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REVIEW

Exosome engineering in cell therapy and drug delivery

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Abstract

Cell-derived exosomes have opened new horizons in modern therapy for advanced drug delivery and therapeutic applications, due to their key features such as low immunogenicity, high physicochemical stability, capacity to penetrate into tissues, and the innate capacity to communicate with other cells over long distances. Exosome-based liquid biopsy has been potentially used for the diagnosis and prognosis of a range of disorders. Exosomes deliver therapeutic agents, including immunological modulators, therapeutic drugs, and antisense oligonucleotides to certain targets, and can be used as vaccines, though their clinical application is still far from reality. Producing exosomes on a large-scale is restricted to their low circulation lifetime, weak targeting capacity, and inappropriate controls, which need to be refined before being implemented in practice. Several bioengineering methods have been used for refining therapeutic applications of exosomes and promoting their effectiveness, on the one hand, and addressing the existing challenges, on the other. In the short run, new diagnostic platforms and emerging therapeutic strategies will further develop exosome engineering and therapeutic potential. This requires a thorough analysis of exosome engineering approaches along with their merits and drawbacks, as outlined in this paper. The present study is a comprehensive review of novel techniques for exosome development in terms of circulation time in the body, targeting capacity, and higher drug loading/delivery efficacies.

Keywords Cargo incorporation · Exosome · Extracellular vesicles · Targeted delivery · Therapeutic applications

Introduction

Overview of Extracellular vesicles (EVs)

Synthetic drug delivery systems, including polymeric nanoparticles, dendrimers, micelles, and liposomes, have long been used to promote pharmaceutic's efficiency and therapeutic applicability in clinical settings (Elsharkasy et al. 2020). Despite the significant advantages of liposomes, as the oldest and most widely studied drug delivery vehicle, their applications are restricted due to their limited stability, long-term safety, and activation of an acute

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hypersensitivity reaction (Sercombe et al. 2015). The use of extracellular vehicles (EVs), as a natural carrier system, could overcome the barriers related to liposomes and other synthetic drug delivery systems (Butreddy et al. 2021). Depending on size, origins, morphology, and functions, EVs are classified into EVs and various types of plasma membrane-derived microvesicles. As a subset of EVs, exosomes are now receiving much attention from the scientific communities (Moloudizargari et al. 2022). The exosome formation occurs in three phases (Fig. 1): the budding, multivesicular body (MVB) formation, combination of the plasma membrane with MVBs, and the release of vesicular contents as exosomes (Ha et al. 2016). Compared to the apoptotic bodies (1000–5000 nm) and the microvesicles (50–1000 nm), which are respectively generated by the apoptotic cells and outward budding of the plasma membrane, exosomes biogenesis initiates with inward budding of the plasma membrane, which tends to start with the generation of intraluminal vesicles (ILVs) at early endosomes (Fu et al. 2020). Endocytosis leads to the creation of early endosomes that capture cellular proteins and genetic materials, found in the cytoplasm, and then turn into late endosomes, from which MVBs are generated (Chen et al. 2021a). MVBs are then degraded by lysosomes, or fused with the plasma membrane to free ILVs in the form of exosomes (Fu et al. 2020).

The therapeutic application of exosomes is of interest to many scholars. This includes their being used as (1) biomarkers that help diagnose a disease and the follow-up procedures, (2) drug delivery vehicles or therapeutic agents, and (3) immunomodulators that stimulate or suppress the immune system (Liu and Su 2019). Exosomes represent a mode of intercellular communication through various active biomolecules, including lipids, cytokines, growth factors, metabolites, proteins, and RNAs, during normal and pathological processes (de Abreu et al. 2020; Zhang et al. 2019). Exosomes' ability to modulate cellular communications and intracellular pathways has advanced their potential for controlling many diseases (Kalluri and LeBleu 2020). Exosomes are generated by different cell types and can be isolated either from different extracellular fluids like cerebrospinal fluid, blood, and urine or from cell culture supernatants (Zhang et al. 2019). Due to their presence in all biological fluids, exosomes can be considered a sensitive

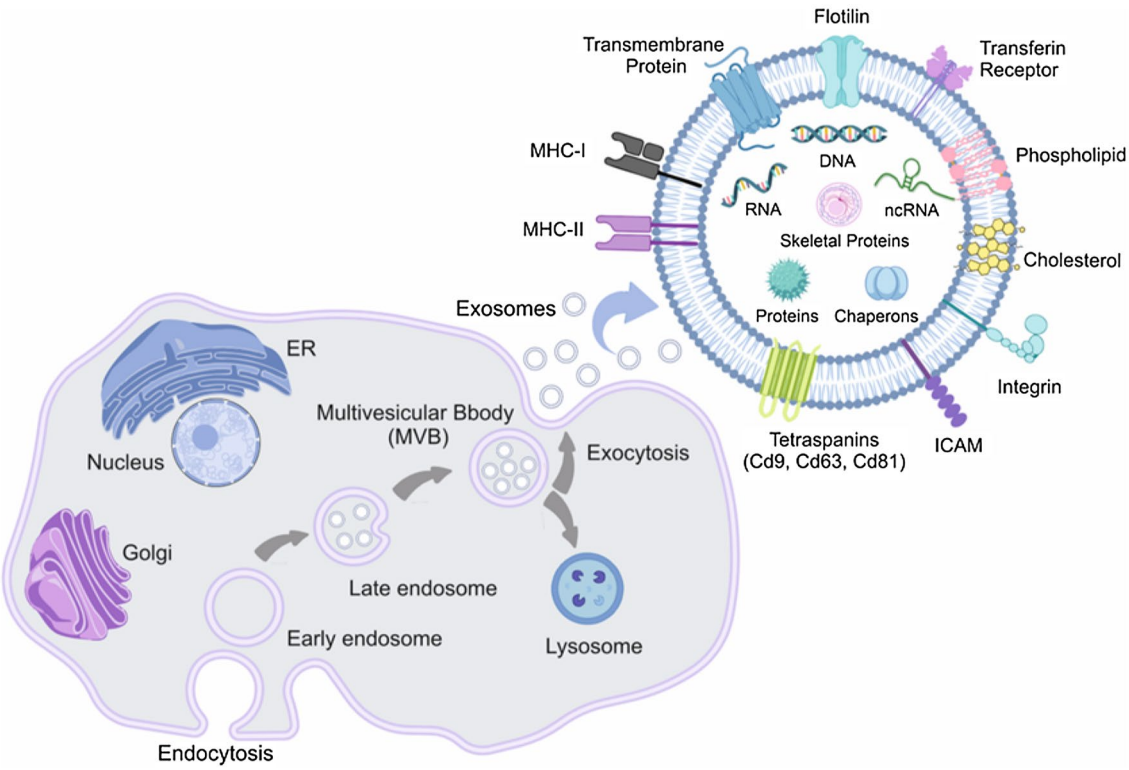

Fig. 1 Exosome biogenesis and its contents. Exosome formation is a function of endocytic membrane invagination and ILV formation inside cells. Early maturation of endosomes leads to the formation of MVBs which are then delivered to lysosomes to be degraded, or cross through microtubules to be combined with the plasma membrane and release exosomes into the extracellular space. In the process of maturation, exosomal cargos (RNAs, proteins, and lipids) are loaded onto ILV via pathways dependent or independent from ESCRT. Source cell cargos can be further delivered to target cells through fusion of direct membrane, endocytosis, and interaction of receptors with ligands

and reliable biomarker for the diagnosis, progression, and effective therapy for a range of diseases, such as tumors, chronic inflammation, metabolic diseases, cardiovascular and neurodegenerative diseases (Shafiee et al. 2021; Zhang et al. 2019). Minimally invasive sample collection, stability, and enrichment of specific exosomal biomarkers are the advantages of using exosomes as diagnostic tools (Wei et al. 2021a, b). Exosomes are attractive drug delivery vehicles, compared to other vehicles, due to their safety, stability, low toxicity, inherent targeting capabilities, high modification flexibility, and toleration by the immune system, even across the biological barriers (Weng et al. 2021). The low immunogenicity of exosomes facilitates their repeated administration, which is currently a major barrier in mRNA, gene, and cell therapies (Einabadi et al. 2020). The stability of exosomes to the drug molecules allows various therapeutic compounds to be transported over long distances under both natural and pathological conditions (Modani et al. 2021). Nevertheless, donor cells selection, exosome surface modification, and drug loading capacity play key roles in exosomal drug delivery (Modani et al. 2021).

Delivering various molecules to the adjacent cells or tissues located in different anatomical sites has made exosomes a unique candidate for vaccine development (Santos and Almeida 2021; Weng et al. 2021). Since antigens appear on exosomes and target cells, they could trigger the appropriate immune responses or act as an adjuvant (Kuçuk et al. 2021; Montaner-Tarbes et al. 2021). It is reported that exosomes are involved in tissue regeneration and homeostasis by affecting the fate decision of some immune cells (Lee et al. 2021; Sadeghi et al. 2020a, b; Taghavi-Farahabadi et al. 2021; Zhao et al. 2019). Also, the anti-inflammatory, proangiogenic and immunoregulatory activities are other unique features of exosomes in the design and development of vaccines (Kuçuk et al. 2021; Sadeghi et al. 2020a, b).

In addition, exosomes have been introduced to address key limitations of cell therapy (Marbán 2018). No risk of immune rejection and malignancy, stability, long-term maintenance, and ability to cross the biological barriers are prominent features that differentiate exosomes from their parent cells. Moreover, the standardization of the exosome manufacturing process is easier than that of cells (Jiang et al. 2020). As lipid bilayer vesicles, exosomes are tough enough to withstand a range of handling extremes and lyophilization (Marbán 2018). They can be used in combination with newly-developed methods or compounds to design carriers for specific particles. Exosomes can also be tailored to be applied to certain tissues or cells as they can move autonomously and reach the damaged tissues (Wei et al. 2021a, b). Furthermore, other determining factors on culture conditions or cell origin must be carefully examined, including biochemical composition, size, and related descriptive information (Wei et al. 2021a, b).

In this review, we summarize an introduction to the basic concepts of exosome, and provide a comprehensive discussion in regard to currently available strategies for exosomal cargo loading, and engineering techniques for targeted delivery and outline the advantages and disadvantages of these modification strategies. In addition, we highlight the ongoing challenges and future directions of this novel field.

Limitations in the therapeutic use of exosomes

The unique properties of exosomes and their ability to carry cargo have made them an ideal candidate for new therapeutic targets; however, some key factors restrict their therapeutic applications, such as barriers related to exosome isolation, characterization, quality check, and probability of functional assays to be reproduced in in-vitro and in vivo conditions, quick systemic circulation clearance, unsatisfactory targeting capability, and indistinct loading effectiveness (Chen et al. 2021a). Thus, the engineered exosomes could be an effective approach to overcome the existing limitations and expand their loading capacity for the desired therapeutic agents (Fu et al. 2020). Several examples of delivering therapeutic cargo by exosomes are presented in Fig. 2. In addition to cargo delivery, various strategies have been considered to improve the targeting of exosomes to successfully reach the recipient cells and facilitate cell uptake capacity (Syn et al. 2017). Some of these approaches are highlighted in the following sections.

Exosome modifications techniques to enhance cargo loading efficiency

To achieve optimal therapeutic cargo delivery and design favorable targeting elements, developing effective loading strategies for exosomes is crucial. Several bioengineering strategies could address the limited loading efficiency and impurity of exosomes (Weng et al. 2021). Diagnostic or therapeutic cargos in exosomes are generally loaded through two processes: exogenous and endogenous. In the exogenous or direct loading process, molecules are loaded onto the purified exosomes after isolation from cells (Kuçuk et al. 2021). This is further subdivided into active and passive loading. Passive loading involves loading the therapeutic cargo into exosomes through diffusion, while active loading is characterized by the disruption of exosome membranes through physically and chemically techniques (Han et al. 2021). The passive loading refers to exosome incubation with the therapeutic cargo. The loading capacity depends on the hydrophobic nature of the cargo molecules and incubation time (Balachandran and Yuana 2019). To overcome the limited loading capacity,

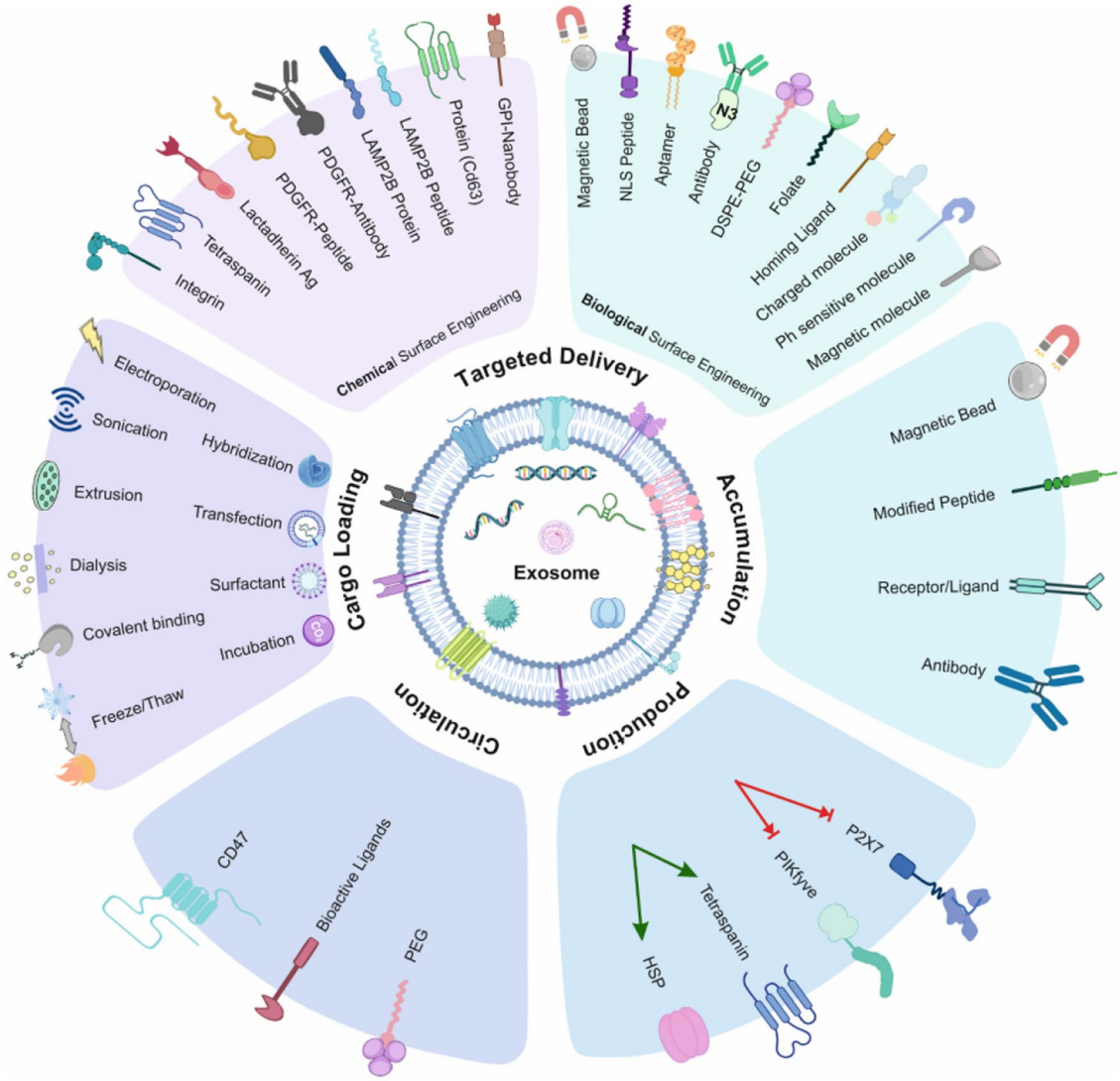

Fig. 2 Summary of exosomal modifications to address their limitation. Cell targeting specificity of exosomes with cell/tissue-specific peptides, tumor-specific receptors/ligands, or antibodies/nanobodies for tumor markers can be increased. For imaging or tracking purposes, exosomes with fluorescent protein or those displaying chemicals on the surface are applied. Moreover, exosome modification is found to decrease their chance of being cleared by liver and increase its concentration in circulation and the target tissue. Exosome stability is also promoted via exosome engineering by means of physical or chemical treatment, as well as surface modification, the result of which is enhanced delivery efficiency. A combined application of these methods is likely to boost cell targeting specificity and delivery efficacy

active cargo loading has been developed using various techniques (Baek et al. 2019). However, these methods are associated with several drawbacks, including exosome aggregation, membrane disruption, and excessive purification steps (Baek et al. 2019). Endogenous loading includes a system in which the therapeutic cargo is directly deposited by a donor cell into the exosome before its shedding. Modifying the parent cells is generally accomplished by incubating specific material with the parent cells. Another approach is gene editing, where parental cells can overexpress desired cargo that will subsequently be encapsulated into the exosomes (Kučuk et al. 2021). In this section, we will discuss exosome engineering methods applied throughout the literature.

Passive diffusion of exosome-secreting cells or exosomes cargos

Incubation of desired cargos with exosomes or exosome-secreting cells, as the simplest cargo loading technique,

results from a concentration gradient in the diffusion of cargos into the exosomes. Hydrophobic and lipid nature of plasma membrane facilitate the spontaneous incorporation of cargos, particularly hydrophobic ones, into exosomes or exosome-secreting cells (Fu et al. 2020). The loading efficiency depends on the cargoe's concentration gradient and its hydrophobicity (Zhang et al. 2020). Simple operation, non-destruction effect on exosome integrity, and maintaining the activity of cargos and exosomes are the biggest strengths of this strategy. However, loading is difficult to control, and pH can also influence loading efficacy. Additionally, drug toxicity is another problem that can impair exosome secretion (Fu et al. 2020; Luan et al. 2017). The efficiency of cargo loading for strategies that are based on incubation can be improved by manipulating concentration, the temperature of incubation, as well as modification volume and time. It can also be promoted by employing some techniques such as transfection and physical modifications (Chen et al. 2021a, b; Fu et al. 2020).

Physical treatments

Table 1 and Fig. 3 compare different physical treatments, including freeze–thaw, surfactant treatment, sonication, extrusion, dialysis, and electroporation, for loading cargos into exosomes. Although physical treatments improve the effectiveness of loading, they potentially damage and contaminate exosomes. This necessitates further analysis of experimental conditions to control micro-pores formation and membrane recombination process (Rayamajhi and Aryal 2020). Physical approaches have found application in exosomes labeling through imaging or the use of fluorescent tags. They are also used with other biological or chemical approaches to maintain exosome's homogeneous population size (Fu et al. 2020).

In situ synthesis and assembly

In situ synthesis and assembly is a non-invasive chemical reaction for loading the molecules into the exosome or their surfaces. In this technique, the exosome is maintained. However, it contains a complicated operation process that is associated with several technological challenges that may hinder its applications (Fu et al. 2020).

Surface engineering

Loading cargos into the exosomes requires bypassing the exosome membrane barrier (Liang et al. 2021). Biodistribution, targeting of specific cells, and the therapeutic use of exosomes depend on their surface properties; thus, the desired characteristics could be achieved using surface engineering techniques (Kučuk et al. 2021). Surface modifications of exosomes could be achieved using chemical modification, genetic engineering, or hybrid membrane engineering (Weng et al. 2021). In genetic engineering, as an appropriate technique for imparting exosomes with new properties, the targeting or ligand molecules are fused with the membrane proteins or lipids and subsequently overexpressed in the donor cells. The plasmid construction and protein overexpression in the donor cells are required for this technique (Liang et al. 2021). Unlike genetic engineering, chemical modification techniques can induce a large number of molecules using non-covalent or covalent interactions, which do not disrupt the exosome membrane. However, the complexity associated with membrane surfaces and issues related to additional steps of purification are key challenges that need to be addressed (Richardson and Ejima 2019). The details of each strategy are discussed in the following sections.

Chemical modification of the exosome membrane Chemical modification to modify exosome surfaces can be divided into non-covalent or covalent interaction strategies (Chen 2021). Covalent interactions are arguably superior to those using non-covalent interactions, as the probability that the interaction is disrupted is lower (Chen 2021). Using the covalent modification, functional groups form covalent bonds with exosomes. For instance, since sulfhydryl is widely presented on the exosomes surface, it is considered as the binding site via the michael addition reaction between maleimide and sulfhydryl (Nan et al. 2022). Exosome surface modification using covalent binding is done using a crosslinking reaction, known as azide-alkyne cycloaddition or click chemistry, and induces no alterations in exosome size and function (Parada et al. 2021a). Using this method, an azide or alkyl group is added to ethe xosome's surface to create active chemical sites to attach targeting moieties in a variety of aqueous buffers such as water, dimethyl sulfoxide (DMSO), and alcohols (Choi et al. 2021). This method is ideal for the biological bonding of small molecules, macromolecules, and polymers to the surface of exosomes via covalent bonds to desired functionality skills (Hood 2016). comparisonion to conventional chemical reactions, click chemistry is more efficient with higher control over the conjugation site (Parada et al. 2021a; Salunkhe et al. 2020), and contributes to loading or encapsulating the therapeutic agents and large plasmids such as CRISPR-Cas9 expression vectors into exosomes (Parada et al. 2021a). In this regard, different chemical strategies can be used to functionalize exosomes surface with amine bearing or thiol bearing functional moiety (Rayamajhi 2021).

However, toxic chemicals requirement is considered as the drawback of using covalent bonds, that raising caution for applying this strategy in therapeutics (Choi et al. 2021). Multivalent electrostatic interactions, hydrophobic

Table 1 A comparison of different strategies for loading cargos into exosomes

Method	Mechanism	Advantages	Disadvantages	Drug loaded/application
Incubation	Drugs and exosomes/parent cell incubation for some time (Gebeyehu et al. 2021)	Simple operation; do not require extra equipment (Chen et al. 2021a)	Low loading efficiency; drug cytotoxicity; impossible to control incorporation efficacy (Kučuk et al. 2021)	Drug (e.g., curcumin, porphyrins, paclitaxel, doxorubicin) si RNAs, catalase (Antimisiaris et al. 2018; Rahbarghazi et al. 2019)
Transfection	Gene edition (Fu et al. 2020)	Stability; high loading efficiency for peptides, proteins, and nucleic acids (Fu et al. 2020)	Time and financial consumption; hard to quantitation; poor controllability; **changes** membrane structure (Fu et al. 2020; Podolak et al. 2010)	Hydrophilic drugs or large molecules (e.g., DNA, RNA), proteins, peptides anti-cancer reagents, signal regulatory protein α (Théry et al. 2002)
Sonication	Extra mechanical shear force for weakening the integrity of exosomal membrane (Antimisiaris et al. 2018)	High loading efficiency; applicable for small RNAs; don't affect the membrane structure (Luan et al. 2017)	Not efficient for hydrophobic drugs; possibility of exosome integrity and cargo aggregation; effect on immune activity; heat generation (Luan et al. 2017)	Paclitaxel, catalase, siRNA, miRNA, ssDNA (Antimisiaris et al. 2018)
Dialysis	Dialyzing of the membrane by stirring to load the drug exosomes (Fu et al. 2020)	More efficient than incubation; increases miRNA and siRNA loading into exosomes (Luan et al. 2017)	Destructive effects on proteins and peptides in exosomes (Podolak et al. 2010)	Drugs, nucleic acid
Electroporation	Micro-pores in the exosome membrane under a short high-voltage pulse (Luan et al. 2017)	Loading with large molecules; useful for loading numerous drugs, specially siRNA or miRNA; ease in control (Chen et al. 2021a)	Disrupts exosome integrity; the risks of RNA aggregation; possibility of protein structure damaging; low loading capacity (Johnsen et al. 2016; Rahbarghazi et al. 2019)	siRNA, drug (porphyrins, doxorubicin, paclitaxel), dextran macromolecules (Antimisiaris et al. 2018; Rahbarghazi et al. 2019)
Extrusion	Loading of mixed exosomes and drugs into a syringe-based lipid extruder with 100–400 nm porous membranes under a controlled temperature (Luan et al. 2017)	Simple and effective; higher encapsulation rate compared to incubation and freeze–thaw cycles; uniform size distribution of exosome (Johnsen et al. 2016)	Possible deformation of membrane; the cytotoxicity and changes in the zeta potential and membrane protein; making exosomes visible to immune cells such as mononuclear phagocytes (Fuhrmann et al. 2015; Luan et al. 2017)	Porphyrins, catalase (Antimisiaris et al. 2018; Rahbarghazi et al. 2019)
Freeze/thaw method	Incubated drugs with exosomes at room temperature and frozen rapidly at − 80 °C or in liquid nitrogen and thawed at room temperature (Sato et al. 2016)	The drug loading capacity is simple and effective for various cargos (Fu et al. 2020)	Possibility of exosome membrane aggregation; lower efficiency than sonication and extrusion; unspecific loading efficiency (Fu et al. 2020)	Catalase, doxorubicin (Antimisiaris et al. 2018; Rahbarghazi et al. 2019)
Surfactant treatment (as saponin and triton)	Membrane permeability via selectively forming the complexes with membrane molecules (e.g. cholesterol) to form a pores structure on the membrane surface (Podolak et al. 2010)	Directly Incorporation of proteins; high encapsulation efficiency under right conditions (Chen et al. 2021a)	Hemolytic activity (Podolak et al. 2010)	Catalase, drug (porphyrins, doxorubicin (Antimisiaris et al. 2018)
In situ assembly and synthesis	Chemical reaction	Maintenance of exosome integrity (Fu et al. 2020)	Suitable for nanomaterials (Fu et al. 2020)	Nanomaterials (Fu et al. 2020)

Table 1 (continued)

Method	Mechanism	Advantages	Disadvantages	Drug loaded/application
Genetic engineering	Insertion, deletion, or modification of target genes at specific sites in the genome (Luan et al. 2017)	Simple (Kuçuk et al. 2021)	Possibility of membrane protein alteration (Kuçuk et al. 2021)	Drugs (e.g., 5-fluorouracil, doxorubicin, paclitaxel) miRNA, anti-miRNA-21, siRNA, imatinib (Liang et al. 2021)
Covalent binding	Biological binding of small molecules, macromolecules, and polymers to the surface of exosomes via covalent bonds (Luan et al. 2017)	Simple, rapid, and effective (Kuçuk et al. 2021)	Possibility of membrane protein alteration (Kuçuk et al. 2021)	Radionuclide and fluorescent agents, drugs (e.g., doxorubicin, cisplatin and 5-fluorouracil (5-FU), peptides or specific nanobodies, curcumin-SPION, siRNA, miRNA (Liang et al. 2021)
Hybridization	Combining exosomes with fusogenic liposomes (Kuçuk et al. 2021)	Enhanced efficacy (Kuçuk et al. 2021)	Decreasing retention of exosome (Kuçuk et al. 2021)	Drug (paclitaxel, gemcitabine HCl, doxorubicin), siRNA, mRNA, methotrexate, camptothecin (Ghitman et al. 2020)
Viral transduction	Overexpress of specific genes in donor cells (Chen et al. 2021b)	Stable loading of genetic-based cargos into exosomes and possible enrichment of the exosome functions (Chen et al. 2021b)	Concerns about safety risk, laborious and time-consuming (Chen et al. 2021b)	Drug [e.g., doxorubicin, cisplatin and 5-fluorouracil (5-FU)], macrolide antibiotics, nucleic acids (Ghitman et al. 2020)

insertion, and magnetic strength are commonly non-covalent strategies to provide stable modification of biological membranes (Armstrong et al. 2017; N'Diaye et al. 2022). In multivalent electrostatic interaction, exosome membranes are coated by a positive charge that divulges moiety and promotes the effectiveness of exosomes targeting towards biological membranes with negative charges (Carreira et al. 2016). Furthermore, the use of newly-produced exosomes with a positive surface charge has been shown to increase the ability of exosomes to be bound into and uptaken by recipient cells (Nakase and Futaki 2015). Cytotoxicity caused by certain cationic nanomaterials through hole formation and membrane thinning is a possible drawback of this methods (Nel et al. 2009). The major downside to this approach is that cells commonly take up cationic nanomaterials through endocytosis and this causes delivered payloads to be lysosomally degraded (Armstrong et al. 2017).

Due to the lipid bilayer of exosomes, hydrophobic interactions is considered as a direct insertion of targeting moieties to the membrane of exosome (Smyth et al. 2014). The transmembrane protein moiety or amine/carboxylic terminated phospholipid of exosome surface can be functionalized with different functional groups. In this regard, functionalized phospholipids can be incorporated into the membrane of exosome by simple incubation following hydrophobic insertion strategy (Rayamajhi 2021). With the help of hydrophobic sequestration, exosomes could be loaded with small lipophilic drugs, such as anti-inflammatory, chemotherapeutic, and photosensitizers agents (Armstrong et al. 2017). For example, this approach is commercially used in exosome membrane stains, such as commonly used dyes BODIPY TR ceramide, DiI, and PKH-67. However, it needs a simple coincubation to be used under loading-efficient ambient conditions that correlate positively with the hydrophobicity of the exogenous species (Fuhrmann et al. 2015).

Targeting drug delivery can also be achieved by exosomes manipulation through magnetic force (Qi et al. 2016), we will discuss this method in section of *exosome engineering for targeted delivery to specific tissues or cells*.

Hybrid membrane engineering The exosomal membrane can spontaneously fuse with other plasma membranes (Liang et al. 2021). Hybridization is a surface modification method that exosomes combined with fusogenic liposomes, which is facilitated by the lipid nature of the exosome's membrane (Choi et al. 2021). Exosome-liposome hybridization strategy have been applied to optimize the exosomal surface characterization to modify immunogenicity, improve colloidal stability, increasing their half-life in blood, and target cell uptake (Choi et al. 2021). The using an exosome-liposomes hybrid system called EXOPLEXs, large molecules can be delivered efficiently without compromising the exosome membrane structure (Goh et al.

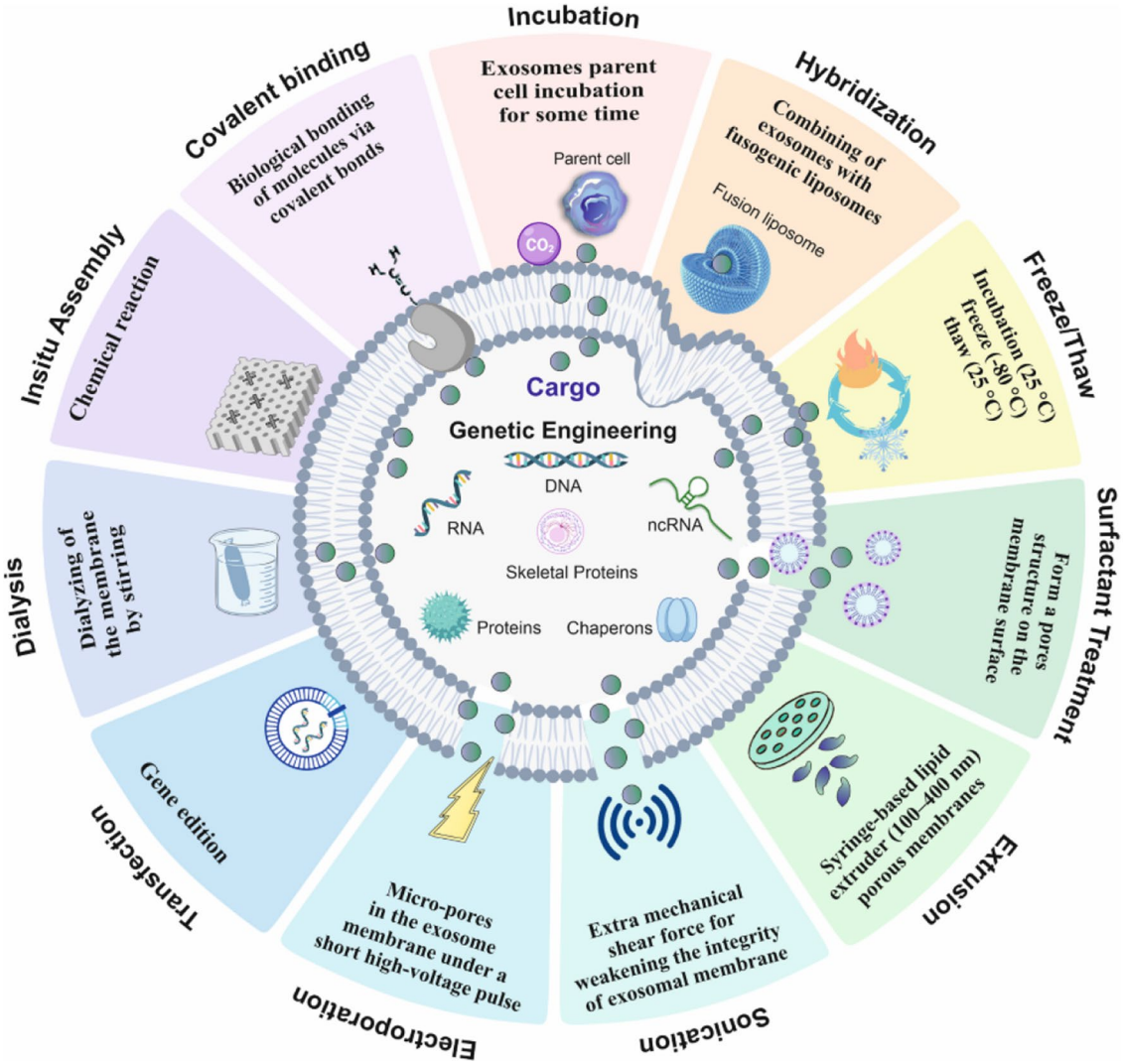

Fig. 3 Physical treatment methods of exosomes for improving therapeutic efficacy. Cargo loading into exosomes is performed through direct physical treatments. This is further facilitated through exosomal membrane pores generated by surfactant treatment, sonication, and electroporation. In the same vein, during membrane recombination processes, cargo loading is enhanced via extrusion, freeze–thaw treatment, and dialysis

2018). A hybrid membrane strategy is possible to modify the exosome surface by fusion with liposomes containing multiple ligands or polyethylene glycol (PEG) or to deliver the CRISPR-Cas9 system for targeted gene editing (Liang et al. 2021). The researcher evaluated several methods to encapsulate the CRISPR–Cas9 technology into extracellular vesicles, and found that exosome-liposomes hybrid system could become a unique technique to deliver the CRISPR–Cas9 in in vivo and in-vitro models (Shafiei et al. 2021).

Since the lipid composition has a major role to target cell uptake, exosomes hybridization can modify plasma membranes and facilitate their transfer into the target cell (Liang et al. 2021). For instance, it was shown that, exosomes hybridized with neutral or anionic liposomes had a higher cell uptake capability by carcinoma cell (Choi et al. 2021). Moreover, hybridization increases exosomes size, that contribute to decreases the in vivo retention, on the other hand it can improve the large cargos or drug encapsulation efficiency which is not possible in native exosome due to their small size (Choi et al. 2021). Additionally, the hybridization through PEG, can protect the hybrid system from immune cells via forming a hydration layer. Therefore, the engineered exosomes have higher stability and a longer turnaround time (Weng et al. 2021). Attenuation of exosome biological functions, due to altering the integrity and direction of membrane proteins, is considered as a drawback of this method (Choi et al. 2021). In addition, after coincubation, exosomes

separation from unbound lipid vesicles is fundamentally impossible (Gorshkov et al. 2022).

Genetic engineering

Using the gene modification techniques, target genes have been promoted to insertion, deletion, or modification at specific sites in the genome and have improved exosome functionality (Damasceno et al. 2020). Generally, this approach is achieved by loding cell with expression vectors (plasmid/virus) with target genes which is fused with various presented proteins in the exosomal membrane (Jia et al. 2021). Transfected cells were able to secrete exosomes with the targeting peptides on their surface (Richardson and Ejima 2019).

The viral transduction-based strategy is considered for delivery systems, due to stable and definite transfection properties (Chen et al. 2021b). Retrovirus, lentivirus, adenovirus, and adeno-associated virus have been extensively used as viral vectors for gene delivery. After virus infection, infected cells overexpress specific genes or regulate transcription, which could be loaded into exosomes. Following encapsulation, exosomes transport biologically-active viral components to distant non-infectious cells (Sancho-Albero et al. 2020). Viruses can enter the exosome biogenesis pathway and viral RNA genome, microRNAs, and proteins are incorporated into exosomes (Sancho-Albero et al. 2020). Therefore, by manipulating this process, exosomes can be modified to target the delivery of the drugs or genes of interest (Gilligan and Dwyer 2017). Viral transduction is suitable for a loading of variety of cells, which is inefficient with chemical transfection (Chen et al. 2021b). However, the viral transduction-base strategy is laborious and time-consuming, and its mechanism is unclear. In addition, the risk of pathogenicity and teratogenicity of viruses in exosomes requires further studies (Chen et al. 2021b). Thus, tissue specificity, non-immunogenicity, and non-toxicity are key factors of gene delivery vectors that determine their clinical application (Chen et al. 2021b).

Therapeutic genome editing enhances the ability of genome editing instruments to modify flawed genes correlated with the pathology of diseases. One such technology CRISPR/Cas9 is widely used for the treatment of infectious diseases, genetic diseases, and tumors because it is highly specific and efficient (Duan et al. 2021). CRISPR/Cas9 contains two components including Cas9, which is an RNA-guided endonuclease that can cleave double-stranded DNA, and a 20-nucleotide-long synthetic guide RNA (sgRNA) responsible for programming Cas9 sequence specificity for DNA cleavage (White et al. 2017). Cell genome can be modified at the location of interest by delivering the Cas9 nuclease complexed with a sgRNA into a cell, which allows for removal or in vivo editing of existing genes. Choosing an appropriate delivery vehicle to unlock the enormous translational potential of CRISPR/Cas9 for in vivo gene therapy is the major drawback of this approach, though considerable developments have been made in this area (Duan et al. 2021). To deliver CRISPR/Cas9 payload, an ideal vector, either viral or nonviral, can be used that is consistent, safe, non-immunogenic, and effective yet minimizes off-target activity and maintains targeting specificity. However, restrictions arising from the application of viral and nonviral vectors in gene therapy are solved using exosomes as a promising alternative delivery platform for CRISPR/Cas9 (McAndrews et al. 2021).

Exosome engineering for targeted delivery to specific tissues or cells

While some research suggests exosomes are ineffective at targeting cells, others show that they are excellent carriers for targeted delivery (Chen et al. 2021b). Exosomes derived from different cells and under specific conditions may be home to the specific sites. Exosome targeting as drug delivery platforms, can be enhanced by selecting specific exosome donors or bioengineering techniques (Chen et al. 2021b). In this regard, the exosome surface can be modified with homing-molecules through ligands, magnetic materials, charge affinity and pH-responsive motifs (Fu et al. 2020). Finally, by packing the drug into the modified exosomes, a targeted carriers to desired cell/organ can be achived that have play a better effect in clinical treatment (He et al. 2021). Due to drug accumulation in the target sites, the efficacy of exosomes could be improved, and the off-target effects reduced (Mosquera-Heredia et al. 2021). In the following sections, the techniques for improving the targeted delivery are discussed (see Table 2).

Ligand–receptor binding-based targeted delivery

Generally, various methods of cell–exosome interaction have been proposed. Exosomes enter the target cells through endocytic mechanisms such as micro- and macro-pinocytosis or phagocytosis and clathrin-/caveolin-mediated endocytosis. They, otherwise, release their content via extracellular proteases-mediated cleavage. Exosomes are internalized into the cells by fusing with the cellular membrane and activating specific signal pathways by ligand–receptor interaction (Gomari et al. 2018). These specific mechanisms endow their potential targeting capacity for delivering an extensive range of molecules. Today, targeted delivery based on ligand mediation is considered as a promising approach to drug delivery (Fu et al. 2020). Exosomes displaying targeting ligands are modified through various molecule conjugation approaches such as transfection and chemical modification (Liang et al. 2021).

Table 2 Exosome modification strategies to improve exosome targeting efficiency

Type of strategy	Source of EV	Loading method	Therapeutic cargo	Conjugated molecules	Targeting peptide/protein	Target receptor/organ	Administration route	Finding	References
Ligand–receptor binding (non-specific binding)	HEK293 cells	Transfection	Imatinib, BCR-ABL siRNA	Lamp2b	Fragment of Interleukin 3 (IL3)	Chronic myeloid leukemia	I.P.	↑ Targeting delivery ↓ Tumor growth in vitro and in vivo	Bellavia et al. (2017)
	HEK293 cells	Transfection	N/A	N/A	Vesicular stomatitis virus glycoprotein (VSVG)	DC, inducing pluripotent stem cells (iPSs)	In vitro	New genetically platform for exosome loading	Meyer et al. (2017)
	HEK293 cells	Transfection	5-fluorouracil anti-miRNA-21	Lamp2b	zHER affibody	Colorectal cancer (HCT-116)	I.V.	Reverse chemoresistance and improve cancer treatment efficiency	Liang et al. (2020a, b)
	MSC	Transfection	MSC exosomes	Lamp2b	Ischemic myocardium-targeting peptide CSTSMLKAC (IMTP) peptide	Cardiomyocytes (H9c2)	I.V.	↓ Inflammation, apoptosis, and fibrosis ↑ Vasculogenesis, and cardiac function	Wang et al. (2018)
	HEK293 cells	Transfection	Tpd50 siRNA	Lamp2b	DARPin	HER2-positive cells (SKBR3)	In vitro	↑ Targeting delivery of RNAi to HER2-positive cancer cells	Limoni et al. (2019)
	DC	Transfection	Curcumin	Lamp2b	Internalizing RGD peptide (iRGD)	αv-Integrin positive breast cancer cells	I.V.	↓ Tumor growth without overt toxicity	Tian et al. (2018)
	Cardiosphere-derived cells (CDCs)	Transfection	MSC exosomes	Lamp2b	CMP peptide	Cardiomyocytes	I.V. or intramyocardial	↑ Targeted delivery of MSC's exosomes	Mentkowski and Lang, (2019)
	HEK293T cells	Transfection	KRAS siRNA	Lamp2b	Internalizing RGD (iRGD) peptide	Adenocarcinoma, human alveolar basal epithelial cells (A549)	I.V.	↓ Tumor growth	Zhou et al. (2019)

Table 2 (continued)

Type of strategy	Source of EV	Loading method	Therapeutic cargo	Conjugated molecules	Targeting peptide/protein	Target receptor/organ	Administration route	Finding	References
	Immature DC (imDCs)	Transfection	Dox	Lamp2b	Internalizing RGD (iRGD) peptide	Breast cancer	I.V.	↑ Targeting delivery of Dox to tumor tissues ↓ Tumor growth without toxicity	Tian et al. (2014)
	HEK293T cells	Transfection	SOX2 siRNA	Lamp2b	Tlyp-1	Non-small cell lung cancer, A549 stem cells	In vitro	High transfection efficiency into lung cancer cell	Bai et al. (2020)
	Primary chondrocyte	Transfection	miRNA-140	Lamp2b	CAP peptide	Chondrocytes	Intra-articularly	↓ Osteoarthritis progression	Liang et al. (2020a, b)
	DC	Transfection	Kartogenin (KGN)	Lamp2b	E7 peptide	SF-MSCs	Intra-articular	Cartilage regeneration	Xu et al. (2021)
	DC	Transfection	miRNA-124	Lamp2b	RVG peptide	Cortical neural progenitors	I.V.	Promote neurogenesis after ischemia	Alvarez-Erviti et al. (2011)
	HEK293 cell	Transfection	Curcumin	Lamp2b	RBP (a RAGE-binding peptide)	RAGE over expressed cell	Intratracheal instillation	↑ Targeting delivery ↓ Inflammation	Kim et al. (2021a)
	HEK293T cells	Transfection	N/A	Lamp2b	RNA-binding protein HuR (human antigen R)	Lysosome	I.V.	↓ Liver fibrosis, miR-155 and other inflammatory genes in the liver injury mouse model	Li et al. (2020)
	Human renal epithelial cells	Transfection	Antisense miRNA oligonucleotides against miR-21 (AMO-21)	Lamp2b	Transferrin receptor-binding (T7) peptide	Glioblastoma	I.V.	↑ Targeting delivery to brain ↓ Tumor growth in glioblastoma mouse model	Kim et al. (2020)
	HEK293 cells	Transfection	Dox	Lamp2b	Designed Ankyrin Repeated Proteins (DARPins)	HER2-positive breast cancer cells	In vitro	↑ Uptake of targeted exosomes by HER2 + cells	Gomari et al. (2018)

Table 2 (continued)

Type of strategy	Source of EV	Loading method	Therapeutic cargo	Conjugated molecules	Targeting peptide/protein	Target receptor/organ	Administration route	Finding	References
	HEK293T cells, mouse liver AML12 cell	Transfection	miR-155	CD9	HuR (human antigen R), an RNA-binding protein	THP1 cell	I.V.	↑ RNA cargo encapsulation into engineered exosomes	Li et al. (2018a, b)
	HEK 293 T cells	Transfection	miRNA-26a	CD63	ApoA-1	Hepatocellular carcinoma (HepG2)	In vitro	↓ Tumor cell migration and proliferation	Liang et al. (2018)
	293F cells	Transfection	Antigen	CD63	OVA antigen	CD8 + T cells	I.V.	↑ Immunogenicity of cancer vaccines	Kanuma et al. (2017)
Ligand–receptor binding (specific binding)	HEK293 cells	Transfection	N/A	Phosphatidylserine-binding domains of lactadherin (C1C2	Anti-HER2 single chain variable fragments (scFv)	HER2 expressing cells	In vitro	↑ Targeting delivery ↑ Drug efficiency	Longatti et al. (2018)
	HEK293 cells	Transfection	N/A	Phosphatidylserine-binding domains of lactadherin (C1C2)	Anti-EGFR nanobody	Epiderma l growth factor receptors (EGFR)	In vitro	↑ Specific binding and uptake of exosome by EGFR-overexpressing tumor cells, without affecting exosome characteristic-tion	Kooijmans et al. (2018)
	HEK293 cells	Transfection	microRNA (miRNA)	Platelet-derived growth factor receptor (PDGFR)	GE11 peptide	Epidermal growth factor receptor (EGFR)-expressing breast cancer cells	I.V.	↑ Targeting delivery	Ohno et al. (2013)
	Neuro2A cells	Transfection	N/A	Glycosylphosphatidylinositol (GPI)	Anti-EGFR nanobodies	EGFR-expressing tumour cells	In vitro	A method for targeting delivery	Kooijmans et al. (2016)
	Bone marrow stromal cells	Chemical modification	N/A	Aldehyde	Aptamer	Bone	I.V.	↑ Bone mass, bone healing in a femur fracture mouse model	Luo et al. (2019)

Table 2 (continued)

Type of strategy	Source of EV	Loading method	Therapeutic cargo	Conjugated molecules	Targeting peptide/protein	Target receptor/organ	Administration route	Finding	References
	Macrophage	Chemical modification	PTX	Hydrophobic Insertion (DSPE-PEG-AA)	Aminoethylanisamide-polyethylene glycol (AA-PEG)	Sigma receptors overexpressed in lung cancer cells	I.V.	↑ Antineoplastic efficacy ↓ Tumor growth ↑ Survival time	Kim et al. (2018a, b)
	Human colorectal cancer cells	Chemical modification	Dox	1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC)	A33 antibodies	A33 positive cells	I.V.	↑ Tumor targeting ↓ Tumor growth ↑ survival time	Li et al. (2018a, b)
	MSCs	Chemical modification	Curcumin	Dibenzocyclooctyne-sulfo-*N*-hydroxysuccinimidyl ester (DBCO-sulfo-NHS)	cyclo(Arg-Gly-Asp-D-Tyr-Lys) peptide [c(RGDyK)]	integrin $\alpha_v\beta_3$ of cerebral vascular endothelial cells	I.V.	↓ Inflammatory response, and cellular apoptosis ↑ Targeting delivery to lesion region	Tian et al. (2018)
	HEK293T cells	Chemical modification	Erastin	DSPE-PEG-folate	Folate	Breast cancer (MDA-MB-231)	I.V.	Targeted induction of ferroptosis	Pi et al. (2018)
	Human tongue squamous cells	Chemical modification	Bcl-2 siRNA and paclitaxel	DSPE-PEG	Biotin, folate	Breast cancer cells	I.V.	↑ Tumor targeting and in vivo tumor imaging	Zhu et al. (2017)
	Human umbilical vein endothelial cells	Chemical Modification	DOX	1,2-distearoyl-sn-glycero-3-phosphoethanolamin epoly(ethylene glycol) (DSPE-PEG)	Biotin, avidin	Tumor cells	I.V.	↑ Targeting delivery with increased efficacy	Wang et al. (2017a, b)
	Blood	Chemical modification	Photosensitizer	C16	NLS peptide	Carcinoma (4T1), colorectal cancer (CT26)	I.V.	↑ Nuclear-targeted and photodynamic therapy	Cheng et al. (2019)
	MCF-7 cells	Chemical modification	Quantum dot photothermal agent	DSPE-PEG-RGD	Arg-Gly-Asp (RGD)	Breast cancer (integrin αvβ3-poitive MCF-7)	I.V.	Photothermal therapy	Cao et al. (2019)
	Leukemia cell line K562	Chemical modification	Mannosamine	DSPE-PEG-RGD	Arg-Gly-Asp (RGD)	αvβ3 overexpressing cells (HUVEC)	Injection into zebrafish embryos	↑ Angiogenesis with targeted imaging	Wang et al. (2017a, b)

Table 2 (continued)

Type of strategy	Source of EV	Loading method	Therapeutic cargo	Conjugated molecules	Targeting peptide/protein	Target receptor/organ	Administration route	Finding	References
	Bovine serum	Chemical modification	N/A	*N*-hydroxysuccinimide activated polyethylene glycol (NHS-PEG)	α-d-mannose	Mannose receptors on dendritic cells (DCs)	Intradermal	↑ Targeted uptake in DC ↑ Exosome accumulation in lymph node ↑ Immune response	Choi et al. (2019)
	Immature DC (imDCs)	Chemical modification	DOX	Diacyl lipid-(PEG)2	sgc8 aptamer	leukemia cells	In vitro	↑ Targeting delivery	Zou et al. (2019)
	Glioblastoma	Chemical modification	N/A	Palmitic anhydride	High-affinity ligand ($Lewis^Y$) for DC-SIGN	DC-SIGN	In vitro	↑ DC targeting	Dusoswa et al. (2019)
	Murine melanoma cells	Transfection and chemical modification	N/A	Streptavidin-lactadherin	CpG DNA	DC	Intratumoral or intradermal	↑ Exosome delivery to murine DC ↑ Antitumor efficacy	Morishita et al. (2016)
	DC	Chemical modification	PTX	Cholesterol-PEG (poly ethylene glycol)	Nucleolin-targeting aptamer AS1411	Breast cancer (MDA-MA-231)	I.V.	↑ Tatgeting delivery	Wan et al. (2018)
	L929 cells	Chemical modification	Methotrexate, KLA (Lys-Leu-Ala)	Lipid	ApoA-1 mimetic peptide	Glioma	I.V.	Selective brain tumor treatment	Ye et al. (2018)

Table 2 (continued)

Type of strategy	Source of EV	Loading method	Therapeutic cargo	Conjugated molecules	Targeting peptide/protein	Target receptor/organ	Administration route	Finding	References
pH gradient/surface charge target	HEK293 cells	Chemical modification	DOX	Octadecylamine, NHS and 1-(3-(dimethylamino) propyl)-3- ethylcarbodiimide hydrochloride (EDC·HCl)	Hyaluronic acid (HA)	CD44 expressing cancer cells	N/A	↑ Drug accumulation in drug resistant breast cancer cells ↓ Tumor growth ↑ Survival time	Liu et al. (2019)
	Serum	Chemical modification	Dox	Biotin-*N*-hydrosuccinimide ester (Biotin-NHS)	I-motif	Low pH environment	In vitro	↑ Drug releasing in an acidic pH-responsive manner ↑ Anti-proliferation activity in multi-drug-resistance breast cancer cell line (MCF-7/MDR)	Kim et al. (2018a, b)
	Mouse plasma	Chemical modification	N/A	Amine-reactive *N*-hydroxysuccinimide (NHS) ester	Molecule fluorophores	N/A	Intradermal	↑ Renal clearance with minimum non-specific tissue uptake ↑ Exosome accumulation in lymphatic system	Hwang et al. (2019)
Magnetism-guided target	Blood	Chemical modification	Dox	Transferrin	Ferroferric oxide	Cancer cells	I.V.	↑ Targeting delivery ↓ Tumor growth	Qi et al. (2016)
	Blood	Chemical modification	N/A	Carboxylated chitosan; transferrin	Superparamagnetic iron oxide nanoparticles (SPION)	Pancreas islet; tumor cells	I.V.	↓ Tumor growth	Zhuang et al. (2020)
	Blood; MSCs	Chemical modification	Curcumin	Neuropillin-1-targeted peptide	Superparamagnetic iron oxide nanoparticle (SPION)	Glioma	I.V.	↑ Targeting delivery and cross the blood–brain barrier (BBB)	Jia et al. (2018)

IV intravenous injection, *IP* intraperitoneal injection, *MSCs* mesenchymal stem cells, *NA* not applicable, *DC* dendritic cells, *Dox* doxorubicin, *PTX* paclitaxel

The genetic modification can be used through transfecting genes encoding targeting moiety (e.g., peptides, receptors and antibodies) that is fused with different membrane proteins of exosome (Choi et al. 2021). As shown in Table 2, generally two categories of transmembrane proteins are used for surface modification. First, the non-specific proteins that are presented on the different exosomes. Lysosome-associated membrane protein 2b (Lamp2b) and the tetraspanin superfamily proteins as the most important examples of these proteins are often chosen for exosomal modification (Armstrong et al. 2017). The N-terminus of Lamp2b, as an extracellular surface protein, can be appended with targeting sequences. Additionally, the tetraspanin protein family such as CD63/CD9/CD81 with four transmembrane domains are widly used for modification and protein fusion (Armstrong et al. 2017).

As a second categories of transmembrane proteins for exosome modification, can be referred to receptor membrane proteins that are present on specific exosomes [e.g. the epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), human epidermal growth factor receptor 2 (HER2), and Glycosylphosphatidylinositol (GPI)] (Chen et al. 2021b).

A wide range of bioactive ligands have been embedded in exosomes through modification strategies (Nan et al. 2022). Using antibodies is the most direct strategy for improving exosome targeting. Nevertheless, due to some limitation of antibodies including large size, complex structure and immune response induction, simpler fragments of antibodies such as single domain antibodies (sdAbs) or single chain variable fragments (scFvs) hav been widely used (Pham et al. 2021). In comparison, targeting peptide due to small size and lower immunogenicity have been utilized as carriers to target tumor-associated receptors (De et al. 2014). Receptor-targeting peptides can be used for improving effective accumulation of drugs at the site of interest (Liu et al. 2021). For example, as a shown in Table 2, RGD (Arg-Gly-Asp) peptide, a tripeptide motif, through specific binding to target integrin receptors and mesenchymal-epithelial transition factor (c-Met) binding peptides via target c-Met binding have been shown interesting results in glioblastoma and breast cancer targeted therapy (Tian et al. 2014; Zhou et al. 2019). Nucleic acid aptamers, as chemical antibodies, are synthetic single stranded DNA or RNA molecules with high affinity to their targets. Aptamers widely utilized in exosomal surface modification for targeted delivery due to their advantages including small size, low immunogenicity and simple chemical modification (Table 2) (Nan et al. 2022). Although, the specificity of this approach may offer some interesting in vivo opportunities, but the major downside is the synthetic challenge and cost of presenting functional ligands on the exogenous material, that should be adderessed (Armstrong et al. 2017). The loss of in vivo targeting efficacy due to immune response clearance, or enzymatic cleavage is considered as another limitations of aptamers in clinical use (Dutta and Paul 2022).

Chemical strategy

Despite the little information available, some chemical approaches can be used to display various natural and synthetic ligands via lipid assembly or conjugation reactions (He et al. 2021). Click chemistry which has described in previose section are applied to conjugate the small or macromolecules that would act as ligands on target cells (Said Hassane et al. 2006). Many different kinds of targeting moieties can be introduced by click chemistry for delivery systems. The molecules most investigated for targeting are folates, biotin, carbohydrates or polysaccharides (e.g. hyaluronic acid), cell-binding peptides (e.g. integrin ligands and cell-penetrating peptides), proteins (RGD peptides, cell-penetrating peptide (CPP)), monoclonal-Abs and oligonucleotides and aptamer (Taiariol et al. 2021). The select of these molecules is related to the varied size ranges, and composition of homing-molecules (Dutta and Paul 2022).

The pH gradient/surface charge-driven targeted delivery is another methods. As an instance, because of the acidic microenvironment around the tumor cells, due to excessive lactate formation and intracellular glycolysis, the pH level in tumor microenvironment is lowered compared to normal tissues, thereby conjugation of exosomes with pH-responsive systems of drug delivery has been considered as a controlled manner at a specific site and time (Table 2) (Fu et al. 2020; Yu et al. 2014).

The surface charge or lipophilicity of exosomes is involved in their cellular internalization, distribution, and targeting to desired organ/cells (Fu et al. 2020). Therefor, by optimizing the surface charge, the targeting efficiency of exosome could be controled toward the desired organs (Blanco et al. 2015). It is shown that positively charged exosomes mainly locate in the lungs, while anionic exosomes predominately accumulate in liver or kidney (Hwang et al. 2019). It has also been reported that, the fate of exosomes can be controlled with inherent charges of fluorescence probe. For instance, systemic administration of zwitterionic fluorophore-coated exosomes result in renal clearance with minimum non-specific uptake in major organs (Blanco et al. 2015). Additionally, surface charge modification of nanoparticles can be affected in different immunological processes. It is documented that cationic nanoparticles preferentially induce lung dendritic cell mediated immune responses wherase anionic formulations uptacked by alveolar macrophage exhibit less immunity

induction (Hwang et al. 2019). In addition, since nanoparticles with negative charge can evade from mononuclear phagocyte system, therefore, the specific surface charge of nanoparticles may affect their serum-protein interactions, circulation time, and homing (Hwang et al. 2019).

Physical strategies

Using physical strategies, the targeted delivery is expected to be realized using an external magnetic field. This way, to achive targeting delivery of drugs, the equipped exosomes with superparamagnetic nanoparticles are directed towards desired locations through a external magnetic force (Hwang et al. 2019). Equipped exosomes with magnetic particles like superparamagnetic iron oxide nanoparticles (SPIONs), is injected into the patient's blood circulation system and an external magnetic force is applied at the specific site (Table 2) (Ahmad et al. 2013). Simplicity and widespread use are the prominent features of this approach, but the short lifetime of magnetic materials and the unintended side effect of the magnetic nanoparticles on the exosomes' function require further research (Fu et al. 2020).

Exosome engineering for prolonging circulation

Exosome size allows them to diffuse passively into tumors through the enhanced permeability and retention (EPR) effect. However, several studies showed conflicting results regarding biodistribution and the arrival of exosomes at the target tissues (Aryani and Denecke 2016; Park 2013; Takahashi et al. 2013). Although cell source has a pivotal role in exosome biodistribution pattern, a significant proportion of injected exosomes is distributed systemically in the lung, liver, spleen, and gastrointestinal tract. Macrophage capture has a major role in exosome clearance of circulation (Chen et al. 2021b). These challenges might arise from a lack of sufficient information related to distribution, half-life, blood level, and urine clearance of exosomes (Yang et al. 2018). Biodistribution analysis of exosomes is critical to evaluate the effective dose and potential side effects associated with exosome applications (Das et al. 2018). Several reports demonstrated new strategies to modify exosome surface structures to effectively track exosomes in vivo and improve their biodistribution (Hu et al. 2015; Zhang et al. 2021a, b).

The biodistribution of exosomes can be modulated by engineering various factors such as bioactive ligands or synthetic molecules. Targeting of exosomes, not only increase the efficiency of exosome delivery, before being taken by the phagocyte cells, but also reduce out-of-target side effects through reducing the therapeutic dosage (Baek et al. 2019). Other strategies are needed to enhance the stability of exosomes (Meng et al. 2020). Further clinical applications of exosomes can focus on their manipulation to increase their lifetime in circulation while reducing their immune clearance (Chen et al. 2021a, b). This approach can be obtained through mimic the mechanisms used by cancer cells to hide from the immune system, via the expression of CD47, PD-L1, CD31, and CD24 molecules (Parada et al. 2021b). Phagocytosis inhibition through integration of some molecules associated with the "don't eat me" (such as CD47, CD24, CD31, CD44, PD-L1, β2M, App1 and DHMQ), would allow greater systemic bioavailability of the modified exosome due to their longer residence time in circulation (Parada et al. 2021b). For example, exosomes containing CD47 facilitate protection against phagocytosis by interacting with the α-ligand signal-regulating protein (SIRPα), and can be encouraging techniques to lengthen the biodistribution of exosomes. In addition, modified exosomes with some synthetic materials such as polyethylene glycol (PEG), can be used to coating the exosome to regulate their pharmacokinetics and biodistribution. PEGs, as a low toxicity chemical polymer, have demonstrated membrane-protective effects in a variety of cells or organs against various insults (Ferrero-Andrés et al. 2020). Nevertheless, cellular binding might be intervened because of PEG's shielding properties (H. Chen et al. 2021b). In addition, anti-PEG IgM is another challenge that contributor to the accelerated blood clearance of PEGylated nanoparticles (Mima et al. 2015).

Exosome engineering for large-scale production

One of the major drawbacks of using exosomes as delivery agents is their low extraction yield efficiency and consequently low encapsulation agents. Most exosome isolation techniques are labor-intensive, complex, and inefficient (Akuma et al. 2019). Scalable production and isolation of exosomes with high yield and purity while maintaining their structure is the main challenge associated with exosome-based therapies. Additionally, the isolation method must be cost-effective, and compatible with a high-throughput production process (Maumus et al. 2020). Generally, two major strategies have been used to increase exosome production (Jafari et al. 2020). First, some strategies, including genetic engineering to overexpress activator genes involved in exosome biogenesis and downregulate the related genes in exosome recycling pathways. Second, cell culture manipulation, and treatment with specific drugs (Jafari et al. 2020). In addition, the three-dimensional culture system can be another effective strategy to increase exosome production for the clinic (H. Chen et al. 2021b). In the following, we will discuss different types of methods to highly pure exosome isolation.

Genetic manipulation

As discussed earlier, exosome production can be improved through the manipulation of key genes involved in exosome biogenesis and recyclin. Some key genes that contribute to plasma membrane binding, trafficking, packaging, and secreting exosomes can be genetically modified through downregulation or overexpression, and this leads to an increase in the efficiency of exosome production (Jafari et al. 2020). For example, genetic manipulation via biogenesis activation (e.g., overexpression of heat shock protein (HSP), tetraspanin) and inhibition of exosome recycling [e.g., negative regulation of phosphoinositide kinase, FYVE-type zinc finger (PIKfyve)] can significantly increase exosomes secretion (Chen et al. 2021b).

It was reported that, overexpression of HSP 20, as a protective protein against different pathological conditions and stress, resulted in increase exosome formation via interaction with tumor susceptibility gene 101 (Tsg101) (Jafari et al. 2020). The tetraspanins proteins are involved in cellular signaling and ESCRT-independent exosome biogenesis. The overexpression of TSPAN6 and tetraspanin CD9 can release more exosomes, through interactions with multifunctional cytosolic adaptor (Guix et al. 2017; Schiller et al. 2018). Negative regulation of PIKfyve in the human prostate cancer epithelial cell line, have positively affect in the exosome production (Hessvik et al. 2016).

Exosome production can be manipulated by modifying the environment and cellular components involved at the beginning, middle, and end of the endolysosomal pathway to enhance ultimate function (Phan et al. 2018). For example, activation of P2X7 receptors (P2X7R) is associated with endosomal content sorting, fusion with the multivesicular body, and exosome secretion (Qu and Dubyak 2009). NSF-binding protein receptors (SNARES) and tumor suppressor activated pathway-6 (TSAP6) are respectively involved in the integration of the multinodular body into the plasma membrane and exosome release regulation (Phan et al. 2018). As part of the exosome trafficking activity, these proteins can mediate multivesicular body integration to the plasma membrane and more exosome secretion. Overexpression of regulatory lipids, such as the phospholipase D2 (PLD2), gene secondary messengers involved in endocytosis and exocytosis, improves cells' exosome secretion (Laulagnier et al. 2004). It was shown that PLD2 activity in cells was correlated to the amount of exosome released (Laulagnier et al. 2004).

Exosomes mimics/mimetics

The small production of exosomes by parent cells and low loading efficiency, as a major barrier to their translation to the clinic, can be addressed by exosome-mimetic vesicles. Given that not all of the exosome's components are required for their proper functioning, engineered exosome-mimics could be a novel platform for the delivery of functional components and drug molecules (Kooijmans et al. 2012). These engineered exosomes can be generated via serial extrusion or cell membrane-cloaked nanoparticles or assembly of liposomes harboring only crucial components of natural exosomes (Kooijmans et al. 2012; Modani et al. 2021). The cells or plasma membrane are extruded through 100—400 nm porous membranes to generate spherical nanovesicles or membrane-enclosed polymer nanoparticles (Wang et al. 2021). Exosome mimetics can be easily produced with a 100-fold higher yield than naturally exosomes which represents them as advantageous in clinical-scale production (Jang and Gho 2014). Exosome mimetics have stability, distribution, and immuno-compatibility similar to exosomes but with less complexity than exosomes. These membrane-bounded exosomes can also be modified to improve their cellular uptake and targeting properties (Wang et al. 2021). Besides, the inclusion of specific peptides onto the cell membranes makes exosome-mimics as suitable vehicles to deliver pharmaceutics in an effective and safe manner (Kooijmans et al. 2012). It is also possible to easily modify exosomes by fusing modified cells-derived exosomes with liposomes embedded with antibodies, peptides, or PEG (Das et al. 2018). The use of exosome mimetics would be more controllable and scalable for clinical settings (Aryani and Denecke 2016). However, exosomal components that are likely to be required for the assembly of functional exosome mimetics are not yet well defined (Kooijmans et al. 2012).

Biomaterial and modification in culture method

3D-culture method Cell-to-cell contact supports cell differentiation and immunomodulation potential, which is not appropriately reflected in the 2D culture methods (Brennan et al. 2020). Therefore, better physiological in-vitro conditions can be achieved either using 3D matrices or scaffold-free (i.e., spheroids) (Egger et al. 2018). Using a 3D culture, the limited surface area can be maximized for exosome yield, but the resulting value falls far apart from the large-scale production value. The microcarriers and hollow-fiber bioreactors are currently used to expand large-scale cells in a 3D environment (Maumus et al. 2020; Vymetalova et al. 2020). A higher cell yield in a shorter time with less contamination risk is the advantage of this method (Maumus et al. 2020; Phan et al. 2018). Microcarriers are small beads manufactured from various materials with different pore sizes and surface characteristics that could support high-scale yield within a shorter incubation time (Maumus et al. 2020). Since the cells are much more metabolically active in this method, more nutrients are required to change the culture medium frequently (Maumus et al. 2020). It has been

reported that 3D spherical culture, in addition to improving exosome production, induces the therapeutic potential of MSCs, including anti-inflammatory and proangiogenic functions (Lee and Kang 2020; Zimmermann and McDevitt 2018).

Biomaterials Biomaterials affect the secretion of exosomes and their biological function (Wu et al. 2021). As a bioactive scaffold, they are involved in cell culture and improve the engraftment and function of transplanted cells by providing a desirable microenvironment (Zhang et al. 2021a, b). Cell incorporation into the structured and modified biomaterials provides a protective microenvironment and mimics the natural extracellular matrix (ECM) (Xu et al. 2019). Due to the important role of the biomaterials on lineage specification, the mechanical, chemical, electrical, and morphological properties need to be embedded in the design of new scaffolds (Xu et al. 2019).

Current challenges in exosome-based therapies

The heterogeneity of exosomes

Because cells release large numbers of exosomes with diverse biological effects, the biggest challenge at this stage is addressing the heterogeneity of secreted exosomes. Exosome-based therapy requires a better understanding of the biogenesis, composition, and heterogeneity of exosomes (Willms et al. 2018). Although exosomes derived from similar cells were expected to be of identical composition, the results showed that these exosomes could have different molecular compositions, as well as targeting moiety. Exosome heterogeneity introduces an extra level of complexity in their design and dose standardization and delivery in clinical approaches. Exosome heterogeneity can be explained by ESCRT (endosomal sorting complex required for transport)-dependent and -independent pathways, as a key mediator of MVBs biogenesis (Yang et al. 2018). Therefore, deeper research of the heterogeneity and cargo composition of the exosome is critical not only to identify suitable subpopulations for specific therapeutic purposes but also to prevent the side effects associated with heterogeneity. So, improving the sensitivities and characteristics of exosome heterogeneity detection methods is critical for a better understanding of exosome characterization in both physiological and pathophysiological processes, and finally, accelerates the expansion of their therapeutic and diagnostic applications (Willms et al. 2018).

In addition to the high-scale production of exosomes, their purity and physicochemical properties are affected by choice of isolation methods. The subpopulations of the exosomes collected using different separation methods are variable. This heterogeneity can be effective in the therapeutic potentials of isolated exosomes. Thus, optimizing the isolation method is important not only to preserve the properties of the exosomes but also to reduce the associated side effects (Yamashita et al. 2018).

Choice of cells

Cells with varying functions are reported to secret exosomes, but the question of what the ideal cell is for our research is yet to be answered (Wei et al. 2021a, b). Due to the importance of the composition and surface markers of exosomes in their function, depending on the source cell, therapeutic approaches can benefit significantly from the biological characteristics of exosomes isolated from different cell types (Luan et al. 2017). The in vivo behavior of exosomes is subjected to the characteristics of parent cell. For example evidence shown the different pattern distribution from transplanted bone marrow dendritic cells, melanoma and muscle cell-derived exosomes in the spleen, lung and liver, respectively (Hwang et al. 2019). It was also reported that neutrophil-derived exosomes have blood–brain barrier penetration capability, and can be used for drug delivery enter into brain and target to glioma (Nan et al. 2022).

In addition, the functional characteristics of exosomes is depending on their origin (Lee et al. 2022). The use of tumor-derived exosomes to deliver therapeutic agents such as chemotherapeutic or anti-cancer agents or to develop vaccines for immunotherapy can be interesting from different aspects. Tumor exosomes can induce the immune system against tumor cells by carrying tumor-associated antigens as well as MHC class I molecules. The self-tolerance in tumour microenvironment dampens the therapeutic effect of T cell responses (Perocheau et al. 2021). Due to counteract tumour immunosuppressive microenvironment by activation of immune response, tumour-derived exosomes can address limitation in current immunotherapies (Perocheau et al. 2021). Additionally, due to tumor- specific targeting capabilitie and preferential tropism of tumour-derived exosomes towards their parent cell type, choosing appropriate sources could be important for further studies (Xu et al. 2020). Nevertheless, tumor exosomes are risky and may potentially threaten patients' health. Hence, the application of tumor exosomes can be avoided since different cell types give rise to exosomes (Luan et al. 2017). Exosomes are released from different cell lines, but the rate at which they are released and the extent to which they are susceptible to modifications vary significantly (García-Manrique et al. 2018). The red blood cell (RBC)-derived exosomes were

suggested as a delivery vehicle with several advantages (Kim et al. 2021a, b). The blood units are easily available source from blood banks and patients. Since RBCs are enucleated cell types, so reduced gene-related risks including horizontal gene transfer are expected. In addition, the possibility of immunogenic responses risks can be minimized through matching blood types between donors and recipients (Kim et al. 2021a, b). Another economically practical and scalable source of exosomes for alternative therapeutic options is agricultural products such as fruits and milk. Theses exosomes loaded with various drugs are considered as a strategy for the mass production of exosomes. These exosomes may be highly productive and have safety profiles, but they fail to boosting host immune system (Luan et al. 2017). Immune cell-derived exosomes have received great attention for drug delivery and vaccination. Exosomes derived from monocytes and macrophages have longer stability by escaping phagocytosis, which increases their efficiency. Also, exosomes derived from DC for vaccine delivery cells have been shown to facilitate tumor rejection by transferring peptide-MHC complexes to other DCs, not in contact with the same antigen (Luan et al. 2017). Among various cell types, mesenchymal stem cells (MSCs) are also considered the most promising sources of exosomes for clinical application in that they can be isolated from many tissues and have a high ex vivo expansion capacity (Lee et al. 2021; Sadeghi et al. 2020a, b). In addition, their immunomodulatory effect is important in autologous and allogenic therapeutic applications.

Choosing loading procedures

Specifically targeted designer exosomes that represent certain cargos through genetic engineering and some chemical/mechanical methods can prove very helpful in meeting medical challenges in today's world (Kalluri and LeBleu 2020; Liao et al. 2019; Zhang et al. 2019). Different loading strategies of exosomes not only expand loading efficiency but also can partially resolve the integrity and biological limitation of exosomes (Xu et al. 2020). Therefore, the appropriate method or new development strategy must be carefully evaluated, considering advantages and limitations. For example, multiple loading methods and a combination of several strategies are effective in increasing the loading potential (Xu et al. 2020). However, despite great progress, the specific exosome modification to enhance the targeting ability is unclear and needs to be studied (Xu et al. 2020). Also, the possible risk of changing exosome content or protein composition, impaired biological responses, and promiscuous interactions during modification should also be considered (136). Therefore, care must be taken in choosing the transformation method to achive better encapsulation or loading efficiency with the least influence on the exosome composition, integrity and morphology (Liu and Su 2019).

Additionally, all these loading modifications can be affected by exosome quality, purity, and their storage conditions. Therefore, future studies shoulb be focused to control these factors and estimate the therapeutic dose of exosomes for drug delivery (Mosquera-Heredia et al. 2021).

Exosome administration routes

Given that the biological effect of exosomes is exerted by their uptake by target cells, knowledge of the biological distribution of exosomes is required for therapeutic application. Different administration routes are effective for rapid clearance, biological distribution, and therapeutic effects of exosomes (Zhang et al. 2020). Due to the lack of proper lymphatic and vascular drainage in solid tumors, the *intravenous injection* can be effective in the extravasation and retention of the exosomes on the tumor side. Also, the short half-life index of circulating exosomes is one of the major limitations of this route administration (Kuçuk et al. 2021). Additionally, the accumulation of intravenously injected exosomes in the liver, spleen, and lung may be due to increased vascular permeability resulting from injury and inflammation (Yamashita et al. 2018). Although, the use of PEGylating can address this limitation by preventing the rapid clearance of exosomes from circulation (Kuçuk et al. 2021). *Local injection* and direct injection of loaded exosomes with a therapeutic or targeting agent is a suitable administration route for the specific delivery of therapeutic agents to desired sites (Kuçuk et al. 2021). The possibility of loading larger doses of exosomes is approached by the *intraperitoneal route*; however, due to the large area of the peritoneal cavity, injected exosomes rapidly dilute and spread to more distant sites (Kuçuk et al. 2021). Although *oral administration* is easy and convenient, enzymatic activity, severe acid–base changes, intestinal barrier, and intestinal microflora are problems regarding exosome delivery to the target tissue (Kuçuk et al. 2021). The *intranasal administration* is a more effective route, particularly in overcoming the challenges associated with drug delivery across the blood–brain barrier (BBB). This route avoids intestinal and hepatic metabolism of the exosome, thereby preserving exosomal vesicles in brain tissue (Kuçuk et al. 2021). The non-invasive administration by *inhalation* is one of the most effective routes of therapeutic agents for various lung diseases. The effectiveness of this administration route is closely related to the properties and amount of drug uptake by receptor cells, respiratory tract geometry, breathing pattern, and mucociliary clearance (Sajnani et al. 2021). Particularly, in COVID-19, inhalation of MSC-derived exosomes significantly promoted lung repair (Sajnani et al. 2021).

Conclusion

The ideal properties of exosomes make them unique carriers for drug delivery purposes. However, in the field of exosome-based therapy, there are several challenges such as short circulating half-life, low targeting, and poor efficiency that limit their applications. Exosome engineering and incorporation of cargo have proven to be successful in engineering exosomes with desirable diagnostic and therapeutic attributes. The engineering technology with homing peptides or specific ligands and component modifications facilitates exosomes' biodistribution and improves their therapeutic efficacy. The bioengineering approaches can also enhance targeted delivery outcomes and allow using reduced doses of therapeutics, which is critical to their clinical application. Nevertheless, there are still obstacles that need to be removed. For example, various exosome functionalities arising from different sources and the number of exosomes to get a desired therapeutic effect have not been fully studied. Also, the heterogeneity of diseases is a critical key that may affect the therapeutic outcome, and knowledge about exosome modification to have a high degree of specificity for a specific target is unclear (Von Schulze and Deng 2020). Also, cell surface markers of particular interest need to be identified to develop highly specific exosomes that can be used as effective drug carriers. Despite some potential setbacks and challenges, exosomes promise a potent application in clinical setting and further studies are required to assess the safety and efficacy of a new generations of exosome, and to assess the differences between exosomes secreted by different cell types, the composition of these vesicles, and their biological destiny after delivery into the body.

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Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose and declare no conflict of interest.

References

Ahmad M, Minhas MU, Sohail M, Faisal M, Rashid H (2013) Comprehensive review on magnetic drug delivery systems: a novel approach for drug targeting. J Pharm Altern Med 2(4):13–21

Akuma P, Okagu OD, Udenigwe CC (2019) Naturally occurring exosome vesicles as potential delivery vehicle for bioactive compounds. Front Sustain Food Syst 3:23

Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJ (2011) Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nat Biotechnol 29(4):341–345

Antimisiaris SG, Mourtas S, Marazioti A (2018) Exosomes and exosome-inspired vesicles for targeted drug delivery. Pharmaceutics 10(4):218

Armstrong JP, Holme MN, Stevens MM (2017) Re-engineering extracellular vesicles as smart nanoscale therapeutics. ACS Nano 11(1):69–83

Aryani A, Denecke B (2016) Exosomes as a nanodelivery system: a key to the future of neuromedicine? Mol Neurobiol 53(2):818–834

Baek G, Choi H, Kim Y, Lee HC, Choi C (2019) Mesenchymal stem cell-derived extracellular vesicles as therapeutics and as a drug delivery platform. Stem Cells Transl Med 8(9):880–886

Bai J, Duan J, Liu R, Du Y, Luo Q, Cui Y et al (2020) Engineered targeting tLyp-1 exosomes as gene therapy vectors for efficient delivery of siRNA into lung cancer cells. Asian J Pharm Sci 15(4):461–471

Balachandran B, Yuana Y (2019) Extracellular vesicles-based drug delivery system for cancer treatment. Cogent Med 6(1):1635806

Bellavia D, Raimondo S, Calabrese G, Forte S, Cristaldi M, Patinella A et al (2017) Interleukin 3-receptor targeted exosomes inhibit in vitro and in vivo chronic myelogenous leukemia cell growth. Theranostics 7(5):1333

Blanco E, Shen H, Ferrari M (2015) Principles of nanoparticle design for overcoming biological barriers to drug delivery. Nat Biotechnol 33(9):941–951

Brennan MÁ, Layrolle P, Mooney DJ (2020) Biomaterials functionalized with MSC secreted extracellular vesicles and soluble factors for tissue regeneration. Adv Func Mater 30(37):1909125

Butreddy A, Kommineni N, Dudhipala N (2021) Exosomes as naturally occurring vehicles for delivery of biopharmaceuticals: insights from drug delivery to clinical perspectives. Nanomaterials 11(6):1481

Cao Y, Wu T, Zhang K, Meng X, Dai W, Wang D et al (2019) Engineered exosome-mediated near-infrared-II region V2C quantum dot delivery for nucleus-target low-temperature photothermal therapy. ACS Nano 13(2):1499–1510

Carreira SC, Armstrong J, Seddon A, Perriman A, Hartley-Davies R, Schwarzacher W (2016) Ultra-fast stem cell labelling using cationised magnetoferritin. Nanoscale 8(14):7474–7483

Chen D (2021) Potential of surface modified exosomes for extended intravitreal drug delivery. University of Waterloo, Waterloo

Chen H, Wang L, Zeng X, Schwarz H, Nanda HS, Peng X, Zhou Y (2021a) Exosomes, a new star for targeted delivery. Front Cell Dev Biol 8;9:751079

Chen H, Wang L, Zeng X, Schwarz H, Nanda HS, Peng X, Zhou Y (2021b) Exosomes, a new star for targeted delivery. Front Cell Dev Biol 8;9:751079

Cheng H, Fan J-H, Zhao L-P, Fan G-L, Zheng R-R, Qiu X-Z et al (2019) Chimeric peptide engineered exosomes for dual-stage light guided plasma membrane and nucleus targeted photodynamic therapy. Biomaterials 211:14–24

Choi ES, Song J, Kang YY, Mok H (2019) Mannose-modified serum exosomes for the elevated uptake to murine dendritic cells and lymphatic accumulation. Macromol Biosci 19(7):1900042

Choi H, Choi Y, Yim HY, Mirzaaghasi A, Yoo J-K, Choi C (2021) Biodistribution of exosomes and engineering strategies for targeted delivery of therapeutic exosomes. Tissue Eng Regener Med 18(4):499–511

Damasceno PKF, de Santana TA, Santos GC, Orge ID, Silva DN, Albuquerque JF et al (2020) Genetic engineering as a strategy to improve the therapeutic efficacy of mesenchymal stem/stromal cells in regenerative medicine. Front Cell Dev Biol 8:737

Das CK, Jena BC, Banerjee I, Das S, Parekh A, Bhutia SK, Mandal M (2018) Exosome as a novel shuttle for delivery of therapeutics across biological barriers. Mol Pharm 16(1):24–40

De G, Ko J-K, Tan T, Zhu H, Li H, Ma J (2014) Amphipathic tail-anchoring peptide is a promising therapeutic agent for prostate cancer treatment. Oncotarget 5(17):7734

de Abreu RC, Fernandes H, da Costa Martins PA, Sahoo S, Emanueli C, Ferreira L (2020) Native and bioengineered extracellular vesicles for cardiovascular therapeutics. Nat Rev Cardiol 17(11):685–697

Duan L, Ouyang K, Wang J, Xu L, Xu X, Wen C et al (2021) Exosomes as targeted delivery platform of CRISPR/Cas9 for therapeutic genome editing. ChemBioChem 22(24):3360–3368

Dusoswa SA, Horrevorts SK, Ambrosini M, Kalay H, Paauw NJ, Nieuwland R et al (2019) Glycan modification of glioblastoma-derived extracellular vesicles enhances receptor-mediated targeting of dendritic cells. J Extracell Vesicles 8(1):1648995

Dutta A, Paul S (2022) Advancement in Exosome-based cancer therapeutics: a new era in cancer treatment. Front. Nanotechnol 4:939197

Egger D, Tripisciano C, Weber V, Dominici M, Kasper C (2018) Dynamic cultivation of mesenchymal stem cell aggregates. Bioengineering 5(2):48

Einabadi M, Ai J, Kargar M, Kafilzadeh F, Taghdiri Nooshabadi V, Jamali H (2020) Mesenchymal cell-derived exosomes as novel useful candidates for drug delivery. Arch Neurosci 7(2)

Elsharkasy OM, Nordin JZ, Hagey DW, de Jong OG, Schiffelers RM, Andaloussi SE, Vader P (2020) Extracellular vesicles as drug delivery systems: why and how? Adv Drug Deliv Rev 159:332–343

Ferrero-Andrés A, Closa D, Roselló-Catafau J, Folch-Puy E (2020) Polyethylene glycol 35 (PEG35) modulates exosomal uptake and function. Polymers 12(12):3044

Fu S, Wang Y, Xia X, Zheng JC (2020) Exosome engineering: current progress in cargo loading and targeted delivery. NanoImpact 20:100261

Fuhrmann G, Serio A, Mazo M, Nair R, Stevens MM (2015) Active loading into extracellular vesicles significantly improves the cellular uptake and photodynamic effect of porphyrins. J Control Release 205:35–44

García-Manrique P, Matos M, Gutiérrez G, Pazos C, Blanco-López MC (2018) Therapeutic biomaterials based on extracellular vesicles: classification of bio-engineering and mimetic preparation routes. J Extracell Vesicles 7(1):1422676

Gebeyehu A, Kommineni N, Meckes Jr DG, Sachdeva MS (2021) Role of exosomes for delivery of chemotherapeutic drugs. Crit Rev Ther Drug Carrier Syst 38(5):53–97

Ghitman J, Biru EI, Stan R, Iovu H (2020) Review of hybrid PLGA nanoparticles: future of smart drug delivery and theranostics medicine. Mater Des 193:108805

Gilligan KE, Dwyer RM (2017) Engineering exosomes for cancer therapy. Int J Mol Sci 18(6):1122

Goh WJ, Zou S, Lee CK, Ou Y-H, Wang J-W, Czarny B, Pastorin G (2018) EXOPLEXs: chimeric drug delivery platform from the fusion of cell-derived nanovesicles and liposomes. Biomacromol 19(1):22–30

Gomari H, Moghadam MF, Soleimani M (2018) Targeted cancer therapy using engineered exosome as a natural drug delivery vehicle. Onco Targets Ther 11:5753

Gorshkov A, Purvinsh L, Brodskaia A, Vasin A (2022) Exosomes as natural nanocarriers for RNA-based therapy and prophylaxis. Nanomaterials 12(3):524

Guix FX, Sannerud R, Berditchevski F, Arranz AM, Horré K, Snellinx A et al (2017) Tetraspanin 6: a pivotal protein of the multiple vesicular body determining exosome release and lysosomal degradation of amyloid precursor protein fragments. Mol Neurodegener 12(1):1–21

Ha D, Yang N, Nadithe V (2016) Exosomes as therapeutic drug carriers and delivery vehicles across biological membranes: current perspectives and future challenges. Acta Pharmaceutica Sinica B 6(4):287–296

Han Y, Jones TW, Dutta S, Zhu Y, Wang X, Narayanan SP et al (2021) Overview and update on methods for cargo loading into extracellular vesicles. Processes 9(2):356

He J, Ren W, Wang W, Han W, Jiang L, Zhang D, Guo M (2021) Exosomal targeting and its potential clinical application. Drug Deliv Transl Res 12(10):2385–2402

Hessvik NP, Øverbye A, Brech A, Torgersen ML, Jakobsen IS, Sandvig K, Llorente A (2016) PIKfyve inhibition increases exosome release and induces secretory autophagy. Cell Mol Life Sci 73(24):4717–4737

Hood JL (2016) Post isolation modification of exosomes for nanomedicine applications. Nanomedicine 11(13):1745–1756

Hu L, Wickline SA, Hood JL (2015) Magnetic resonance imaging of melanoma exosomes in lymph nodes. Magn Reson Med 74(1):266–271

Hwang DW, Jo MJ, Lee JH, Kang H, Bao K, Hu S et al (2019) Chemical modulation of bioengineered exosomes for tissue-specific biodistribution. Adv Ther 2(11):1900111

Jafari D, Malih S, Eini M, Jafari R, Gholipourmalekabadi M, Sadeghizadeh M, Samadikuchaksaraei A (2020) Improvement, scaling-up, and downstream analysis of exosome production. Crit Rev Biotechnol 40(8):1098–1112

Jang SC, Gho YS (2014) Could bioengineered exosome-mimetic nanovesicles be an efficient strategy for the delivery of chemotherapeutics? Nanomedicine 9(2):177–180

Jia G, Han Y, An Y, Ding Y, He C, Wang X, Tang Q (2018) NRP-1 targeted and cargo-loaded exosomes facilitate simultaneous imaging and therapy of glioma in vitro and in vivo. Biomaterials 178:302–316

Jia X, Tang J, Yao C, Yang D (2021) Recent progress of extracellular vesicle engineering. ACS Biomater Sci Eng 7(9):4430–4438

Jiang Y, Zhang P, Zhang X, Lv L, Zhou Y (2020) Advances in mesenchymal stem cell transplantation for the treatment of osteoporosis. Cell Prolif 54(1):e12956

Johnsen KB, Gudbergsson JM, Skov MN, Christiansen G, Gurevich L, Moos T, Duroux M (2016) Evaluation of electroporation-induced adverse effects on adipose-derived stem cell exosomes. Cytotechnology 68(5):2125–2138

Kalluri R, LeBleu VS (2020) The biology, function, and biomedical applications of exosomes. Science 7;367(6478):eaau6977

Kanuma T, Yamamoto T, Kobiyama K, Moriishi E, Masuta Y, Kusakabe T et al (2017) CD63-mediated antigen delivery into extracellular vesicles via DNA vaccination results in robust CD8+ T cell responses. J Immunol 198(12):4707–4715

Kim JY, Song J, Jung H, Mok H (2018a) I-motif-coated exosomes as a pH-sensitive carrier for anticancer drugs. Appl Biol Chem 61(6):599–606

Kim MS, Haney MJ, Zhao Y, Yuan D, Deygen I, Klyachko NL et al (2018b) Engineering macrophage-derived exosomes for targeted

paclitaxel delivery to pulmonary metastases: in vitro and in vivo evaluations. Nanomed Nanotechnol Biol Med 14(1):195–204
Kim G, Kim M, Lee Y, Byun JW, Lee M (2020) Systemic delivery of microRNA-21 antisense oligonucleotides to the brain using T7-peptide decorated exosomes. J Control Release 317:273–281
Kim G, Lee Y, Ha J, Han S, Lee M (2021a) Engineering exosomes for pulmonary delivery of peptides and drugs to inflammatory lung cells by inhalation. J Control Release 330:684–695
Kim H, Jang H, Cho H, Choi J, Hwang KY, Choi Y et al (2021b) Recent advances in exosome-based drug delivery for cancer therapy. Cancers 13(17):4435
Kooijmans SA, Vader P, van Dommelen SM, van Solinge WW, Schiffelers RM (2012) Exosome mimetics: a novel class of drug delivery systems. Int J Nanomed 7:1525
Kooijmans SA, Aleza CG, Roffler SR, van Solinge WW, Vader P, Schiffelers RM (2016) Display of GPI-anchored anti-EGFR nanobodies on extracellular vesicles promotes tumour cell targeting. J Extracell Vesicles 5(1):31053
Kooijmans SA, Gitz-Francois JJ, Schiffelers RM, Vader P (2018) Recombinant phosphatidylserine-binding nanobodies for targeting of extracellular vesicles to tumor cells: a plug-and-play approach. Nanoscale 10(5):2413–2426
Kučuk N, Primožič M, Knez Ž, Leitgeb M (2021) Exosomes engineering and their roles as therapy delivery tools, therapeutic targets, and biomarkers. Int J Mol Sci 22(17):9543
Laulagnier K, Grand D, Dujardin A, Hamdi S, Vincent-Schneider H, Lankar D et al (2004) PLD2 is enriched on exosomes and its activity is correlated to the release of exosomes. FEBS Lett 572(1–3):11–14
Lee B-C, Kang K-S (2020) Functional enhancement strategies for immunomodulation of mesenchymal stem cells and their therapeutic application. Stem Cell Res Ther 11(1):1–10
Lee B-C, Kang I, Yu K-R (2021) Therapeutic features and updated clinical trials of mesenchymal stem cell (MSC)-derived exosomes. J Clin Med 10(4):711
Lee J, Lee J-H, Chakraborty K, Hwang J, Lee Y-K (2022) Exosome-based drug delivery systems and their therapeutic applications. RSC Adv 12(29):18475–18492
Li Y, Gao Y, Gong C, Wang Z, Xia Q, Gu F et al (2018a) A33 antibody-functionalized exosomes for targeted delivery of doxorubicin against colorectal cancer. Nanomed Nanotechnol Biol Med 14(7):1973–1985
Li Z, Zhou X, Wei M, Gao X, Zhao L, Shi R et al (2018b) In vitro and in vivo RNA inhibition by CD9-HuR functionalized exosomes encapsulated with miRNA or CRISPR/dCas9. Nano Lett 19(1):19–28
Li Z, Zhou X, Gao X, Bai D, Dong Y, Sun W et al (2020) Fusion protein engineered exosomes for targeted degradation of specific RNAs in lysosomes: a proof-of-concept study. J Extracell Vesicles 9(1):1816710
Liang G, Kan S, Zhu Y, Feng S, Feng W, Gao S (2018) Engineered exosome-mediated delivery of functionally active miR-26a and its enhanced suppression effect in HepG2 cells. Int J Nanomed 13:585
Liang G, Zhu Y, Ali DJ, Tian T, Xu H, Si K et al (2020a) Engineered exosomes for targeted co-delivery of miR-21 inhibitor and chemotherapeutics to reverse drug resistance in colon cancer. J Nanobiotechnol 18(1):1–15
Liang Y, Xu X, Li X, Xiong J, Li B, Duan L et al (2020b) Chondrocyte-targeted microRNA delivery by engineered exosomes toward a cell-free osteoarthritis therapy. ACS Appl Mater Interfaces 12(33):36938–36947
Liang Y, Duan L, Lu J, Xia J (2021) Engineering exosomes for targeted drug delivery. Theranostics 11(7):3183
Liao W, Du Y, Zhang C, Pan F, Yao Y, Zhang T, Peng Q (2019) Exosomes: the next generation of endogenous nanomaterials for advanced drug delivery and therapy. Acta Biomater 86:1–14
Limoni SK, Moghadam MF, Moazzeni SM, Gomari H, Salimi F (2019) Engineered exosomes for targeted transfer of siRNA to HER2 positive breast cancer cells. Appl Biochem Biotechnol 187(1):352–364
Liu C, Su C (2019) Design strategies and application progress of therapeutic exosomes. Theranostics 9(4):1015
Liu J, Ye Z, Xiang M, Chang B, Cui J, Ji T et al (2019) Functional extracellular vesicles engineered with lipid-grafted hyaluronic acid effectively reverse cancer drug resistance. Biomaterials 223:119475
Liu M, Fang X, Yang Y, Wang C (2021) Peptide-enabled targeted delivery systems for therapeutic applications. Front Bioeng Biotechnol 9:701504
Longatti A, Schindler C, Collinson A, Jenkinson L, Matthews C, Fitzpatrick L et al (2018) High affinity single-chain variable fragments are specific and versatile targeting motifs for extracellular vesicles. Nanoscale 10(29):14230–14244
Luan X, Sansanaphongpricha K, Myers I, Chen H, Yuan H, Sun D (2017) Engineering exosomes as refined biological nanoplatforms for drug delivery. Acta Pharmacol Sin 38(6):754–763
Luo Z-W, Liu Y-W, Rao S-S, Yin H, Huang J, Chen C-Y et al (2019) Aptamer-functionalized exosomes from bone marrow stromal cells target bone to promote bone regeneration. Nanoscale 11(43):20884–20892
Marbán E (2018) The secret life of exosomes: what bees can teach us about next-generation therapeutics. J Am Coll Cardiol 71(2):193–200
Maumus M, Rozier P, Boulestreau J, Jorgensen C, Noël D (2020) Mesenchymal stem cell-derived extracellular vesicles: opportunities and challenges for clinical translation. Front Bioeng Biotechnol 8:997
McAndrews KM, Xiao F, Chronopoulos A, LeBleu VS, Kugeratski FG, Kalluri R (2021) Exosome-mediated delivery of CRISPR/Cas9 for targeting of oncogenic KrasG12D in pancreatic cancer. Life Sci Alliance 19;4(9):e202000875
Meng W, He C, Hao Y, Wang L, Li L, Zhu G (2020) Prospects and challenges of extracellular vesicle-based drug delivery system: considering cell source. Drug Deliv 27(1):585–598
Mentkowski KI, Lang JK (2019) Exosomes engineered to express a cardiomyocyte binding peptide demonstrate improved cardiac retention in vivo. Sci Rep 9(1):1–13
Meyer C, Losacco J, Stickney Z, Li L, Marriott G, Lu B (2017) Pseudotyping exosomes for enhanced protein delivery in mammalian cells. Int J Nanomed 12:3153
Mima Y, Hashimoto Y, Shimizu T, Kiwada H, Ishida T (2015) Anti-PEG IgM is a major contributor to the accelerated blood clearance of polyethylene glycol-conjugated protein. Mol Pharm 12(7):2429–2435
Modani S, Tomar D, Tangirala S, Sriram A, Mehra NK, Kumar R et al (2021) An updated review on exosomes: biosynthesis to clinical applications. J DrugTarget 29(9):925–940
Moloudizargari M, Asghari MH, Jørgensen MM, Reiter RJ, Kabelitz D (2022) Extracellular vesicles in cancer immunosurveillance. Front Immunol 13:993967
Montaner-Tarbes S, Fraile L, Montoya M, Del Portillo H (2021) Exosome-based vaccines: pros and cons in the world of animal health. Viruses 13:1499 (**s Note: MDPI stays neutral with regard to jurisdictional claims in published…**)
Morishita M, Takahashi Y, Matsumoto A, Nishikawa M, Takakura Y (2016) Exosome-based tumor antigens—adjuvant co-delivery utilizing genetically engineered tumor cell-derived exosomes with immunostimulatory CpG DNA. Biomaterials 111:55–65

Mosquera-Heredia MI, Morales LC, Vidal OM, Barceló E, Silvera-Redondo C, Vélez JI, Garavito-Galofre P (2021) Exosomes: potential disease biomarkers and new therapeutic targets. Biomedicines 9(8):1061

N'Diaye E-R, Orefice NS, Ghezzi C, Boumendjel A (2022) Chemically modified extracellular vesicles and applications in radiolabeling and drug delivery. Pharmaceutics 14(3):653

Nakase I, Futaki S (2015) Combined treatment with a pH-sensitive fusogenic peptide and cationic lipids achieves enhanced cytosolic delivery of exosomes. Sci Rep 5(1):1–13

Nan W, Zhang C, Wang H, Chen H, Ji S (2022) Direct modification of extracellular vesicles and its applications for cancer therapy: a mini-review. Front Chem 11;10:910341

Nel AE, Mädler L, Velegol D, Xia T, Hoek E, Somasundaran P et al (2009) Understanding biophysicochemical interactions at the nano–bio interface. Nat Mater 8(7):543–557

Ohno S-I, Takanashi M, Sudo K, Ueda S, Ishikawa A, Matsuyama N et al (2013) Systemically injected exosomes targeted to EGFR deliver antitumor microRNA to breast cancer cells. Mol Ther 21(1):185–191

Parada N, Romero-Trujillo A, Georges N, Alcayaga-Miranda F (2021a) Camouflage strategies for therapeutic exosomes evasion from phagocytosis. J Adv Res 8;31:61–74

Parada N, Romero-Trujillo A, Georges N, Alcayaga-Miranda F (2021b) Camouflage strategies for therapeutic exosomes evasion from phagocytosis. J Adv Res 31:61–74

Park K (2013) Facing the truth about nanotechnology in drug delivery. ACS Nano 7(9):7442–7447

Perocheau D, Touramanidou L, Gurung S, Gissen P, Baruteau J (2021) Clinical applications for exosomes: are we there yet? Br J Pharmacol 178(12):2375–2392

Pham TC, Jayasinghe MK, Pham TT, Yang Y, Wei L, Usman WM et al (2021) Covalent conjugation of extracellular vesicles with peptides and nanobodies for targeted therapeutic delivery. J Extracell Vesicles 10(4):e12057

Phan J, Kumar P, Hao D, Gao K, Farmer D, Wang A (2018) Engineering mesenchymal stem cells to improve their exosome efficacy and yield for cell-free therapy. J Extracell Vesicles 7(1):1522236

Pi F, Binzel DW, Lee TJ, Li Z, Sun M, Rychahou P et al (2018) Nanoparticle orientation to control RNA loading and ligand display on extracellular vesicles for cancer regression. Nat Nanotechnol 13(1):82–89

Podolak I, Galanty A, Sobolewska D (2010) Saponins as cytotoxic agents: a review. Phytochem Rev 9(3):425–474

Qi H, Liu C, Long L, Ren Y, Zhang S, Chang X et al (2016) Blood exosomes endowed with magnetic and targeting properties for cancer therapy. ACS Nano 10(3):3323–3333

Qu Y, Dubyak GR (2009) P2X7 receptors regulate multiple types of membrane trafficking responses and non-classical secretion pathways. Purinergic Signal 5(2):163–173

Rahbarghazi R, Jabbari N, Sani NA, Asghari R, Salimi L, Kalashani SA et al (2019) Tumor-derived extracellular vesicles: reliable tools for Cancer diagnosis and clinical applications. Cell Commun Signal 17(1):1–17

Rayamajhi S (2021) Engineering synthetic and natural vesicular system for tumor-targeted drug delivery

Rayamajhi S, Aryal S (2020) Surface functionalization strategies of extracellular vesicles. J Mater Chem B 8(21):4552–4569

Richardson JJ, Ejima H (2019) Surface engineering of extracellular vesicles through chemical and biological strategies. Chem Mater 31(7):2191–2201

Sadeghi S, Mosaffa N, Hashemi SM, Naghizadeh MM, Ghazanfari T (2020a) The immunomodulatory effects of mesenchymal stem cells on long term pulmonary complications in an animal model exposed to a sulfur mustard analog. Int Immunopharmacol 80:105879

Sadeghi S, Soudi S, Shafiee A, Hashemi SM (2020b) Mesenchymal stem cell therapies for COVID-19: current status and mechanism of action. Life Sci 1; 262:118493

Said Hassane F, Frisch B, Schuber F (2006) Targeted liposomes: convenient coupling of ligands to preformed vesicles using "click chemistry." Bioconjug Chem 17(3):849–854

Sajnani SLP, Zhang Y, Vllasaliu D (2021) Exosome-based therapies for mucosal delivery. Int J Pharm 25;608:121087

Salunkhe S, Basak M, Chitkara D, Mittal A (2020) Surface functionalization of exosomes for target-specific delivery and in vivo imaging and tracking: strategies and significance. J Control Release 326:599–614

Sancho-Albero M, Medel-Martínez A, Martín-Duque P (2020) Use of exosomes as vectors to carry advanced therapies. RSC Adv 10(40):23975–23987

Santos P, Almeida F (2021) Exosome-based vaccines: history, current state, and clinical trials. 14;12:711565

Sato YT, Umezaki K, Sawada S, Mukai S-A, Sasaki Y, Harada N et al (2016) Engineering hybrid exosomes by membrane fusion with liposomes. Sci Rep 6(1):1–11

Schiller LT, Lemus-Diaz N, Ferreira RR, Böker KO, Gruber J (2018) Enhanced production of exosome-associated AAV by overexpression of the tetraspanin CD9. Mol Therapy-Methods Clin Dev 9:278–287

Sercombe L, Veerati T, Moheimani F, Wu SY, Sood AK, Hua S (2015) Advances and challenges of liposome assisted drug delivery. Front Pharmacol 6:286

Shafiee A, Moradi L, Lim M, Brown J (2021) Coronavirus disease 2019: a tissue engineering and regenerative medicine perspective. Stem Cells Transl Med 10(1):27–38

Shafiei M, Ansari MNM, Razak SIA, Khan MUA (2021) A comprehensive review on the applications of exosomes and liposomes in regenerative medicine and tissue engineering. Polymers 13(15):2529

Smyth T, Petrova K, Payton NM, Persaud I, Redzic JS, Graner MW et al (2014) Surface functionalization of exosomes using click chemistry. Bioconjug Chem 25(10):1777–1784

Syn NL, Wang L, Chow EK-H, Lim CT, Goh B-C (2017) Exosomes in cancer nanomedicine and immunotherapy: prospects and challenges. Trends Biotechnol 35(7):665–676

Taghavi-Farahabadi M, Mahmoudi M, Rezaei N, Hashemi SM (2021) Wharton's Jelly mesenchymal stem cells exosomes and conditioned media increased neutrophil lifespan and phagocytosis capacity. Immunol Invest 50(8):1042–1057

Taiariol L, Chaix C, Farre C, Moreau E (2021) Click and bioorthogonal chemistry: the future of active targeting of nanoparticles for nanomedicines? Chem Rev 122(1):340–384

Takahashi Y, Nishikawa M, Shinotsuka H, Matsui Y, Ohara S, Imai T, Takakura Y (2013) Visualization and in vivo tracking of the exosomes of murine melanoma B16-BL6 cells in mice after intravenous injection. J Biotechnol 165(2):77–84

Théry C, Zitvogel L, Amigorena S (2002) Exosomes: composition, biogenesis and function. Nat Rev Immunol 2(8):569–579

Tian Y, Li S, Song J, Ji T, Zhu M, Anderson GJ et al (2014) A doxorubicin delivery platform using engineered natural membrane vesicle exosomes for targeted tumor therapy. Biomaterials 35(7):2383–2390

Tian T, Zhang H-X, He C-P, Fan S, Zhu Y-L, Qi C et al (2018) Surface functionalized exosomes as targeted drug delivery vehicles for cerebral ischemia therapy. Biomaterials 150:137–149

Von Schulze A, Deng F (2020) A review on exosome-based cancer therapy. J Cancer Metastasis Treat 6:42

Vymetalova L, Kucirkova T, Knopfova L, Pospisilova V, Kasko T, Lejdarova H et al (2020) Large-scale automated hollow-fiber

bioreactor expansion of umbilical cord-derived human mesenchymal stromal cells for neurological disorders. Neurochem Res 45(1):204–214

Wan Y, Wang L, Zhu C, Zheng Q, Wang G, Tong J et al (2018) Aptamer-conjugated extracellular nanovesicles for targeted drug delivery preparation of cancer-targeted extracellular nanovesicles. Can Res 78(3):798–808

Wang J, Li W, Lu Z, Zhang L, Hu Y, Li Q et al (2017a) The use of RGD-engineered exosomes for enhanced targeting ability and synergistic therapy toward angiogenesis. Nanoscale 9(40):15598–15605

Wang J, Li W, Zhang L, Ban L, Chen P, Du W et al (2017b) Chemically edited exosomes with dual ligand purified by microfluidic device for active targeted drug delivery to tumor cells. ACS Appl Mater Interfaces 9(33):27441–27452

Wang X, Chen Y, Zhao Z, Meng Q, Yu Y, Sun J et al (2018) Engineered exosomes with ischemic myocardium-targeting peptide for targeted therapy in myocardial infarction. J Am Heart Assoc 7(15):e008737

Wang K, Li Y, Ren C, Wang Y, He W, Jiang Y (2021) Extracellular vesicles as innovative treatment strategy for amyotrophic lateral sclerosis. Fron Cell Dev Biol 9:754630–754630

Wei H, Chen Q, Lin L, Sha C, Li T, Liu Y et al (2021a) Regulation of exosome production and cargo sorting. Int J Biol Sci 17(1):163

Wei W, Ao Q, Wang X, Cao Y, Liu Y, Zheng SG, Tian X (2021b) Mesenchymal stem cell-derived exosomes: a promising biological tool in nanomedicine. Front Pharmacol 11:1954

Weng Z, Zhang B, Wu C, Yu F, Han B, Li B, Li L (2021) Therapeutic roles of mesenchymal stem cell-derived extracellular vesicles in cancer. J Hematol Oncol 14(1):1–22

White MK, Kaminski R, Young WB, Roehm PC, Khalili K (2017) CRISPR editing technology in biological and biomedical investigation. J Cell Biochem 118(11):3586–3594

Willms E, Cabañas C, Mäger I, Wood MJ, Vader P (2018) Extracellular vesicle heterogeneity: subpopulations, isolation techniques, and diverse functions in cancer progression. Front Immunol 9:738

Wu Z, He D, Li H (2021) Bioglass enhances the production of exosomes and improves their capability of promoting vascularization. Bioact Mater 6(3):823–835

Xu Y, Chen C, Hellwarth PB, Bao X (2019) Biomaterials for stem cell engineering and biomanufacturing. Bioact Mater 4:366–379

Xu M, Yang Q, Sun X, Wang Y (2020) Recent advancements in the loading and modification of therapeutic exosomes. Front Bioeng Biotechnol 11;8:586130

Xu X, Liang Y, Li X, Ouyang K, Wang M, Cao T et al (2021) Exosome-mediated delivery of kartogenin for chondrogenesis of synovial fluid-derived mesenchymal stem cells and cartilage regeneration. Biomaterials 269:120539

Yamashita T, Takahashi Y, Takakura Y (2018) Possibility of exosome-based therapeutics and challenges in production of exosomes eligible for therapeutic application. Biol Pharm Bull 41(6):835–842

Yang Y, Hong Y, Cho E, Kim GB, Kim I-S (2018) Extracellular vesicles as a platform for membrane-associated therapeutic protein delivery. J Extracell Vesicles 7(1):1440131

Ye Z, Zhang T, He W, Jin H, Liu C, Yang Z, Ren J (2018) Methotrexate-loaded extracellular vesicles functionalized with therapeutic and targeted peptides for the treatment of glioblastoma multiforme. ACS Appl Mater Interfaces 10(15):12341–12350

Yu X, Yang X, Horte S, Kizhakkedathu JN, Brooks DE (2014) A pH and thermosensitive choline phosphate-based delivery platform targeted to the acidic tumor microenvironment. Biomaterials 35(1):278–286

Zhang Y, Liu Y, Liu H, Tang WH (2019) Exosomes: biogenesis, biologic function and clinical potential. Cell Biosci 9(1):1–18

Zhang Y, Bi J, Huang J, Tang Y, Du S, Li P (2020) Exosome: a review of its classification, isolation techniques, storage, diagnostic and targeted therapy applications. Int J Nanomed 15:6917

Zhang S, Zhu D, Mei X, Li Z, Li J, Xie M et al (2021a) Advances in biomaterials and regenerative medicine for primary ovarian insufficiency therapy. Bioact Mater 6(7):1957–1972

Zhang X, Zhang H, Gu J, Zhang J, Shi H, Qian H et al (2021b) Engineered extracellular vesicles for cancer therapy. Adv Mater 33(14):2005709

Zhao J, Li X, Hu J, Chen F, Qiao S, Sun X et al (2019) Mesenchymal stromal cell-derived exosomes attenuate myocardial ischaemia-reperfusion injury through miR-182-regulated macrophage polarization. Cardiovasc Res 115(7):1205–1216

Zhou Y, Yuan Y, Liu M, Hu X, Quan Y, Chen X (2019) Tumor-specific delivery of KRAS siRNA with iRGD-exosomes efficiently inhibits tumor growth. ExRNA 1(1):1–7

Zhu L, Dong D, Yu Z-L, Zhao Y-F, Pang D-W, Zhang Z-L (2017) Folate-engineered microvesicles for enhanced target and synergistic therapy toward breast cancer. ACS Appl Mater Interfaces 9(6):5100–5108

Zhuang M, Chen X, Du D, Shi J, Deng M, Long Q et al (2020) SPION decorated exosome delivery of TNF-α to cancer cell membranes through magnetism. Nanoscale 12(1):173–188

Zimmermann J, McDevitt T (2018) Engineering the 3D MSC spheroid microenvironment to enhance immunomodulation. Cytotherapy 20(5):S106

Zou J, Shi M, Liu X, Jin C, Xing X, Qiu L, Tan W (2019) Aptamer-functionalized exosomes: elucidating the cellular uptake mechanism and the potential for cancer-targeted chemotherapy. Anal Chem 91(3):2425–2430