic dielectric properties and size as well as applied electric field magnitude and frequency. Exosomes, a nano-sized particle are attracted towards the high electric field regions while larger particles migrate towards low electric field. This method needs electrothermal heating which limits its use but can be explored for positives of rapid and high-throughput characteristics [29,30].

4.9. Deterministic Lateral Displacement (DLD) separation

Devices or chips that facilitate DLD separation have specific critical sizes for particle separation. The principle is that the flow path of particles greater than the critical size will be changed and rest will flow unaltered. Though it is easy to use and is label free, researchers are facing challenges on separation and clogging [31,32].

5. Characterization techniques

Characterization of exosomes is essential to understand the exosomal properties and functions along with size range, structure, and surface proteins as they influence drug loading and delivery. The conventional exosomes characterization techniques can detect the morphology, size, quantity, and surface protein contents. However, the conventional exosome characterization techniques still have shortcomings, such as complicated equipment, low sensitivity, and high reagent consumption. These conventional exosome detection techniques are classified as optical methods namely dynamic light scattering and nanoparticle tracking analysis whereas non-optical methods include transmission electron microscopy, atomic force microscopy and enzyme-linked immunosorbent assay. Fortunately, with the rapid development of microfluidics detection techniques, we can achieve high throughput with high precision and low reagent consumption. Microfluidics-based exosome detection techniques include fluorescence correlation microscopy (FCM), colorimetric detection, surface plasmon resonance (SPR) detection, and nuclear magnetic resonance (NMR) detection.[33]. Listed below are some of the common techniques used in the characterization of exosomes.

5.1. Dynamic Light Scattering (DLS)

Dynamic Light Scattering (DLS), known as photon coherence spectroscopy, is a physical characterization method used to measure particle size and distribution in suspension. DLS works on the principle of light scattering by particles detected by a camera and optical signal is analyzed to detect the particles. This technique is used to determine the size of exosomes but cannot be employed to obtain source information. If sample contains uniform exosomes, this method can be used to obtain accurate size information. However, limitation of this method is for samples with different particles sizes, larger particles interfere with small particles detection and thus obtaining size distribution is not ideal using this method [34,35].

5.2. Nanoparticle Tracking Analysis (NTA)

Nanoparticle Tracking Analysis (NTA) is combination of light scattering and Brownian motion of particles to analyze the particle size of a sample. It tracks the Brownian motion of particles individually and simultaneously estimates the hydrodynamic diameters. It can detect different sizes in the samples as each particle is imaged in different regions. Also, NTA can analyze fluorescence and hence can detect the antigen present on exosomes by fluorescence labelling [36,37].

5.3. Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy (TEM) uses accelerated electron beam to determine the structure and morphology of particles. TEM gives the image of exosomes and hence size can be measured from it. However, morphology of exosomes can be affected during TEM sample preparation. Cryo-TEM is upgradation which eliminates sample preparation effects [38,39].

5.4. Atomic Force Microscopy (AFM)

Atomic Force Microscopy (AFM) is used to measure the relative size distribution and map the mechanical properties of exosomes. AFM uses surface scanning with tip of cantilever beam and provides sub-nanometer resolution imaging. AFM can be used to quantify and detect the structure, biomechanics and abundance simultaneously [40].

5.5. Enzyme-linked Immunosorbent Assay (ELISA)

ELISA is plate-based assay for detection and quantification of peptides, proteins, antibodies and hormones. Recently, it has been used to detect exosomes but requires large volumes of samples and has low sensitivity. Immunosorbent assay using ELISA has been developed which gives absolute cancer exosome count with high accuracy [41].

5.6. Fluorescence Correlation Microscopy (FCM)

Microfluidics-based Fluorescence Correlation Microscopy (FCM)

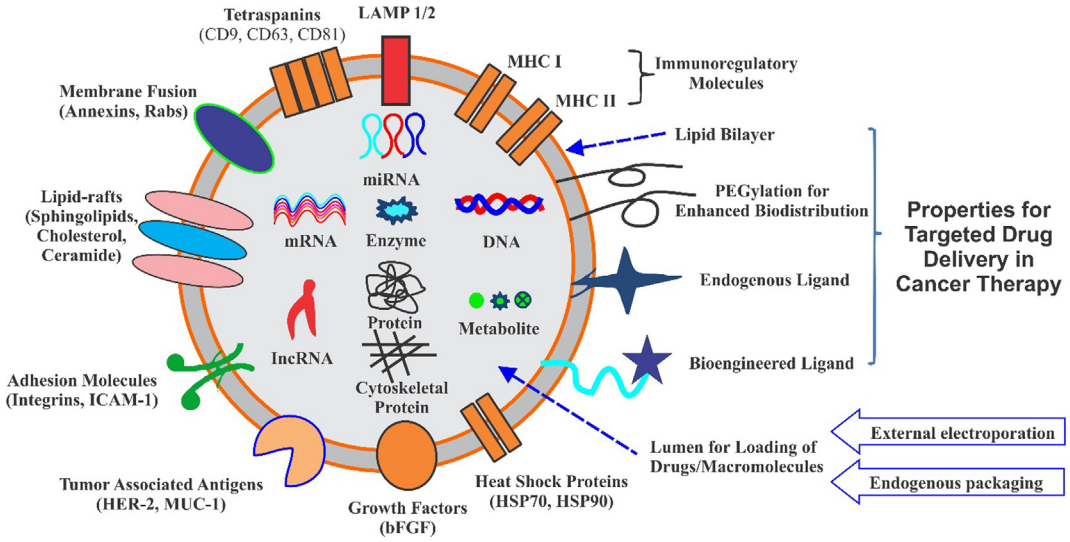

Fig. 3. Schematic of an exosomes showing different internal components and membrane proteins. The internal components are either part of the exosome since their origin or can be loaded into exosomes using different methods. Several membrane proteins are expressed on the surface of exosomes, and additionally functional groups such as polyethylene glycol (PEG), specific ligands can be expressed to obtain a targeted exosome.
Adapted from [3]

uses specific antibody to capture exosomes, captured exosomes are stained with fluorescent dye and are quantified using plate reader. Microfluidic chip can be fabricated for immunocapture and quantitative analysis of exosomes based on FCM [42].

5.7. Colorimetric detection

Colorimetric detection is based on the color depth of a chromogenic substance to detect a sample. It is used in association with microfluidic systems to capture the exosomes or ELISA for detection and quantification of exosomes. Researcher have successfully applied this method in detection of exosomes from cancer cells [43,44].

5.8. Surface Plasmon Resonance (SPR) detection

Surface Plasmon Resonance (SPR) is based on resonant oscillation between conduction electrons at the interface between negative dielectric constant material and a positive dielectric constant material. To enhance the detection performance, a nano plasmonic exosome (nPLEX) made by optimizing a nano substrate is used to detect exosomes. Based on nPLEX chip, we will be able to functionalize each nano pore with antibodies so that distinct nanopores can bind certain exosomes. It detects various exosomes and quantifies multiple exosomal proteins by microfluidics based SPR chip [45,46].

5.9. Nuclear Magnetic Resonance (NMR) detection

Nuclear Magnetic Resonance (NMR) is widely used in chemical analysis. However, researchers have developed microNMR systems to quantify the number and the proteins present in exosomes. Systems captures the microvesicles with immunomagnetic nanoparticles and can be used to detect the exosomes after concentrating it by filtration. Signal-to-noise ratio is used to estimate the number of exosomes [47,48].

6. Exosomes as drug delivery system

For exosomes to be effectively used as drug delivery systems, therapeutic agents need to be efficiently loaded into the exosomes. Therapeutic agents are incorporated into exosomes by two key approaches: active or passive loading/encapsulation [49–51]. Passive drug loading methods are relatively simple and straightforward. Passive loading can be achieved either by incubation of drug with exosomes or incubation of drug with donor cells. In incubation with exosomes, the drugs diffuse into the exosomes based on concentration gradient whereas in incubation with donor cells, the cells are initially treated with drugs and these cells later secrete exosomes loaded with the drug. Active drug loading method offers enhanced drug loading efficiency and facilitates loading of large molecules. Active drug loading can be achieved either by sonication, extrusion, electroporation, or drug conjugation techniques [52]. In the sonication method, the membrane integrity of exosomes is compromised to allow the drug to diffuse into exosomes without affecting membrane-bound proteins. Similarly, extrusion also disrupts membrane diffusion that is enabled by syringe-based lipid extruder. In the electroporation method, the exosomes are suspended in a conductive solution and subjected to an electric field. The electric field disrupts the phospholipid bilayer and introduces temporary small pores through which the drug diffuses into the exosomes. Alternatively, drugs can be conjugated to the surface of exosomes *via* click chemistry or antibody binding. In click chemistry, the drug molecules are directly attached to the surfaces of exosomes *via* covalent bonds whereas in antibody binding highly specific antibodies are used to bind to a specific antigen on the exosome surface. The above-mentioned drug loading methods all produce different loading capacities and the choice to be employed is dependent on the properties of the cargo such as hydrophilicity, lipophilicity, and molecular weight. Also, apart from the loading efficiency, exosome membrane integrity and stability are of utmost importance in drug delivery [50–52]. Fig. 3 below shows a schematic of exosomes with different components that can be loaded into the exosome using the different methods discussed above. We will briefly discuss recent research articles that employed various approaches to load and deliver drugs using exosomes as drug delivery systems. Table 3 below lists recent research publications wherein exosomes were used as drug delivery vehicles against different diseases.

6.1. Small molecules

Extensive research has been done using exosomes as a vehicle for therapeutic drug delivery. Particularly, exosomes isolated from cancer cells were loaded with anti-cancer agents and explored for their potential as anti-cancer drug delivery systems. Doxorubicin (Dox) is an

Table 3
Summary of recently published papers wherein exosomes were used as drug delivery systems.

Drug/Biologics	Cancer/Disease Type	Size/Charge	Source of Exosomes	Isolation/Purification Method	Animal Studies/Cell Culture	Reference
Small molecules						
Doxorubicin	Colon cancer	85.1 ± 1.5 nm	Colon cancer cells (LIM1215)	Ultracentrifugation	Colon cancer cells (LIM1215), Abelson murine leukemia virus-induced tumor (RAW 264.7); Male BALB/c mice: Xenograft model mice, distribution study, anti-tumor efficacy, histological analysis	[54]
	Breast cancer	100 nm	Human embryonic kidney cells (HEK293)	Centrifugation; Filtration	HER2-positive cells (SKBR3); Female BALB/c mice: immunohistochemistry, distribution, mouse xenograft	[55,56]
	Hepatocarcinoma, Breast cancer	260 ± 15 nm	Hepatoma cell (Bel7402), Melanoma cells (B16-F10)	Differential ultracentrifugation	Murine hepatocarcinoma cell (H22), mouse breast cancer cells (4T1) and human hepatocarcinoma cells (Bel7402); Male/female BALB/c mice and male C57BL/6 mice: lung metastasis cancer model, Biodistribution, tumor penetration, DOX accumulation, anti-cancer activity	[57]
Paclitaxel	Pulmonary metastases	304.5 ± 3.9 nm/−4.4 ± 0.1 mV	Murine macrophage cells (RAW 264.7)	Size exclusion chromatography	Cellosaurus cell line (3LL-M27); C57BL/6 mice: pulmonary metastases, biodistribution, therapeutic efficacy	[58]
	Breast cancer	150 nm	Murine macrophage cells (RAW 264.7)	Ultracentrifugation	Murine breast cancer cells (4T1); BALB/c mice: xenograft tumors, H&E staining	[59]
		149 nm	Mesenchymal stem cells	Differential ultracentrifugation, filtration	Breast cancer cells (MDA-MB-231 and MCF-7); BALB/c mice: Fluc activity	[60,62]
	Glioblastoma Therapy	75 nm	Embryonic stem cells (H9)	Filtration, ultracentrifugation	HCC cells (HepG2), melanoma cells (B16), breast cancer cells (MDA-MB-231), and prostatic cancer cells (DU145); Athymic nude mice: Subcutaneous Xenograft and Orthotopic Glioma- Bearing Nude Mouse Model, biodistribution, histopathological examination	[61]
Gemcitabine	Pancreatic cancer	70–150 nm	Pancreatic cancer cells (Panc-1)	Ultracentrifugation	Pancreatic cancer cells (Panc-1) and lung cancer cells (A549); male BALB/c mice: biodistribution, mouse xenograft tumor model	[64]
Dopamine	Parkinson's disease	70–100 nm	Orbit venous plexus blood	Ultracentrifugation	mouse brain cell (bEnd.3); Kumming mice: distribution in brain, immunohistochemistry	[65]
Biologics						
Trastuzumab emtansine	Breast cancer, Gastric cancer	–	HER2-positive SKBR-3 and EFM-192A breast cancer cells	Serial ultracentrifugation	human breast cancer cell line (EFM-192A) and (SKBR-3)	[69]
CRISPR/Cas9	Gene therapy	30–100 nm	Human embryonic kidney cells (HEK293)	Ultracentrifugation	Human embryonic kidney cells (HEK293)	[85]
	Ovarian cancer	100 nm	Human embryonic kidney cells (HEK293), ovarian cancer cell (SKOV3)	Centrifugation, filtration	ovarian cancer cell (SKOV3); female BALB/C nude mice: tumor xenograft, western blotting analysis, immunofluorescence	[86]
siRNA	Pancreatic cancer	107.0 ± 8.2 nm	Human embryonic kidney cells (HEK293)	Differential centrifugation, ultracentrifugation	Pancreatic cancer cells (Panc-1)	[70]
	Breast Cancer	30–130 nm	Human embryonic kidney cells (HEK293)	Sequential centrifugation	Breast cancer cells (MDA-MB-231 and SKBR3)	[71]
		263.71 ± 24.84 nm/−28.63 ± 0.33	Autologous breast cancer cells	Ultracentrifugation	human umbilical vein endothelial cells (HUVEC) and mouse breast cancer (4T1) cells; female BALB/c mice: metastasis model, distribution, efficacy, western blot immunofluorescence	[74]
miRNA	Breast cancer	179.4 ± 65.5 nm/− 14.67 ± 1.53 mV	Macrophages (THP-1)	Sequential centrifugation	Breast cancer cells (MDA-MB-231); female BALB/c mice: xenograft-tumor model, biodistribution, Tumor suppression, Histology and immunohistochemistry analysis	[75]
	Cardiac diseases	104 nm	Human peripheral blood	Centrifugation	Cardiac muscle cells (H9C2); c57BL/6 mice: distribution, western blot, echocardiography	[90]
	Angiogenic therapy	115 nm	Mesenchymal stem cells	Ultracentrifugation	C57BL/6 mice (MCAO/R model)	[82]
	Cartilage and bone regeneration	37 nm	Rabbit bone and human bone marrow mesenchymal stem cells	Centrifugation	human bone marrow mesenchymal stem cells (hBMSCs); Sprague Dawley rats: bone regeneration, immunohistochemical (IHC) staining	[83,84]

anti-cancer agent commonly used to treat a broad range of cancers. However, prolonged exposure to Dox causes significant cytotoxicity and limits its continued use. Schindler et al. have shown that Dox loaded exosomes (Exo-Dox) were readily up taken by cells and re-distributed from the endosomes to nucleus as compared to free Dox and liposomal Dox (Myocet® and Doxil®). Further, the Exo-Dox displayed enhanced *in vitro* potency in multiple cell lines including human embryonic kidney cells (HEK293), human umbilical vein endothelial cell (HUVEC), and hiPS cardiomyocytes, compared to free Dox and liposomal Dox [53]. In another recent study by Li et al., antibody-functionalized exosomes for targeted delivery of Dox against colorectal cancer were developed and evaluated *in vivo*. In this study, exosomes were isolated from A33-positive colorectal cancer (LIM1215) cells (A33-Exo) by ultracentrifugation and loaded with Dox. Additionally, super magnetic iron oxide nanoparticles (US) with A33 antibodies were bound to A33-positive exosomes to form complex exosomes (A33Ab-US-Exo/Dox) that target A33-positive colon cancer cells. After administration of the modified exosomes, the results showed good affinity and anti-proliferative effect in LIM1215 cells. *In vivo* studies exhibited excellent tumor targeting ability and tumor inhibition with prolonged survival of mice and reduced cardiotoxicity after intravenous administration. They successfully demonstrated that exosomes functionalized with targeting ligands and high-density antibodies can be an effective approach for cancer targeting [54].

Gomari et al. used mesenchymal stromal cells derived exosomes modified by loading Dox to target breast cancer cells *in vivo*. For targeting HER2+ TUBO breast cancer cells, the authors expressed the ligand, LAMP2b-DARPin, on the surface of exosomes as it is known to bind to HER2+ but not HER2- cells. In a HER2+ TUBO breast cancer model, the authors show that the modified exosomes led to an increase in the accumulation of Dox in tumor site and resulted in a significant tumor growth reduction compared to untargeted Dox-loaded exosomes and free Dox [55,56]. Yong et al. developed biocompatible tumor-cell-exocytosed exosome-biomimetic porous silicon nanoparticles (PSiNPs) as drug carriers for targeted cancer chemotherapy. Exosome-enclosed doxorubicin loaded PSiNPs (Dox@E-PSiNPs) were generated by exocytosis of the endocytosed DOX-loaded PSiNPs from tumor cells. *In vivo* studies showed an increase in anti-cancer activity and tumor growth reduction in subcutaneous (H22 tumor bearing mice), orthotopic (4T1 breast cancer) and metastatic (B16-F10 lung metastasis cancer) tumor models supporting the efficiency of this system [57].

Various researchers evaluated the exosomal drug delivery of paclitaxel (PTX), another commonly used anti-cancer agent. Kim et al. used macrophage-derived exosomes for PTX delivery to pulmonary metastases. They used exosomes released by primary bone-marrow derived macrophages and incorporated aminoethylanisamide-polyethylene glycol (AA-PEG) moiety to target the sigma receptor, which is over-expressed in lung cancer cells. Experimental results showed that engineered exosomes possessed a high loading capacity (~33%) along with cancer targeting, potent inhibition of pulmonary metastases growth and prolonged survival compared to non-targeted exosomes [58]. In another study by Wang et al., exosomes derived from M1-polarized macrophages were loaded with PTX and administered to treat breast cancer (4T1 cells) in a mouse model. M1-Exos provided pro-inflammatory environment which enhanced the anti-tumor activity *via* caspase-3 mediated pathway [59]. In another example, exosomes derived from human bone marrow-derived mesenchymal stem cells were used for PTX loading and were tested against triple negative breast cancer (TNBC) (MDA-MB-231) cells. *In vitro* and *in vivo* results showed significant cytotoxicity and tumor growth inhibition [60]. Zhu et al. focused on assessing the effect of PTX drug alone and with exosome system (cRGD-Exo-PTX) on glioblastoma. For the glioblastoma therapy, the authors used embryonic stem cells-derived exosomes and loaded them with PTX. *In vitro* and *in vivo* results summarized that the cRGD-Exo-PTX significantly improved curative effects of PTX *via* enhanced targeting in glioblastoma therapy [61]. Various other studies show the specific and efficient targeting of exosomes loaded with small molecules including PTX against lung cancer (A549) [62], ovarian cancer (SKOV3) [63] and breast cancer (MDA-hyb1) cells [62].

Other drugs used for exosomes drug delivery are gemcitabine (Gem) for pancreatic cancer and dopamine for treating Parkinson's disease. Exosomes loaded with gemcitabine (Exo-Gem) were evaluated in pancreatic tumor-bearing mice and results showed suppressed tumor growth with prolonged survival and minimal damage to normal tissues [64]. Parkinson's disease, most common neurodegenerative disorders is challenging to treat owing to blood-brain barrier blocking passage for most of the drugs. Exosomes, besides enhancing the drug properties, are employed to carry small-molecules across the blood-brain barrier. *In vitro* and *in vivo* studies of dopamine-loaded exosomes showed better therapeutic efficacy in Parkinson's disease mouse model and lower systemic toxicity after intravenous administration compared to dopamine alone [65].

6.2. Large molecules

In addition to small molecules, exosomal drug delivery systems are used to deliver large molecules such as proteins and peptides. In a recent study by Tian et al., exosomes were used for the treatment of ischemic stroke in which the artery of brain is blocked. Briefly, exosomes were conjugated with c(RGDyK) peptide on the surface using bio-orthogonal chemistry to target the lesion region of the ischemic brain. Curcumin was loaded into these engineered exosomes to suppress the inflammatory response and cellular apoptosis in the lesion region. *In vivo* results showed encouraging targeting ability and therapeutic efficacy of the cRGD-Exo delivery system [66]. Another study by Liu et al. investigated the use of exosomes in neuronal recovery in ischemic stroke by inhibiting neuronal p53/Caspase-3. Enkephalin, a pentapeptide, is a neurotransmitter and is used to promote neuron regeneration. *In vivo* delivery of exosomal system in rats carried out using a transient middle cerebral artery occlusion (tMCAO)/reperfusion model showed exosomes to cross the blood-brain barrier and decrease the levels of lactate dehydrogenase (LDH), p53 and caspase-3. In addition, it improved brain neuron density and neurological score suggesting neurological recovery after stroke [67].

Kim et al. evaluated whether genetic modification of exosomes to express a targeting ligand will enhance the delivery to the target tissue and reduce systemic toxicity. To investigate this theory, exosomes were modified to express the cardiac-targeting peptide and their ability to deliver to heart cells and tissues in both *in vitro* and *in vivo* was evaluated. Exosomes were isolated from HEK293 cells by differential centrifugation. After isolation, exosomes were genetically modified by introducing cardiac-targeting peptide (CTP)-Lamp2b on the exosomal membrane (CTP-Exo) and expression of CTP-Lamp2b peptide was stabilized by attaching glycosylation sequences. They used exosomes expressing only Lamp2b on exosomal membranes (CTL-Exo) as a control. *In vitro* studies showed a significant increase in delivery of CTP-Exo in H9C2 rat cardiomyocytes compared to CTL-Exo. Moreover, *in vivo* studies showed significant increase in delivery of CTP-Exo (15% more) than CTL-Exo suggesting that genetic modification with targeting peptides does increase exosomes delivery, reduce systemic toxicity (similar accumulation levels in spleen and liver between CTL and CTP-Exo), and can be explored as therapeutic tool for heart diseases [68].

Delivery of trastuzumab-emtansine (T-DM1), an antibody-drug conjugate, was examined against HER2-postive cancer by Barok et al. Exosomes were isolated from several HER2+ (SKBR-3 and EFM-192A breast cancer), HER2- (MCF-7 breast cancer) and gastric cancer (SNU-216) cell lines *via* ultracentrifugation and were treated with T-DM1. The results showed that T-DM1 exosomes bind to HER2+ but not HER2- cancer cells. Further, T-DM1-containing exosomes resulted in growth inhibition and activation of caspases-3 showing the binding of T-DM1 to HER2+ cancer cells [69].

6.3. Nucleic acids

Exosomes naturally carry nucleic acids such as DNA and RNA to targeted cells and induces genetic modifications in both biological and pathogenic processes as we discussed earlier. Because of the natural ability to carry genetic material, exosomes drew major interest in drug delivery strategies involving genetic therapy that alter gene expression in certain diseases and improve genetic therapy.

6.3.1. Small interference RNA (siRNA)

In genetic therapy, siRNA is used to disrupt genes of interest, but they have low stability and tend to degrade in systemic circulation. Exosomes, however, help in overcoming this challenge by protecting and delivering siRNA to targeted cells and act as therapeutic vehicles. Several studies have been performed to investigate successful delivery of siRNA to target cells. In a recent study by Faruqu et al., siRNA was loaded into exosomes and successfully delivered to cancer cells *in vitro*. Exosomes were collected from HEK-293 cells by differential centrifugation and ultracentrifugation onto sucrose cushion. siRNA was fluorescently labeled and loaded into exosomes by electroporation and excess siRNA was removed using gel filtration. *In vitro* results showed promising exosome yield, encapsulation efficiency of siRNA, and ability of exosomes to deliver into cancer cells [70]. Similar exosomal system (LAMP2B-DARPin bearing exosomes) was developed by Limoni et al. to bind specifically to HER2/Neu and deliver the siRNA molecule against TPD52 gene into SKBR3 cells. The siRNA loaded exosomes successfully downregulated the TPD52 gene expression by 70% [71].

Park et al. determined the efficiency of serum-derived exosomes in delivering tyrosinase-related protein-2 (TRP-2) peptides to lymph nodes. Exosomes loaded with TRP-2 shows strong release of proinflammatory cytokines (TNF-α and IL-6) from both macrophages (RAW264.7) and murine dendritic cell (DC2.4 [72]. Serum-derived exosomes were loaded with siRNA, functional in modulating lipopolysaccharide (LPS)-induced lung inflammation *in vivo*, and were delivered *via* intratracheal instillation into lung macrophages avoiding the immune system which is major challenge in deliver [73]. Zhao et al. developed biomimetic nanoparticles (cationic bovine serum albumin (CBSA) conjugated siRNA, siS100A4, and exosome membrane coated nanoparticles, CBSA/siS100A4@Exosome) to deliver to the lung premetastatic niche. Exosomes were isolated by ultracentrifugation and BSA-siRNA-Exo were prepared by incubation and extrusion method. The exosomal system exhibited gene-silencing effects that significantly inhibited the growth of malignant breast cancer cells [74].

6.3.2. microRNA (miRNA)

miRNA is non-coding RNA and with non-protein nucleotides is found in eukaryotic cells. They bind to complementary sequences on targeted mRNA and control the post-transcriptional gene expression. Since, miRNA is naturally present in exosomes, they are used as therapeutic vehicles to carry miRNA.

In a recent study, Gong et al. investigated the exosomal delivery facilitated by co-administration of Dox and cholesterol-modified miRNA 159 (Cho-miR159) to TNBC cells. Exosomes (Exo) were derived from continuous protein kinase C activation in monocyte-derived macrophages. These exosomes had the modified version of disintegrin and metalloproteinase 15 (A15) on membranes (A15-Exo). *In vitro* studies showed synergistic therapeutic effect of A15-Exo co-loaded with Dox and Cho-miR159 in MDA-MB-231 TNBC cells whereas *in vivo* studies showed effective silencing of TCF-7 gene and demonstrated improved anti-cancer effects and low cytotoxicity [75]. Liang et al. engineered exosomes to co-administer 5-Fluorouracil (5-FU) and microRNA-21 inhibitor oligonucleotide–(miR-21i) to HER2 expressing cancer cells. After systemic administration, the dual drug loaded exosomes downregulated miR-21 induced cell cycle arrest, reduced tumor proliferation, increased apoptosis and rescued PTEN and hMSH2 expressions, regulatory targets of miR-21. Further, the combinational delivery of miR-21i and 5-FU within exosomes effectively reversed drug resistance and significantly enhanced the cytotoxicity in 5-FU-resistant colon cancer cells [76]. Another study used exosomes derived from MDA-MB-231 cells for treating NSCLC. Exosomes (231-Exo) were specifically internalized by NSCLC *via* interaction between overexpressed integrin β_4 (on exosomes) and surface protein C (SPC) on cancer cells. miRNA-126 was loaded in exosomal carriers (miR-126:231-Exo) and suppressed cell proliferation and migration through interrupting PTEN/PI3K/AKT signaling pathway. *In vivo* study showed the inhibition of lung cancer metastasis in mice [77].

Hepatocellular carcinoma (HCC) displays high resistance to commonly used chemotherapeutic agents (e.g. doxorubicin) hence new therapeutic approaches to enhance HCC chemosensitivity is the need of the hour [78]. Lou et al. developed adipose tissue-derived MSCs (AMSCs) to deliver miRNA-199a (miR-199a) for improving HCC chemosensitivity. Experimental studies such as cell proliferation and apoptosis assays for chemosensitivity and mTOR levels in HCC cells by western blot analysis showed that Exo-miR-199a significantly sensitized HCC cells to Dox by targeting mTOR and subsequently, inhibiting the mTOR pathway [79]. Another study by O'Brien et al. focused on delivering potential tumor suppressor microRNA, miR-379 for *in vivo* therapy of breast cancer. They administered extracellular vesicle encapsulated miR-379 and found significant reduction in the rate of tumor formation and growth in T47D breast cancer cells expressing miR-379 [80]. Naseri et al. used exosomes isolated from bone marrow-derived mesenchymal stem cells (MSCs) and delivered anti-miR-142-3p. They observed reduction in the mi-R142-3p and miR-150 levels and increase in the transcription of the regulatory genes, APC and P2X7R, in TUBO breast cancer cell lines. The MSC derived exosomal system penetrated the tumor site and were able to deliver the inhibitory oligonucleotides by downregulating the expression levels of the above mentioned microRNAs [81].

Also, exosomal delivery of miRNA proves boon for angiogenic therapy. In particular, miR-210 has shown improvement in angiogenesis for brain tissue repair after cerebral ischemia. Zhang et al. formulated exosomes conjugated with c(RGDyK) peptide and loaded with cholesterol modified miR-210 (RGD-exo: miR-210). The intravenous administration of the exosomal system resulted in increase of miR-210 in the lesion region of the ischemic brain. Furthermore, it was observed that repeated administration of RGD-exo: miR-210 every alternate day for 14 days resulted in the upregulation of integrin β_3, vascular endothelial growth factor (VEGF), and CD34 which promotes wound-healing in ischemia. They also observed enhancement in animal survival rate advocating the targeted delivery of miR-210 to ischemic brain and acting as angiogenic agent [82].

Recent studies demonstrated role of exosomes in cartilage regeneration and enhancing their regenerative effect. In one of the studies, they combined exosomes with microRNA-140 (miR-140) to enhance the cell differentiation by inducing membrane fusion and consequent miR-140 release into the cytoplasm. The Exo-miR-140 system was formulated by the freeze and thaw method. The effects of exosomal system on bone marrow stem cells (BMSCs) was evaluated by cell adhesion and morphology, and their uptake efficiency by real-time polymerase chain reaction (RT-PCR). The RT-PCR results showed the differentiation of BMSCs into chondrocytes involved in cartilage healing process exploiting the tissue engineering route [83]. A similar exosomal system loaded with miR-375 (Exo-miR-375) was formulated to facilitate the bone regeneration in rat model of calvarial defect. Administration of Exo-miR-375 improved the osteogenic differentiation of BMSCs to promote bone regeneration [84].

6.3.3. Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) system

In recent years, researchers have used CRISPR/Cas9 systems to treat various genetic diseases such as cancers *via* repairing, deleting or silencing certain genetic mutations relating to the disease. For the

CRISPR/Cas9 system to be efficient in editing the gene, targeted delivery of CRISPR/Cas9 system to the receptor cells is essential. Lin et al. used exosome-liposome hybrid nanoparticles to deliver CRISPR/Cas9 system into MSCs. Exosomes were derived from HEK293FT cells and isolated by ultracentrifugation. Hybrid exosomes were produced *via* simple incubation of exosomes with liposomes and large DNA molecule was encapsulated into exosomes and transferred exosomes to MSCs. They were successful in using the hybrid nanoparticles to deliver into MSCs and regulate the target gene expression [85]. Another study by Kim et al. suggests that cancer-derived exosomes possessed better tumor targeting potential compared to epithelial cell-derived exosomes. Briefly, delivery of CRISPR/Cas9 was evaluated using exosomes derived from SKOV3 cells and HEK293 cells and found that cancer-derived exosomes serve as potential vehicles for effective *in vivo* delivery *via* selective accumulation because of cell tropism. CRISPR/Cas9-loaded exosomes inhibited expression of poly (ADP-ribose) polymerase-1 (PARP-1), resulting in the induction of apoptosis in ovarian cancer. The CRISPR/Cas9-mediated genome editing enhanced the chemosensitivity to cisplatin, showing synergistic cytotoxicity [86].

7. Challenges in using exosomes for drug delivery

Contrasting conventional nanoparticulate systems such as liposomes or polymeric nanoparticles, exosomes can possibly avoid the endosomal pathway and lysosomal degradation and deliver cargos directly into the cytoplasm. However, inefficient understanding of exosome nature and their role in overall disease and health conditions makes it difficult to foresee long-term safety and therapeutic effect. There exist many challenges in a clear understanding of exosomes regarding therapeutic cargo loading and assembly for drug delivery.

Isolation and separation of exosomes with high purity on a large scale is essential if exosomes are to be used as drug delivery carriers in large-scale. Presently, there is no distinct ideal purification technique for isolation and separation of exosomes with high purity [87]. The multitude of existing separation and isolation techniques not only yield low quantities of exosomes but also are expensive for large scale production [88]. To achieve targetability of exosomes, ligands are attached to the surface through chemical conjugation and these active targeting molecular combinational techniques and systems needs to be examined for safety and efficacy.

Even though extensive biology is known, exosomes consist of heterogenous components that exhibit different cellular functions. If inappropriate donor cells are chosen to derive exosomes they may show immunogenicity effects. Further, exosomes contribution in tumor progression or membrane antigens release is of huge interest in tumor growth. For example, exosomes that transport caspase-3 may inhibit cell death by apoptosis or enhance tumor cell survival by averting chemotherapeutics drug accumulation [89]. Exosomes role in disease must be investigated in detail to enable clinical translation and allow for safe use of exosomes as drug delivery vehicles in cancer treatment. Clearly, further research is required into the composition of exosomes and their efficacy as a drug carrier.

8. Conclusions and outlook

The advent of exosomes and their functions in biology are one of the most exciting breakthroughs in the medical field in recent years. Exosomes, a nano-vesicle produced by most cell types, performs an exceptional role in cell-cell communication and can deliver various cargos of drug molecules, proteins, and nucleotides. As a relatively new carrier system, exosomes have various advantages such as unique structure, distinct physiochemical characteristics, low immunogenicity and toxicity and inherent targeting ability. Few exosomal systems have entered clinical trials but component characterization and immune reactions need to be clearly identified. Despite the great development, to realize the full potential of exosomes as drug carriers, there are many challenges to address which include standardized separation and purification method, low yield after isolation, and poor stability. Recent literature shows continued exploration and promise of exosomes as drug delivery carriers for various diseases including solid tumors, bone regeneration, cardiac diseases, Parkinson's amongst others. With increased research on exosomes, we are hopeful that the challenges will be addressed soon and the clinical translation realized.

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.

References

[1] V.V. Tarasov, A.A. Svistunov, V.N. Chubarev, S.A. Dostdar, A.V. Sokolov, A. Brzecka, O. Sukocheva, M.E. Neganova, S.G. Klochkov, S.G. Somasundaram, C.E. Kirkland, G. Aliev, Extracellular vesicles in cancer nanomedicine, Semin. Cancer Biol. (2019), https://doi.org/10.1016/j.semcancer.2019.08.017.
[2] D. Ha, N. Yang, V. Nadithe, Exosomes as therapeutic drug carriers and delivery vehicles across biological membranes: current perspectives and future challenges, Acta Pharm. Sin. B. 6 (2016) 287–296, https://doi.org/10.1016/j.apsb.2016.02.001.
[3] G. Kibria, E.K. Ramos, Y. Wan, D.R. Gius, H. Liu, Exosomes as a drug delivery system in cancer therapy: potential and challenges, Mol. Pharm. 15 (2018) 3625–3633, https://doi.org/10.1021/acs.molpharmaceut.8b00277.
[4] P.D. Robbins, A.E. Morelli, Regulation of immune responses by extracellular vesicles, Nat. Rev. Immunol. 14 (2014) 195–208, https://doi.org/10.1038/nri3622.
[5] W. Liao, Y. Du, C. Zhang, F. Pan, Y. Yao, T. Zhang, Q. Peng, Exosomes: the next generation of endogenous nanomaterials for advanced drug delivery and therapy, Acta Biomater. 86 (2019) 1–14, https://doi.org/10.1016/j.actbio.2018.12.045.
[6] S.G. Antimisiaris, S. Mourtas, A. Marazioti, Exosomes and exosome-inspired vesicles for targeted drug delivery, Pharmaceutics 10 (2018), https://doi.org/10.3390/pharmaceutics10040218.
[7] X.-C. Jiang, J.-Q. Gao, Exosomes as novel bio-carriers for gene and drug delivery, Int. J. Pharm. 521 (2017) 167–175, https://doi.org/10.1016/j.ijpharm.2017.02.038.
[8] N.P. Hessvik, A. Llorente, Current knowledge on exosome biogenesis and release, Cell. Mol. Life Sci. 75 (2018) 193–208, https://doi.org/10.1007/s00018-017-2595-9.
[9] E.J. Bunggulawa, W. Wang, T. Yin, N. Wang, C. Durkan, Y. Wang, G. Wang, Recent advancements in the use of exosomes as drug delivery systems 06 Biological Sciences 0601 Biochemistry and Cell Biology, J. Nanobiotechnol. 16 (2018) 1–13, https://doi.org/10.1186/s12951-018-0403-9.
[10] S.A.A. Kooijmans, P. Vader, S.M. van Dommelen, W.W. van Solinge, R.M. Schiffelers, Exosome mimetics: a novel class of drug delivery systems, Int. J. Nanomedicine. 7 (2012) 1525–1541, https://doi.org/10.2147/IJN.S29661.
[11] I. Parolini, C. Federici, C. Raggi, L. Lugini, S. Palleschi, A. De Milito, C. Coscia, E. Iessi, M. Logozzi, A. Molinari, M. Colone, M. Tatti, M. Sargiacomo, S. Fais, Microenvironmental pH is a key factor for exosome traffic in tumor cells, J. Biol. Chem. (2009), https://doi.org/10.1074/jbc.M109.041152.
[12] A. Piccin, W.G. Murphy, O.P. Smith, Circulating microparticles: pathophysiology and clinical implications, Blood Rev. (2007), https://doi.org/10.1016/j.blre.2006.09.001.
[13] C. Subra, D. Grand, K. Laulagnier, A. Stella, G. Lambeau, M. Paillasse, P. De Medina, B. Monsarrat, B. Perret, S. Silvente-Poirot, M. Poirot, M. Record, Exosomes account for vesicle-mediated transcellular transport of activatable phospholipases and prostaglandins, J. Lipid Res. (2010), https://doi.org/10.1194/jlr.M003657.
[14] S. Fontana, L. Saieva, S. Taverna, R. Alessandro, Contribution of proteomics to understanding the role of tumor-derived exosomes in cancer progression: State of the art and new perspectives, Proteomics (2013), https://doi.org/10.1002/pmic.201200398.
[15] R.J. Simpson, H. Kalra, S. Mathivanan, Exocarta as a resource for exosomal research, J. Extracell. Vesicles. (2012), https://doi.org/10.3402/jev.v1i0.18374.
[16] S. Mathivanan, C.J. Fahner, G.E. Reid, R.J. Simpson, ExoCarta 2012: database of exosomal proteins, RNA and lipids, Nucleic Acids Res. (2012), https://doi.org/10.1093/nar/gkr828.
[17] J. Skog, T. Würdinger, S. van Rijn, D.H. Meijer, L. Gainche, W.T. Curry, B.S. Carter, A.M. Krichevsky, X.O. Breakefield, Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers, Nat. Cell Biol. (2008), https://doi.org/10.1038/ncb1800.
[18] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell (2004), https://doi.org/10.1016/S0092-8674(04)00045-5.
[19] F. Jacob, J. Monod, Genetic regulatory mechanisms in the synthesis of proteins, J. Mol. Biol. (1961), https://doi.org/10.1016/S0022-2836(61)80072-7.
[20] S. Frank, A. Aguirre, J. Hescheler, L. Kurian, A lncRNA Perspective into (Re) building the heart, Front. Cell Dev. Biol. (2016), https://doi.org/10.3389/fcell.2016.00128.
[21] Z. Qin, V.A. Ljubimov, C. Zhou, Y. Tong, J. Liang, Cell-free circulating tumor DNA

in cancer, Chin. J. Cancer. (2016), https://doi.org/10.1186/s40880-016-0092-4.
[22] B.J. Tauro, D.W. Greening, R.A. Mathias, H. Ji, S. Mathivanan, A.M. Scott, R.J. Simpson, Comparison of ultracentrifugation, density gradient separation, and immunoaffinity capture methods for isolating human colon cancer cell line LIM1863-derived exosomes, Methods (2012), https://doi.org/10.1016/j.ymeth.2012.01.002.
[23] S. Kamerkar, V.S. Lebleu, H. Sugimoto, S. Yang, C.F. Ruivo, S.A. Melo, J.J. Lee, R. Kalluri, Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer, Nature (2017), https://doi.org/10.1038/nature22341.
[24] X. Doldán, P. Fagúndez, A. Cayota, J. Laíz, J.P. Tosar, Electrochemical sandwich immunosensor for determination of exosomes based on surface marker-mediated signal amplification, Anal. Chem. (2016), https://doi.org/10.1021/acs.analchem.6b02421.
[25] A. Cheruvanky, H. Zhou, T. Pisitkun, J.B. Kopp, M.A. Knepper, P.S.T. Yuen, R.A. Star, Rapid isolation of urinary exosomal biomarkers using a nanomembrane ultrafiltration concentrator, Am. J. Physiol. – Ren. Physiol. (2007), https://doi.org/10.1152/ajprenal.00434.2006.
[26] K. Takov, D.M. Yellon, S.M. Davidson, Comparison of small extracellular vesicles isolated from plasma by ultracentrifugation or size-exclusion chromatography: yield, purity and functional potential, J. Extracell. Vesicles. (2019), https://doi.org/10.1080/20013078.2018.1560809.
[27] N. Bohmer, N. Demarmels, E. Tsolaki, L. Gerken, K. Keevend, S. Bertazzo, M. Lattuada, I.K. Herrmann, Removal of cells from body fluids by magnetic separation in batch and continuous mode: influence of bead size, concentration, and contact time, ACS Appl. Mater. Interfaces (2017), https://doi.org/10.1021/acsami.7b10140.
[28] K. Lee, H. Shao, R. Weissleder, H. Lee, Acoustic purification of extracellular microvesicles, ACS Nano (2015), https://doi.org/10.1021/nn506538f.
[29] A. Aghilinejad, M. Aghaamoo, X. Chen, J. Xu, Effects of electrothermal vortices on insulator-based dielectrophoresis for circulating tumor cell separation, Electrophoresis (2018), https://doi.org/10.1002/elps.201700264.
[30] S.D. Ibsen, J. Wright, J.M. Lewis, S. Kim, S.Y. Ko, J. Ong, S. Manouchehri, A. Vyas, J. Akers, C.C. Chen, B.S. Carter, S.C. Esener, M.J. Heller, Rapid isolation and detection of exosomes and associated biomarkers from plasma, ACS Nano 11 (2017) 6641–6651, https://doi.org/10.1021/acsnano.7b00549.
[31] K.K. Zeming, N.V. Thakor, Y. Zhang, C.H. Chen, Real-time modulated nanoparticle separation with an ultra-large dynamic range, Lab Chip (2016), https://doi.org/10.1039/c5lc01051a.
[32] B.H. Wunsch, J.T. Smith, S.M. Gifford, C. Wang, M. Brink, R.L. Bruce, R.H. Austin, G. Stolovitzky, Y. Astier, Nanoscale lateral displacement arrays for the separation of exosomes and colloids down to 20nm, Nat. Nanotechnol. (2016), https://doi.org/10.1038/nnano.2016.134.
[33] S. Lin, Z. Yu, D. Chen, Z. Wang, J. Miao, Q. Li, D. Zhang, J. Song, D. Cui, Progress in microfluidics-based exosome separation and detection technologies for diagnostic applications, Small 16 (2020) 1903916, https://doi.org/10.1002/smll.201903916.
[34] Y. Dieckmann, H. Cölfen, H. Hofmann, A. Petri-Fink, Particle size distribution measurements of manganese-doped ZnS nanoparticles, Anal. Chem. (2009), https://doi.org/10.1021/ac900043y.
[35] C.M. Hoo, N. Starostin, P. West, M.L. Mecartney, A comparison of atomic force microscopy (AFM) and dynamic light scattering (DLS) methods to characterize nanoparticle size distributions, J. Nanoparticle Res. (2008), https://doi.org/10.1007/s11051-008-9435-7.
[36] R.A. Dragovic, C. Gardiner, A.S. Brooks, D.S. Tannetta, D.J.P. Ferguson, P. Hole, B. Carr, C.W.G. Redman, A.L. Harris, P.J. Dobson, P. Harrison, I.L. Sargent, Sizing and phenotyping of cellular vesicles using Nanoparticle Tracking Analysis, Nanomedicine Nanotechnology, Biol. Med. (2011), https://doi.org/10.1016/j.nano.2011.04.003.
[37] J.G. Walker, Improved nano-particle tracking analysis, Meas. Sci. Technol. (2012), https://doi.org/10.1088/0957-0233/23/6/065605.
[38] T. Pisitkun, R.F. Shen, M.A. Knepper, Identification and proteomic profiling of exosomes in human urine, Proc. Natl. Acad. Sci. U. S. A. (2004), https://doi.org/10.1073/pnas.0403453101.
[39] S. Gurunathan, M.-H. Kang, M. Jeyaraj, M. Qasim, J.-H. Kim, Review of the isolation, characterization, biological function, and multifarious therapeutic approaches of exosomes, Cells (2019), https://doi.org/10.3390/cells8040307.
[40] J. Hardij, F. Cecchet, A. Berquand, D. Gheldof, C. Chatelain, F. Mullier, B. Chatelain, J.M. Dogné, Characterisation of tissue factor-bearing extracellular vesicles with AFM: comparison of air-tapping-mode AFM and liquid peak force AFM, J. Extracell. Vesicles. (2013), https://doi.org/10.3402/jev.v2i0.21045.
[41] D.M. Rissin, C.W. Kan, T.G. Campbell, S.C. Howes, D.R. Fournier, L. Song, T. Piech, P.P. Patel, L. Chang, A.J. Rivnak, E.P. Ferrell, J.D. Randall, G.K. Provuncher, D.R. Walt, D.C. Duffy, Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations, Nat. Biotechnol. (2010), https://doi.org/10.1038/nbt.1641.
[42] S. Fang, H. Tian, X. Li, D. Jin, X. Li, J. Kong, C. Yang, X. Yang, Y. Lu, Y. Luo, B. Lin, W. Niu, T. Liu, Clinical application of a microfluidic chip for immunocapture and quantification of circulating exosomes to assist breast cancer diagnosis and molecular classification, PLoS ONE (2017), https://doi.org/10.1371/journal.pone.0175050.
[43] R. Vaidyanathan, M. Naghibosadat, S. Rauf, D. Korbie, L.G. Carrascosa, M.J.A. Shiddiky, M. Trau, Detecting exosomes specifically: A multiplexed device based on alternating current electrohydrodynamic induced nanoshearing, Anal. Chem. (2014), https://doi.org/10.1021/ac502082b.
[44] L.G. Liang, M.Q. Kong, S. Zhou, Y.F. Sheng, P. Wang, T. Yu, F. Inci, W.P. Kuo, L.J. Li, U. Demirci, S.Q. Wang, An integrated double-filtration microfluidic device for isolation, enrichment and quantification of urinary extracellular vesicles for detection of bladder cancer, Sci. Rep. (2017), https://doi.org/10.1038/srep46224.
[45] R. Gordon, D. Sinton, K.L. Kavanagh, A.G. Brolo, A new generation of sensors based on extraordinary optical transmission, Acc. Chem. Res. (2008), https://doi.org/10.1021/ar800074d.
[46] H. Im, H. Shao, Y. Il Park, V.M. Peterson, C.M. Castro, R. Weissleder, H. Lee, Label-free detection and molecular profiling of exosomes with a nano-plasmonic sensor, Nat. Biotechnol. (2014), https://doi.org/10.1038/nbt.2886.
[47] D. Issadore, C. Min, M. Liong, J. Chung, R. Weissleder, H. Lee, Miniature magnetic resonance system for point-of-care diagnostics, Lab Chip (2011), https://doi.org/10.1039/c1lc20177h.
[48] H. Shao, J. Chung, L. Balaj, A. Charest, D.D. Bigner, B.S. Carter, F.H. Hochberg, X.O. Breakefield, R. Weissleder, H. Lee, Protein typing of circulating microvesicles allows real-time monitoring of glioblastoma therapy, Nat. Med. (2012), https://doi.org/10.1038/nm.2994.
[49] B. Balachandran, Y. Yuana, Extracellular vesicles-based drug delivery system for cancer treatment, Cogent Med. 6 (2019) 1635806, https://doi.org/10.1080/2331205X.2019.1635806.
[50] X. Zhao, D. Wu, X. Ma, J. Wang, W. Hou, W. Zhang, Exosomes as drug carriers for cancer therapy and challenges regarding exosome uptake, Biomed. Pharmacother. 128 (2020) 110237, https://doi.org/10.1016/j.biopha.2020.110237.
[51] X. Luan, K. Sansanaphongpricha, I. Myers, H. Chen, H. Yuan, D. Sun, Engineering exosomes as refined biological nanoplatforms for drug delivery, Acta Pharmacol. Sin. 38 (2017) 754–763, https://doi.org/10.1038/aps.2017.12.
[52] P. Akuma, O.D. Okagu, C.C. Udenigwe, Naturally occurring exosome vesicles as potential delivery vehicle for bioactive compounds, Front. Sustain. Food Syst. 3 (2019) 23 https://www.frontiersin.org/article/10.3389/fsufs.2019.00023.
[53] C. Schindler, A. Collinson, C. Matthews, A. Pointon, L. Jenkinson, R.R. Minter, T.J. Vaughan, N.J. Tigue, Exosomal delivery of doxorubicin enables rapid cell entry and enhanced in vitro potency, PLoS ONE 14 (2019), https://doi.org/10.1371/journal.pone.0214545.
[54] Y. Li, Y. Gao, C. Gong, Z. Wang, Q. Xia, F. Gu, C. Hu, L. Zhang, H. Guo, S. Gao, A33 antibody-functionalized exosomes for targeted delivery of doxorubicin against colorectal cancer, Nanomed. Nanotechnol. Biol. Med. 14 (2018) 1973–1985, https://doi.org/10.1016/j.nano.2018.05.020.
[55] H. Gomari, M.F. Moghadam, M. Soleimani, M. Ghavami, S. Khodashenas, Targeted delivery of doxorubicin to HER2 positive tumor models, Int. J. Nanomedicine. 14 (2019) 5679–5690, https://doi.org/10.2147/IJN.S210731.
[56] H. Gomari, M.F. Moghadam, M. Soleimani, Targeted cancer therapy using engineered exosome as a natural drug delivery vehicle, Onco. Targets. Ther. 11 (2018) 5753–5762, https://doi.org/10.2147/OTT.S173110.
[57] T. Yong, X. Zhang, N. Bie, H. Zhang, X. Zhang, F. Li, A. Hakeem, J. Hu, L. Gan, H.A. Santos, X. Yang, Tumor exosome-based nanoparticles are efficient drug carriers for chemotherapy, Nat. Commun. 10 (2019), https://doi.org/10.1038/s41467-019-11718-4.
[58] M.S. Kim, M.J. Haney, Y. Zhao, D. Yuan, I. Deygen, N.L. Klyachko, A.V. Kabanov, E.V. Batrakova, Engineering macrophage-derived exosomes for targeted paclitaxel delivery to pulmonary metastases: in vitro and in vivo evaluations, Nanomedicine 14 (2018) 195–204, https://doi.org/10.1016/j.nano.2017.09.011.
[59] P. Wang, H. Wang, Q. Huang, C. Peng, L. Yao, H. Chen, Z. Qiu, Y. Wu, L. Wang, W. Chen, Exosomes from M1-polarized macrophages enhance paclitaxel antitumor activity by activating macrophages-mediated inflammation, Theranostics 9 (2019) 1714–1727, https://doi.org/10.7150/thno.30716.
[60] S. Kalimuthu, P. Gangadaran, R.L. Rajendran, L. Zhu, J.M. Oh, H.W. Lee, A. Gopal, S.H. Baek, S.Y. Jeong, S.W. Lee, J. Lee, B.C. Ahn, A new approach for loading anticancer drugs into mesenchymal stem cell-derived exosome mimetics for cancer therapy, Front. Pharmacol. 9 (2018), https://doi.org/10.3389/fphar.2018.01116.
[61] Q. Zhu, X. Ling, Y. Yang, J. Zhang, Q. Li, X. Niu, G. Hu, B. Chen, H. Li, Y. Wang, Z. Deng, Embryonic stem cells-derived exosomes endowed with targeting properties as chemotherapeutics delivery vehicles for glioblastoma therapy, Adv. Sci. 6 (2019), https://doi.org/10.1002/advs.201801899.
[62] C. Melzer, V. Rehn, Y. Yang, H. Bähre, J. von der Ohe, R. Hass, Taxol-loaded MSC-Derived exosomes provide a therapeutic vehicle to target metastatic breast cancer and other carcinoma cells, Cancers (Basel) 11 (2019), https://doi.org/10.3390/cancers11060798.
[63] H. Liu, M. Shen, D. Zhao, D. Ru, Y. Duan, C. Ding, H. Li, The effect of triptolide-loaded exosomes on the proliferation and apoptosis of human ovarian cancer SKOV3 cells, Biomed Res. Int. 2019 (2019) 2595801, https://doi.org/10.1155/2019/2595801.
[64] Y.J. Li, J.Y. Wu, J.M. Wang, X. Bin Hu, J.X. Cai, D.X. Xiang, Gemcitabine loaded autologous exosomes for effective and safe chemotherapy of pancreatic cancer, Acta Biomater. 101 (2020) 519–530, https://doi.org/10.1016/j.actbio.2019.10.022.
[65] M. Qu, Q. Lin, L. Huang, Y. Fu, L. Wang, S. He, Y. Fu, S. Yang, Z. Zhang, L. Zhang, X. Sun, Dopamine-loaded blood exosomes targeted to brain for better treatment of Parkinson's disease, J. Control. Release 287 (2018) 156–166, https://doi.org/10.1016/j.jconrel.2018.08.035.
[66] T. Tian, H.-X. Zhang, C.-P. He, S. Fan, Y.-L. Zhu, C. Qi, N.-P. Huang, Z.-D. Xiao, Z.-H. Lu, B.A. Tannous, J. Gao, Surface functionalized exosomes as targeted drug delivery vehicles for cerebral ischemia therapy, Biomaterials 150 (2018) 137–149, https://doi.org/10.1016/j.biomaterials.2017.10.012.
[67] Y. Liu, N. Fu, J. Su, X. Wang, X. Li, Rapid enkephalin delivery using exosomes to promote neurons recovery in ischemic stroke by inhibiting neuronal p53/caspase-3, Biomed. Res. Int. 2019 (2019) 4273290, https://doi.org/10.1155/2019/4273290.
[68] H. Kim, N. Yun, D. Mun, J.Y. Kang, S.H. Lee, H. Park, H. Park, B. Joung, Cardiac-specific delivery by cardiac tissue-targeting peptide-expressing exosomes, Biochem. Biophys. Res. Commun. 499 (2018) 803–808, https://doi.org/10.1016/j.bbrc.2018.03.227.

[69] M. Barok, M. Puhka, G. Vereb, J. Szollosi, J. Isola, H. Joensuu, Cancer-derived exosomes from HER2-positive cancer cells carry trastuzumab-emtansine into cancer cells leading to growth inhibition and caspase activation, BMC Cancer. 18 (2018) 504, https://doi.org/10.1186/s12885-018-4418-2.
[70] F.N. Faruqu, L. Xu, K.T. Al-Jamal, Preparation of exosomes for siRNA delivery to cancer cells, J. Vis. Exp. 2018 (2018), https://doi.org/10.3791/58814.
[71] S.K. Limoni, M.F. Moghadam, S.M. Moazzeni, H. Gomari, F. Salimi, Engineered exosomes for targeted transfer of siRNA to HER2 positive breast cancer cells, Appl. Biochem. Biotechnol. 187 (2019) 352–364, https://doi.org/10.1007/s12010-018-2813-4.
[72] O. Park, E.S. Choi, G. Yu, J.Y. Kim, Y.Y. Kang, H. Jung, H. Mok, Efficient delivery of tyrosinase related protein-2 (TRP2) peptides to lymph nodes using serum-derived exosomes, Macromol. Biosci. 18 (2018), https://doi.org/10.1002/mabi.201800301.
[73] D. Zhang, H. Lee, X. Wang, A. Rai, M. Groot, Y. Jin, Exosome-mediated small RNA delivery: a novel therapeutic approach for inflammatory lung responses, Mol. Ther. 26 (2018) 2119–2130, https://doi.org/10.1016/j.ymthe.2018.06.007.
[74] L. Zhao, C. Gu, Y. Gan, L. Shao, H. Chen, H. Zhu, Exosome-mediated siRNA delivery to suppress postoperative breast cancer metastasis, J. Control. Release 318 (2020) 1–15, https://doi.org/10.1016/j.jconrel.2019.12.005.
[75] C. Gong, J. Tian, Z. Wang, Y. Gao, X. Wu, X. Ding, L. Qiang, G. Li, Z. Han, Y. Yuan, S. Gao, Functional exosome-mediated co-delivery of doxorubicin and hydrophobically modified microRNA 159 for triple-negative breast cancer therapy, J. Nanobiotechnology. 17 (2019), https://doi.org/10.1186/s12951-019-0526-7.
[76] G. Liang, Y. Zhu, D.J. Ali, T. Tian, H. Xu, K. Si, B. Sun, B. Chen, Z. Xiao, Engineered exosomes for targeted co-delivery of miR-21 inhibitor and chemotherapeutics to reverse drug resistance in colon cancer, J. Nanobiotechnology 18 (2020), https://doi.org/10.1186/s12951-019-0563-2.
[77] H. Nie, X. Xie, D. Zhang, Y. Zhou, B. Li, F. Li, F. Li, Y. Cheng, H. Mei, H. Meng, L. Jia, Use of lung-specific exosomes for miRNA-126 delivery in non-small cell lung cancer, Nanoscale 12 (2020) 877–887, https://doi.org/10.1039/c9nr09011h.
[78] K. Lohitesh, R. Chowdhury, S. Mukherjee, Resistance a major hindrance to chemotherapy in hepatocellular carcinoma: an insight, Cancer Cell Int. 18 (2018), https://doi.org/10.1186/s12935-018-0538-7.
[79] G. Lou, L. Chen, C. Xia, W. Wang, J. Qi, A. Li, L. Zhao, Z. Chen, M. Zheng, Y. Liu, MiR-199a-modified exosomes from adipose tissue-derived mesenchymal stem cells improve hepatocellular carcinoma chemosensitivity through mTOR pathway, J. Exp. Clin. Cancer Res. 39 (2020), https://doi.org/10.1186/s13046-019-1512-5.
[80] K.P. O'Brien, S. Khan, K.E. Gilligan, H. Zafar, P. Lalor, C. Glynn, C. O'Flatharta, H. Ingoldsby, P. Dockery, A. De Bhulbh, J.R. Schweber, K. St John, M. Leahy, J.M. Murphy, W.M. Gallagher, T. O'Brien, M.J. Kerin, R.M. Dwyer, Employing mesenchymal stem cells to support tumor-targeted delivery of extracellular vesicle (EV)-encapsulated microRNA-379, Oncogene 37 (2018) 2137–2149, https://doi.org/10.1038/s41388-017-0116-9.
[81] Z. Naseri, R.K. Oskuee, M.R. Jaafari, M.F. Moghadam, Exosome-mediated delivery of functionally active miRNA-142-3p inhibitor reduces tumorigenicity of breast cancer in vitro and in vivo, Int. J. Nanomed. 13 (2018) 7727–7747, https://doi.org/10.2147/IJN.S182384.
[82] H. Zhang, J. Wu, J. Wu, Q. Fan, J. Zhou, J. Wu, S. Liu, J. Zang, J. Ye, M. Xiao, T. Tian, J. Gao, Exosome-mediated targeted delivery of miR-210 for angiogenic therapy after cerebral ischemia in mice, J. Nanobiotechnol. 17 (2019), https://doi.org/10.1186/s12951-019-0461-7.
[83] G. Won Lee, M. Thangavelu, M. Joung Choi, E. Yeong Shin, H. Sol Kim, J. Seon Baek, Y. Woon Jeong, J. Eun Song, C. Carlomagno, J. Miguel Oliveira, R. Luis Reis, G. Khang, Exosome mediated transfer of miRNA-140 promotes enhanced chondrogenic differentiation of bone marrow stem cells for enhanced cartilage repair and regeneration, J. Cell. Biochem. (2020), https://doi.org/10.1002/jcb.29657.
[84] S. Chen, Y. Tang, Y. Liu, P. Zhang, L. Lv, X. Zhang, L. Jia, Y. Zhou, Exosomes derived from miR-375-overexpressing human adipose mesenchymal stem cells promote bone regeneration, Cell Prolif. 52 (2019), https://doi.org/10.1111/cpr.12669.
[85] Y. Lin, J. Wu, W. Gu, Y. Huang, Z. Tong, L. Huang, J. Tan, Exosome-liposome hybrid nanoparticles deliver CRISPR/Cas9 system in MSCs, Adv. Sci. 5 (2018), https://doi.org/10.1002/advs.201700611.
[86] S.M. Kim, Y. Yang, S.J. Oh, Y. Hong, M. Seo, M. Jang, Cancer-derived exosomes as a delivery platform of CRISPR/Cas9 confer cancer cell tropism-dependent targeting, J. Control. Release 266 (2017) 8–16, https://doi.org/10.1016/j.jconrel.2017.09.013.
[87] K.E. Petersen, E. Manangon, J.L. Hood, S.A. Wickline, D.P. Fernandez, W.P. Johnson, B.K. Gale, A review of exosome separation techniques and characterization of B16–F10 mouse melanoma exosomes with AF4-UV-MALS-DLS-TEM, Anal. Bioanal. Chem. 406 (2014) 7855–7866, https://doi.org/10.1007/s00216-014-8040-0.
[88] D.D. Taylor, S. Shah, Methods of isolating extracellular vesicles impact down-stream analyses of their cargoes, Methods 87 (2015) 3–10, https://doi.org/10.1016/j.ymeth.2015.02.019.
[89] G. Turturici, R. Tinnirello, G. Sconzo, F. Geraci, Extracellular membrane vesicles as a mechanism of cell-to-cell communication: advantages and disadvantages, Am. J. Physiol. – Cell Physiol. 306 (2014), https://doi.org/10.1152/ajpcell.00228.2013.
[90] J.Y. Kang, H. Park, H. Kim, D. Mun, H. Park, N. Yun, B. Joung, Human peripheral blood-derived exosomes for microRNA delivery, Int. J. Mol. Med. 43 (2019) 2319–2328, https://doi.org/10.3892/ijmm.2019.4150.