REVIEW ARTICLE

Designer Exosomes: A New Platform for Biotechnology Therapeutics

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Abstract

Desirable features of exosomes have made them a suitable manipulative platform for biomedical applications, including targeted drug delivery, gene therapy, cancer diagnosis and therapy, development of vaccines, and tissue regeneration. Although natural exosomes have various potentials, their clinical application is associated with some inherent limitations. Recently, these limitations inspired various attempts to engineer exosomes and develop designer exosomes. Mostly, designer exosomes are being developed to overcome the natural limitations of exosomes for targeted delivery of drugs and functional molecules to wounds, neurons, and the cardiovascular system for healing of damage. In this review, we summarize the possible improvements of natural exosomes by means of two main approaches: parental cell-based or pre-isolation exosome engineering and direct or post-isolation exosome engineering. Parental cell-based engineering methods use genetic engineering for loading of therapeutic molecules into the lumen or displaying them on the surface of exosomes. On the other hand, the post-isolation exosome engineering approach uses several chemical and mechanical methods including click chemistry, cloaking, bio-conjugation, sonication, extrusion, and electroporation. This review focuses on the latest research, mostly aimed at the development of designer exosomes using parental cell-based engineering and their application in cancer treatment and regenerative medicine.

Graphic Abstract

Extended author information available on the last page of the article
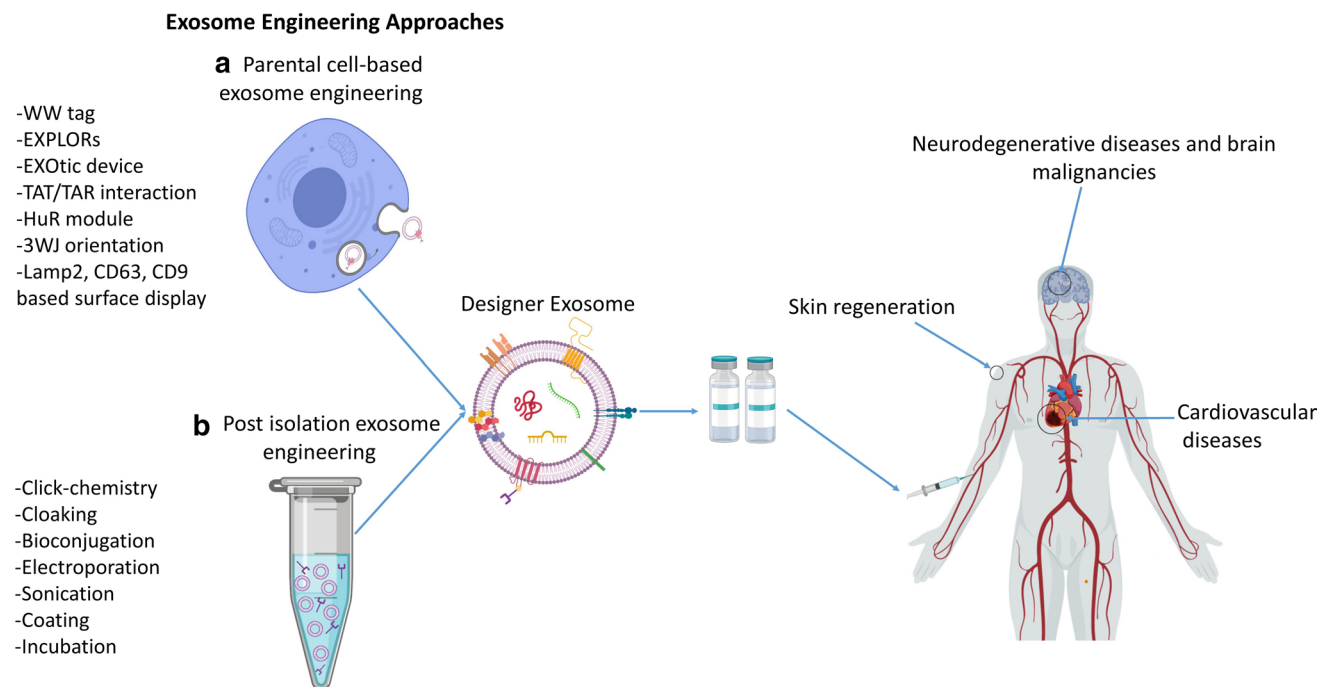
Key Points

Designer exosomes could erase the limitations of natural exosomes, including low targeting capability, low half-life in circulation, and low concentration of functional molecules.

A designer exosome benefits simultaneously from its own functional molecules and the newly loaded molecules.

Anti-cancer designer exosomes could be engineered for targeted delivery of chemotherapeutic drugs and short interfering RNAs to tumor tissue and requires further development of highly specific targeting peptides.

Regenerative designer exosomes from engineered mesenchymal stem cells (MSCs) could be used for healing of wounds, and neural and cardiac tissue healing and regeneration.

1 Introduction

Exosomes comprise a small fraction of extracellular vesicles (EVs), which are produced by all types of cells and secreted into the extracellular environment [1]. Since EVs consist of different heterogeneous populations of vesicles, isolation of a pure population of exosomes requires several purification and characterization steps [2]. The three main populations of EVs have been categorized as exosomes, microvesicles, and apoptotic bodies. These populations of vesicles are different in their origin and size. Exosomes originate from multivesicular bodies (MVBs), which are derived from the budding of the plasma membrane of cells, and apoptotic bodies are formed from cell fragmentation through apoptosis. For the most detailed information on EVs and exosome populations, readers are referred to a recently published review by Willms et al. [3].

Among EVs, exosomes have been widely studied due to their potential application in medicine. Thus far, exosomes have been used in many studies for tissue regeneration, delivery of drugs and genes, and diagnosis of diseases. Naturally, in addition to the well-known cell-to-cell communication, new studies have shown other roles for exosomes, including spreading of various infectious agents, such as the human immunodeficiency virus (HIV) [4, 5], Epstein–Barr virus (EBV) [6], and prions [7]. Exosomes derived from different cells have specific features, composition, and effects on their target cells [8, 9]. In addition, exosomes naturally exhibit the characteristics

of their parental cells [10, 11]. These two main features of exosomes could have many biomedical applications. For instance, researchers use mesenchymal stem cell (MSC)-derived exosomes in regenerative medicine for tissue regeneration and wound healing [12, 13]. On the other hand, exosomes are a new source and platform for the discovery of biomarkers in biofluids for the diagnosis and screening of cancer and other disorders [14, 15].

Structurally, the surfaces of exosomes are rich in transmembrane proteins, receptors, and other functional molecules. In the lumen, there is a vast range of different proteins, lipids, RNAs, DNAs, and metabolites, which vary widely between exosomes derived from different sources [16–18]. Readers are referred to our most recent published review article, in which the molecular composition of exosomes is discussed in depth [19].

Although, extensive research in the recent 2 decades has enriched our knowledge of the biology, chemistry, and physiology of exosomes, a Food and Drug Administration (FDA)-approved exosomal-based therapeutic or diagnostic platform is yet to be established. This is mainly due to the fact that exosomes, as natural vesicles, lack some features for therapeutic development. For instance, for cancer treatment using exosomes, targeting of therapeutic exosomes specifically to cancer cells is essential. In addition, in many applications, researchers need to load a completely new drug, a protein, or RNA into exosomes to impart a therapeutic effect. Besides the challenges of large-scale manufacturing of exosomes, these limitations of natural exosomes have challenged the translational development of exosomes. Fortunately, various biotechnological approaches have led to the emergence of the new field of exosome engineering. Exosome engineering aims to achieve the following goals: (1) targeting the exosomes to a particular type of tissue or cell; (2) loading the exogenous molecules, drugs, proteins, or nucleic acids into exosomes or onto their surface; and (3) enrichment of an endogenous molecule into the lumen of exosomes or on their surface (Fig. 1). Most recently, advanced studies have combined several exosome engineering approaches in order to design highly specialized exosomes called designer exosomes.

These engineered exosomes offer several novel features for special therapeutic applications. Herein, we aimed to classify exosome engineering approaches: parental cell-based and direct exosome engineering. However, different methods have been used in each class, and this could help researchers in various fields of biomedical sciences to recognize possible approaches for creating or improving therapeutic features of exosomes. In this review, we begin with natural exosomes and their potential and limitations in medicine. In the next sections, we focus on the necessity of exosome engineering, different engineering approaches, and applications of designer exosomes.

2 Natural Exosomes Versus Designer Exosomes

In the last 2 decades, synthetic nanoparticles such as liposomes (synthetic lipid bilayer used as a carrier for drugs and genes), aptamers (short single-stranded RNAs or DNAs that strongly bind to specific ligands), dendrimers (highly branched polymers used as carriers of drugs and genes), and many other biomolecules have provided useful tools for selective delivery of drugs and genes to target cells and tissues [1]. Despite this progress, multiple challenges still remain in using these nanoparticles including, bioavailability (systemic circulation access of administered carrier-loaded therapeutics), cost, biocompatibility, undesirable effects such as cytotoxic effects on some cells [1, 20], and short half-life *in vivo*, resulting in quick elimination from the systemic circulation [21, 22].

Since their introduction in the early 1980s by Pan and Johnstone, exosomes have been considered carriers that transport cell waste to the extracellular environment [23]. However, through vast studies on EVs, their various crucial functions have been discovered and experimentally approved for many physiological and pathophysiological conditions. These critical roles of exosomes consist of extensive cell-to-cell communications [24], cancer progression and metastasis [25, 26], embryogenesis [27], organ development [28], wound healing and tissue regeneration [29], cell differentiation [30], immunomodulation [31], antigen presentation [32], and pathogenesis [33–35]. These functions have led to intensive research on their biomedical applications, including tissue regeneration [36], cancer treatment and diagnosis [37], drug delivery [38], gene therapy [39], and vaccine development [40, 41].

Exosomes are natural vesicles with 50–150 nm size and a bilayer membrane, and their luminal contents consist of surface ligands and receptors [20, 22]. Exosomes can be detected and isolated from cell culture supernatants and all body fluids [1, 21]. Compared to synthetic nanovesicles such as liposomes, exosomes have higher stability and bioavailability [42], have a natural origin [43, 44], longer in vivo circulation half-life [45], a role as endogenous functional biomolecules, lack of toxicity, low to no immunogenicity, biocompatibility (because of their natural origin) [46], and ability to pass biological barriers such as the blood–brain barrier (BBB) [47, 48], penetrating massive tissues with their surface ligands and receptors [49]. As a result, exosomes have the potential to be a suitable platform for delivering drugs, genes, and functional biomolecules to target tissues or cells for different applications [21].

However, the application of exosomes in their natural form and composition as therapeutic agents is challenging. Generally, a natural exosome lacks the specific cell- or tissue-targeting feature. The therapeutic application of exosomes as a delivery system necessitates the design of controlled and targeted exosomes. Furthermore, many clinical applications involve the loading of certain additional therapeutic molecules alongside exosomes' own content, which demands different loading techniques to be developed. In this regard, targeted designer exosomes or designer exosomes loaded with tested molecules could potentially address these limitations. In addition to the functional contents, ligands, and receptors of natural exosomes, designer exosomes also benefit from other engineered features [50, 51]. In the following sections, different approaches implemented for the development of designer exosomes are discussed.

3 Exosome Engineering Approaches

The functional molecule can be either loaded into the lumen or displayed on the surface of exosomes for a therapeutic purpose (Fig. 1a). The two major strategies, including parental cell-based and direct exosome engineering, are routinely employed for loading and displaying functional molecules, and mainly differ in the level of engineering applied for their development. In the parental cell-based approach, the starting material is the cells that are the source of the exosomes, and engineering occurs before exosome isolation from cells. In contrast, in the second approach, exosomes are the direct starting material for engineering, and the engineering procedure occurs after exosome isolation. The next two subsections separately discuss different methods employed in the two main exosome engineering approaches (Fig. 1b).

3.1 Parental Cell-Based Exosome Engineering

The parental cell-based approach of exosome engineering, which was first used for discovering the biology of exosomes and not for the purpose of manipulation, consists of genetically engineering cells to produce specifically fabricated exosomes (Fig. 1a). Here, the basics of this approach are discussed via previously published examples, to demonstrate how a functional molecule can be directed to either exosome lumen (loaded) or surface (displayed) using genetic engineering of the parental cells.
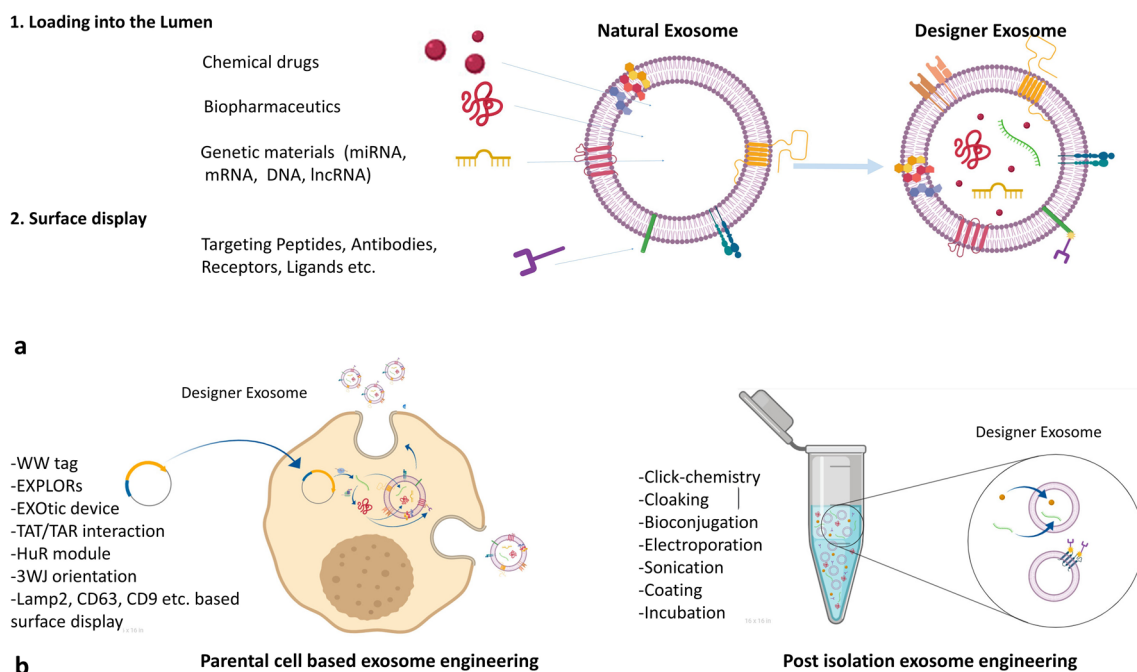
Fig. 1 Exosome engineering approaches. **a** Different therapeutic molecules can be either loaded into lumen or displayed on the exosome surface for clinical purposes. **b** Two main approaches of exosome engineering, parental cell-based and post-isolation engineering, and their different methods. *EXPLORs* Exosomes for Protein Loading via

Optically Reversible protein-protein interactions, *EXOtic* Exosomal Transfer Into Cells, *3WJ* 3-way junction, *Lamp2* lysosome-associated membrane protein 2, *lncRNA* long non-coding RNA, *TAR* trans-activating response element, *TAT* trans-activator of transcription

3.1.1 Exosomal Surface Display Using Parental Cell-Based Approach

Using the parental cell-based approach of exosome engineering, the most common method for directing a protein to the surface of exosomes uses a exosomal signal peptide. For example, lysosome-associated membrane protein 2b (Lamp2b) is an exosomal surface protein with an exosomal signal peptide. Fusion of a protein of interest to Lamp2b is very common for displaying the protein on the surface of exosomes as a targeting moiety, ligand, or receptor. A Lamp2b-based fusion protein can be used to display rabies viral glycoprotein (RVG), a neuron-specific targeting peptide, on the surface. Expression of Lamp2b-RVG fusion proteins in dendritic cells (DCs) displays RVG on the surface of DC-derived exosomes (Fig. 2a). When these exosomes are systemically administered, surface displayed RVG leads to the accumulation of these exosomes in the neurons and brain of mice [43]. Removal of the signal peptide from Lamp2b-RVG significantly reduces surface display of RVG on the engineered exosomes. Altogether, the signal peptide of Lamp2b can be used to display any fusion protein on the surface of exosomes. In addition, introduction of a glycosylation motif into Lamp2b-RVG fusion proteins could further enhance exosome delivery to the neurons and glial cells by protecting the surface-displayed fusion protein from

enzymatic degradation. Therefore, this method allows simultaneous glyco-engineering and display of a protein on the surface of exosomes [52, 53].

Other commonly used molecules for exosomal surface display of fusion proteins include tetraspanins (CD63, CD9, CD81) [54], glycosylphosphatidylinositol (GPI) [55], platelet-derived growth factor receptors (PDGFRs) [56], and lactadherin (C1C2 domain) [57]. In a very similar method to Lamp2b fusion proteins, the NH2-terminal of CD63 can be fused to a protein of interest for the same purpose [58].

Other non-cellular and exosomal proteins such as vesicular stomatitis virus glycoprotein (VSVG) have also been used for surface display of proteins on exosomes. VSVG is a key protein involved in virus envelope formation during microdomain budding from the surface of the plasma membrane. Since a similar domain to VSVG participates in the formation of endosomes and exosomes in the cell, researchers used VSVG to express proteins on the exosomal surface. VSVG contains ectoplasmic, transmembrane, and cytoplasmic domains [59]. Replacement of the ecto- and cytoplasmic domains of VSVG with another protein (e.g., green and red fluorescent proteins [GFPs and RFPs] or luciferase), without any alteration in the signal peptide and the transmembrane domain, results in proper exosomal surface display of the protein. Improved protein surface display and improved exosome uptake to the target cells are two advantages of using

VSVG fusion proteins for exosomal surface display [60]. The stated methods allow proper display of a protein on the surface of exosomes, while some parental cell-based exosome engineering techniques have been developed recently for loading proteins and RNAs into the lumen of exosomes. These methods are discussed in the next subsection.

3.1.2 Loading Proteins into the Lumen of Exosomes Using Parental Cell-Based Engineering

The loading of therapeutic molecules is a common requirement for the production of therapeutic exosomes. The methods of parental cell-based engineering for loading molecules into the lumen of exosomes were developed very recently. These methods recruit the molecule sorting modules (MSMs) for sorting of proteins and RNAs into the lumen. These modules are able to bind to the protein or RNA of interest and direct them to exosomes. However, different MSMs or other modules have been used in different methods.

Two new methods for loading of proteins into the exosomes mimic the natural protein sorting system of cells based on ubiquitination. In the first method, an engineered ubiquitin tag (the last two glycine residues in the C-terminal are removed) has been developed. Removal of the two glycine residues results in enhanced ubiquitinated protein half-life. Fusion of this ub-tag to the proteins of interest, such as Ag85B and ESAT6 (*Mycobacterium tuberculosis* proteins) or enhanced green fluorescent protein (EGFP) and nHer2 (tumor antigens), leads to the loading of the proteins into the lumen of exosomes in human embryonic kidney (HEK293) cells. A recent study showed that the ubiquitin tag acts as a sorting sequence, resulting in efficient loading of proteins into exosomes [61]. In the second method, a short tag (WW tag) with the ability to bind specifically to the L-domain motif of Ndfip1 (which activates HECT domain-containing E3 ubiquitin-protein ligases) is used. The WW tag method is another system that uses ubiquitination for the loading of proteins into exosomes. Fusion of Cre recombinase (as a protein of interest) to the WW tag and simultaneous expression with Ndfip1 leads to recognition of the WW tag by Ndfip1 and activation of E3 ubiquitin ligases and ubiquitination of Cre. Subsequently, the ubiquitinated Cre-WW are loaded into the exosomes. Ndfip1 protein induces the molecular switch for ubiquitination and helps with the exosomal packaging of the Cre-WW fusion protein (Fig. 2b). The validation results of the mentioned method showed that when engineered exosomes containing the Cre-WW fusion protein are taken up by the target cells, Cre-WW is capable of DNA recombination [62].

Another method was recently used for the loading of specific proteins into the exosomes by a non-functional mutant of the HIV-I Nef protein. This engineered mutant of Nef (Nef[mut]) lacks the enzymatic activity and most functions of the intact Nef protein. The Nef protein is associated with lipid-raft microdomains of the plasma membrane [63] and exosomes. Similar to Lamp2b, CD63, and VSVG fusion proteins, Nef[mut] is developed for sorting of proteins to the exosomes [64]. Nef[mut]-GFP fusion protein was successfully loaded into exosomes for monitoring transfection and loading efficiency [65]. Moreover, fusing Nef[mut] to antigens of several pathogens, including Ebola virus VP24, VP40, and NP, influenza virus NP, Crimean-Congo hemorrhagic fever NP, West Nile virus NS3, and hepatitis C virus NS3, resulted in the expression of stable fusion proteins and their efficient loading into exosomes [66].

A recent attempt to load proteins into the lumen of exosomes was reported by Yim et al. [67]. They employed optically reversible protein–protein interaction (PPI) for the development of EXPLORs (exosomes for protein loading via optically reversible PPIs) using photoreceptor cryptochrome 2 (CRY2) and CIBN PPI modules. This method depends on intracellular delivery of proteins by reversible PPIs and their co-localization into exosomes by irradiation with a blue light. In the first step, a reporter protein was fused to CRY2 and CIBN protein was fused to the exosomal membrane CD9 protein (a representative marker of exosomes) to obtain two fusion proteins (CIBN-EGFP-CD9 and mCherry-CRY2). Blue light induced reversible PPI between CIBN and CRY2 indicates the reporter fusion protein (mCherry-CRY2) direction to the inner surface of exosomes through interaction with CIBN in the CIBN-EGFP-CD9 complex (Fig. 2c). The results of the study showed that protein-loaded EXPLORs increased intracellular levels of reporter proteins in the recipient cells [67].

3.1.3 Loading of RNAs into Exosomes Using Parental Cell-Based Engineering

In addition to the parental cell-based exosome engineering methods and devices developed for loading of proteins into exosomes, similar methods have been developed for loading of RNAs. Recently, a novel parental cell-based strategy was established for loading mRNAs, called the EXOtic (Exosomal Transfer Into Cells) device. In the EXOtic device, in addition to the RNA packaging system (CD63-L7Ae, an archaeal ribosomal protein, which can bind to the C/Dbox in the 3′ untranslated region [UTR] of any RNA structure), two other devices known as cytosolic delivery helper (Cx43 S368A, a mutated connexin 43) and targeting module (RVG-Lamp2b) also contribute, which together provide a new method for loading therapeutic mRNAs into exosomes. Using this device, the mRNA of a catalase enzyme carrying a C/Dbox sequence on its 3′ UTR was loaded into exosomes, which are simultaneously targeted to brain tissue by the Lamp2b-RVG targeting module. Briefly, as we discussed earlier, since CD63 is an
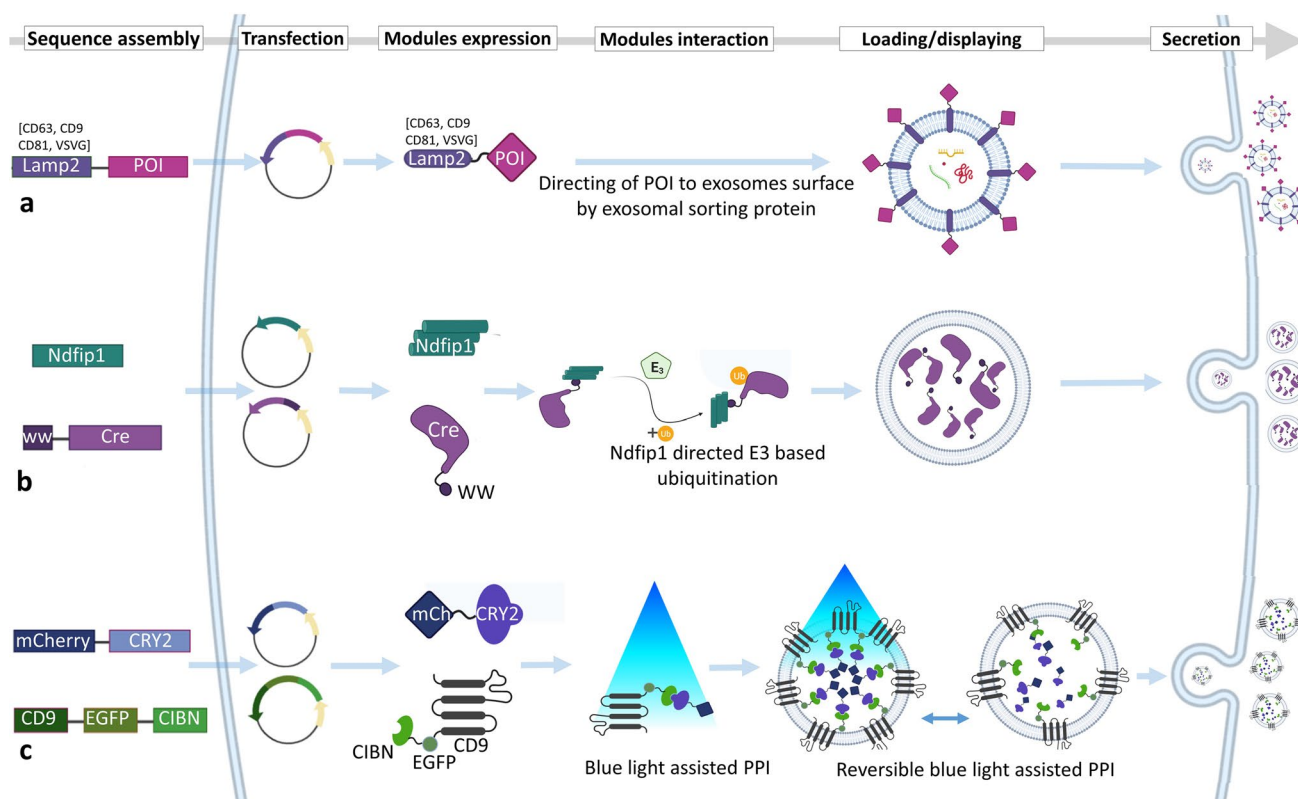
Fig. 2 Parental cell-based exosome engineering for loading proteins into the lumen of exosomes or displaying them on their surface. **a** Surface display of a POI using different sorting modules. The fusion protein composed of the POI and the sorting module is directed to the surface of exosomes by an exosomal signal peptide. **b** Use of WW tag and Ndfip1 for loading Cre enzyme into the exosomes. Ndfip1 recognizes the WW tag and activates the E3 ubiquitin ligases, leading to the ubiquitination of Cre and its subsequent loading into the exosomes. **c** The EXPLOR method for loading mCherry reporter protein into the exosomes. Blue light-assisted PPI between CRY2 and

CIBN results in the formation of a complex between two modules. Sorting of the whole complex into the exosomes is assisted by the CD9 sorting module. After elimination of the blue light, the two parts of the complex separate and mCherry-CRY2 is released to the lumen of exosomes. *CRY2* photoreceptor cryptochrome 2, *EGFP* enhanced green fluorescent protein, *EXPLOR* exosomes for protein loading via optically reversible protein-protein interactions, *Lamp2* lysosome-associated membrane protein 2, *mCh* mCherry, *POI* protein of interest, *PPI* protein–protein interaction, *VSVG* vesicular stomatitis virus glycoprotein

exosomal surface protein, the binding of CD63-L7Ae to the C/Dbox of catalase mRNA (through the L7Ae protein) directs the whole complex (CD63-L7Ae-mRNA) to exosomes. This system loads catalase mRNA into exosomes. The validation of this method showed that Lamp2b-RVG on the surface of these exosomes targets them to brain tissue and subsequently reduces neuroinflammation and neurotoxicity in the mouse model of Parkinson's disease [68]. This is one of the most complex reports on parental cell-based exosome engineering that displays a neuron-targeting protein (RVG) on exosomes and loads a therapeutic mRNA into their lumen simultaneously (Fig. 3a).

Sutaria et al. [69] designed a system for loading miR-199a into exosomes. They fused a modified miR-199a composed of pre-miR-199a, to a trans-activating response element (TAR) sequence (trans-activation response RNA loop) and separately fused a trans-activator of transcription (TAT) peptide (transcription activator peptide of HIV-1) to

Lamp2a (Lamp2a-TAT). When expressed in HEK293 cells, miR-199a-TAR binds to Lamp2a-TAT, and the interaction on the luminal C-terminal of Lamp2a leads to the effective loading of miR-199a into the lumen of exosomes (Fig. 3b). This TAT-TAR protein–RNA interaction strategy can be an efficient tool for loading any therapeutic microRNAs (miR-NAs) into exosomes and microvesicles [69].

In this context, using of RNA binding modules seems to be an efficient method for loading RNA into exosomes. Similar to TAT and L7Ae, HuR is an RNA binding protein that binds strongly to miR-155. In this regard, HuR protein was used as an RNA sorting module for exosome engineering. In one study, HuR was fused to the C-terminal of CD9 in order to be localized in the exosomal lumen. Binding to miR-155 and simultaneous localization of the HuR module inside the exosomes results in the loading of miR-155 into the exosomes. HuR can also bind to adenylate uridylate-rich elements of engineered RNAs [70].

Besides the specific loading of molecules into exosomes by means of MSMs, one approach for nonspecific enrichment of exosomes with a molecule is based on the transfection of parental cells with a gene of interest without any other modules for packaging and sorting. Expression of this gene in the cells results in a general enrichment of the molecules in the exosomes. For instance, when cells are transfected with miRNA mimics, after expression of these miRNAs, they are significantly enriched in both MSCs and their derived EVs [9].

3.2 Direct or Post-Isolation Exosome Engineering Approach

Small nucleic acid molecules (e.g., miRNAs and short interfering RNAs [siRNAs]) and therapeutic molecules such as anticancer drugs can be encapsulated in exosomes by direct exosome engineering. This common type of exosome engineering is technically less complex compared with the parental cell-based methods, and has been widely employed for the application of exosomes as drug delivery or gene delivery vehicles in the last 2 decades. In this approach, common manipulation methods of electroporation [20, 71], extrusion, sonication, incubation, freeze-thaw, bio-conjugation, click chemistry, and cloaking [55, 61] can be used for direct engineering of exosomes after isolation from cells. Given that these methods do not provide a continuous source of engineered exosomes, in contrast to the parental cell-based engineering methods, concise protocols for frequent engineering procedures are warranted for clinical applications. Several informative review articles have been published that describe direct exosome engineering; thus, we refer the readers to these reviews for more information [72–74]. However, a brief review of these methods is presented here in the next sections and in Table 1.

4 Clinical Applications of Designer Exosomes

In the previous section, we described how researchers can engineer exosomes, and the basics of the methods and underlying mechanisms were discussed. In this section, we discuss the latest research on designer exosomes for specific clinical purposes. Indeed, cancer treatment and regenerative medicine seem to be the most important fields in which designer exosomes are being used.

4.1 Targeted Designer Exosomes

Tetraspanins on the surface of exosomes indicate both the origin of exosomes and their desired cells. These proteins cause a general slight attraction between exosomes and specific cells [85]. However, this general attraction of exosomes is not enough for targeted delivery of molecules to specific cells, and it requires the existence of targeting peptides or proteins on the surface of exosomes. In recent years, a substantial number of studies have focused on using exosomes for gene and drug delivery, while only a few of them exploited targeted designer exosomes. In the previous sections, we briefly discussed the basics of exosome targeting approaches. Here, recent studies aimed at developing targeted designer exosomes will be discussed.

In an example of an immunotherapeutic application of designer exosomes, transfection of murine melanoma cells by the *CIITA* gene led to the overexpression of MHC-II on their surfaces, hence, producing exosomes enriched in MHC-II. Next, the engineered exosomes were targeted toward T cells and increased the type 1 T helper cell (T_H1) response against cancer cells. Therefore, the enriched MHC-II molecule in the engineered exosomes acted as a targeting peptide and a therapeutic agent [86]. Alternatively, an antibody light chain can be attached to the exosomal surface to direct exosomes toward T cells [87].

Due to the distinct ability of exosomes to cross the BBB and deliver drugs to the brain, treatment of neurodegenerative disorders and related malignancies remains one of the main goals for targeted designer exosomes. Using targeting modules on the surface of exosomes specific for neurons and brain tissue can efficiently target the engineered exosomes toward brain to deliver therapeutic molecules. In one study, overexpression of folate receptor α (FRα) on the surface of exosomes permitted them to specifically bind to the brain parenchyma [88]. Another study used RVG surface-displayed designer exosomes for the delivery of siRNAs to knockdown β-secretase 1 (BACE1), an essential factor of Alzheimer's disease, which suppressed BACE1 by ~ 40% [43]. Other designer exosomes developed against neurodegenerative diseases will be discussed in the regenerative designer exosomes section.

Exosome targeting can also be accomplished by surface chemical modification in a direct engineering approach. In a study by Nag and Awasthi [89], a method of direct engineering called "cloaking" was applied, which is a membrane anchoring platform and consists of three components: a DMPE phospholipid membrane anchor, a polyethylene glycol (PEG) spacer, and a streptavidin molecule. Briefly, DMPE-PEG-streptavidin inserts into the exosomal membrane via hydrophobic interaction and provides an anchor for the connection of biotinylated ligand proteins [89]. A recent study used both the cloaking platform and parental cell-based surface display methods to develop targeted designer exosomes. These exosomes were engineered to target the injured myocardium via chemical engineering or surface display of an ischemic homing peptide. Comparative analysis revealed the same results of in vivo biodistribution to injured myocardium for both methods [90], verifying the potential of the two different approaches for targeted
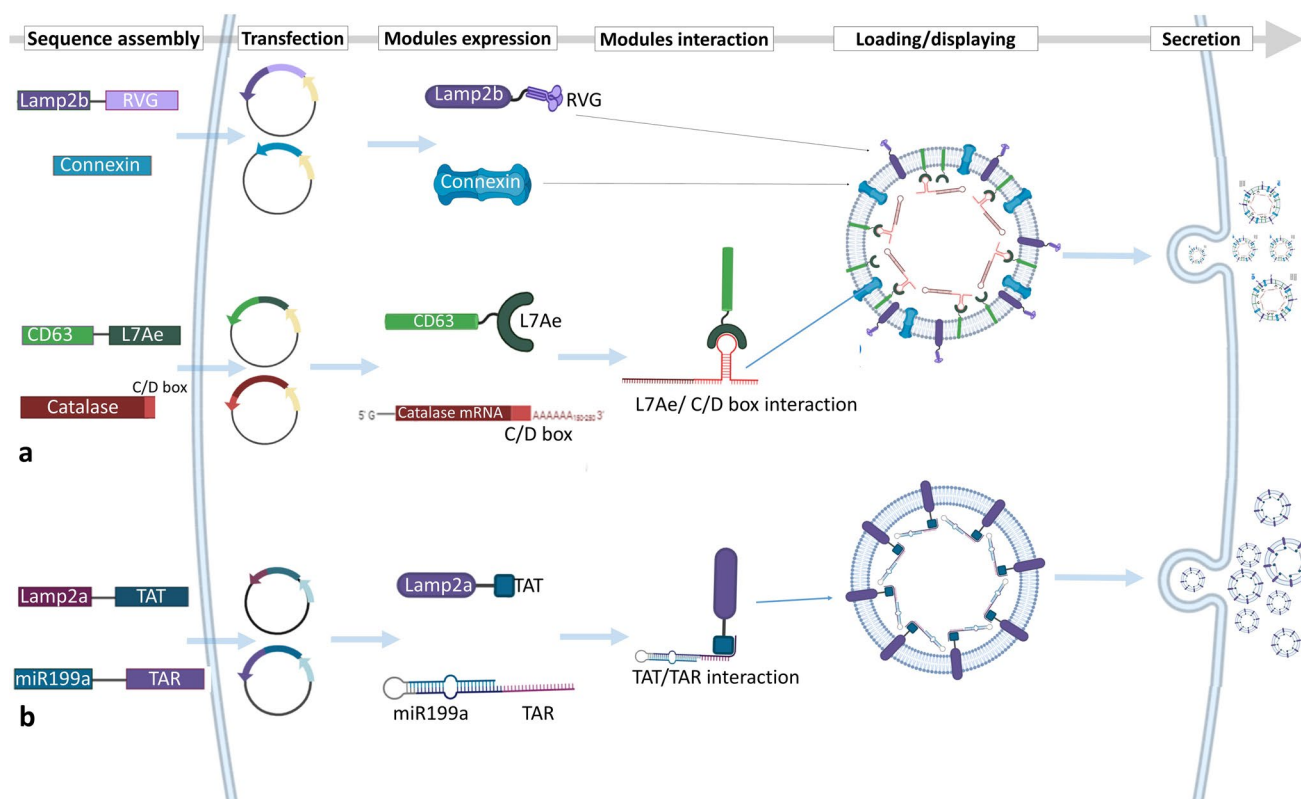
Fig. 3 Parental cell-based exosome engineering for loading RNAs into the lumen of exosomes. **a** The different modules of the EXOtic device for loading catalase mRNA into the lumen of exosomes using the CD63-L7Ae sorting module. Connexin is a packaging helper module, and Lamp2b-RVG is a targeting module. **b** Loading of miR-199a into exosomes using TAT–TAR interaction. Interaction of Lamp2a-TAT as the sorting module with the TAR sequence fused to miR-199a results in the loading of miR-199a into the exosomes. *EXOtic* EXOsomal Transfer Into Cells, *Lamp2* lysosome-associated membrane protein 2, *RVG* rabies viral glycoprotein, *TAR* trans-activating response element, *TAT* trans-activator of transcription

designer exosome development. Although chemical-based engineering methods such as cloaking are more straightforward than the parental cell-based methods, they need to be carried out for every isolated exosome. Overall, the cloaking technology has been recognized as a versatile exosome surface engineering method for development of targeted designer exosomes [78]. Targeting moieties are one of the most important parts of targeted therapy, and Table 2 provides the targeting moieties used for targeted designer exosome development.

4.2 Anti-cancer Designer Exosomes

As described in the beginning of the previous section, in some cases, exosomes have intrinsic homing ability toward certain cells. For instance, exosomes derived from Raw264.7 (macrophage; Abelson murine leukemia virus-transformed) and U937 (of the myeloid lineage) cells show targeting ability toward cancerous endothelial cells [112]. Jang et al. hypothesized that (at least for the exosome-mimetic nanovesicles) the presence of lymphocyte

function-associated antigen 1 (LFA-1) inherited from the donor cell line facilitated binding of the exosomes to endothelial cell adhesion molecules (CAMs), and in turn directed them to the growing tumors with widespread neovascularization [112]. However, significant adverse side effects for healthy proliferating cells, especially in the case of anti-cancer chemotherapeutic agents, have encouraged scientists to design targeted vehicles [101, 105]. In this section, we focus on targeted designer exosomes specifically designed for cancer therapy.

The GE11 peptide has a strong affinity for epidermal growth factor receptor (EGFR). A recent study developed a GE11-based targeted designer exosome for breast cancer treatment. They used a GE11 targeting peptide, hemagglutinin, and myc-tag, fused to the PDGFR, in order to be expressed on the exosome surface [56]. EGFR upregulation in several types of solid cancer represents great potential for cancer treatment through targeting this receptor [113]. GE11 binds to EGFR without any receptor activation; thus, it makes a potential candidate for drug delivery to tumor tissue [56]. In this study, EGFR targeting

Table 1 Studies that used direct or post-isolation exosome engineering approach

EVs source	Loaded molecule	Loading method	Displayed molecule	Displaying method	Mechanism of action	Result	Target cell/organ/tissue	References
A549	Pd catalyst	Incubation	–	–	Pd induce dealkylation and prodrug activation	Tropism for A549 cells and activation of HDAC prodrug	A549	[75]
HFL-1	Erastin	Sonication	FA	Incubation	FA bound to FA-R	Suppress proliferation and migration	MDA-MB-231	[76]
Raw264.7	–	–	CHP	Incubation	Exosome was coated with CHP nanogel	CHP systems induce transporting and effective delivery by exosomes	HeLa	[77]
MCF-7	DOX	Electroporation	FA-EV [TA-Fe^{3+}]	Clocking	EV-[TA-Fe^{3+}] as protective layer contain less negative charge	Tumor targeting and pH-controlled release of drugs	MCF-7	[78]
LIM1215	DOX	Incubation	A33 antibody	By iron oxide nanoparticles	Attachment of A33Ab to A33$^+$ exosomes	Targeting of Ab-exosomes-DOX to A33$^+$ cells	LIM1215	[79]
Macrophage	PTX	Sonication	PEG-AA	Incubation	AA binds to sigma receptor	Targeted drug delivery and accumulated in cancer cells	NSCLC	[80]
HEK293T	Survivin-siRNA	Incubation	PSMA$_{apt}$, EGFR$_{apt}$, FA	Decoration by 3WJ	Aptamers and FA bound to receptors on the cells	Knockdown of survivin gene, inhibits tumor growth	Prostate, breast and colorectal cancer cells	[81]
HUVECs	PTX	Incubation	Biotin-avidin	Incubation	Facilitates PTX loaded exosomes isolation by microfluidics device	As a drug delivery vehicle, accumulates in cancer tissue	HepG2	[82]
DCs	let-7 and VEGF siRNA	Electroporation	AS1411 aptamer	Incubation	AS1411 binding to nucleolin	AS1411-EVs act as a potential drug delivery vehicle for treat cancers	MDA-MB-231	[15]
Neuro2A	–	–	EGa1 nanobody and PEG	Bioconjugation	EGa1 bound to the EGFR and PEG enhance EVs characteristics	Prolonged circulation times, EV accumulation in targeted tissues and improving cargo delivery.	Neuro2A and A431	[83]
H1299 and YRC9 cells	GNPs and DOX	Bioconjugation	–	–	DOX-GNP complex by pH-sensitive linker induce pH-sensitive drug release	Low toxicity and efficient drug delivery to cancer cells and pH-sensitive drug release	H1299	[84]

3WJ 3-way junction, *A431* epidermoid carcinoma cell line, *A549* human lung cancer cell line, *CHP* cardiac homing peptide, *DC* dendritic cell, *DOX* doxorubicin, *EGFR* epidermal growth factor receptor, *EV* extracellular vesicle, *FA* folic acid, *FA-R* folic acid receptor, *GNP* gold nanoparticle, *H1299* human non-small cell lung carcinoma cell, *HDAC* histone deacetylase inhibitor, *HeLa* immortalized human adenocarcinoma of the cervix cells, *HepG2* human hepatoma cell line, *HFL-1* human fetal lung fibroblast cells, *HUVEC* human umbilical vein endothelial cell, *LIM1215* human colorectal carcinoma cell line, *MCF-7* breast cancer cell line, *MDA-MB-231* human breast cancer cell line, *MDA-MB-241* triple negative breast cancer cell line, *Neuro2A* murine neuroblastoma cell line, *NSCLC* non-small cell lung cancer, *Pd* palladium, *PEG* polyethylene glycol, *PTX* paclitaxel, *Raw264.7* murine macrophage tumor cell lines, *siRNA* short interfering RNA, *VEGF* vascular endothelial growth factor, *YRC9* lung cancer cell line

by systematically injected targeted designer exosomes in a Rag2$^{-/-}$ mice model of breast cancer significantly decreased the tumor size via accumulation in tumor tissue. In this regard, the main step prior to the development of targeted designer exosomes is finding suitable and specific targeting peptides. Table 1 summarizes the targeting peptides previously used for designing targeted designer exosomes or for detecting exosome-producing cancer cells [114].

RGE is a specific targeting peptide for NRP-1 on glioma cells. Following the click chemistry-assisted surface display of RGE peptide, targeted designer exosomes were loaded with curcumin and superparamagnetic iron oxide nanoparticles (SPIONs) (RGE-Exo-SPION/Cur). Binding of RGE peptide to the exosome's surface was carried out by a cycloaddition reaction of sulfonyl azide. Briefly, the targeted designer exosomes were generated in two steps: conjugation of the alkyne group with the protein (the phosphatidylethanolamine) on the exosomal membrane with an EDC-NHS condensation reaction and conjugation of RGE with the azido group to the alkyne group by triazole linkages. Parental cell-based assessment showed that systemic administration of these targeted designer exosomes can potentially cross the BBB in a mouse glioma model and accumulate in the tumor tissue. These targeted exosomes can also be used for targeted imaging and diagnosis [111].

4.3 Regenerative Designer Exosomes

Various previously published studies corroborate the regenerative effects of exosomes isolated from progenitor and stem cells. MSC-derived exosomes are well studied, and their regenerative potentials have been experimentally approved in regenerative medicine for the treatment of various diseases. However, the contents of exosomes as the key factor of their regenerative effects are not yet fully identified. RNAs and proteins are the main effectors of the regenerative potential of the exosomes, while miRNAs and mRNAs are the most critical bioactive elements in these exosomes. The content of each exosome depends on the cell type and its physiological state. Recent studies have demonstrated that the exosomes secreted by differentiated cells such as induced pluripotent stem cell-derived cardiomyocytes (iCMs) contain a distinct set of miRNAs, which can modify the post-transcriptional and translational profile and phenotype of the recipient cells more than exosomes secreted by undifferentiated cells. Eventually, exosomes derived from these cells have a substantial effect on the promotion of infarcted heart recovery by providing cardiac protective factors such as clusterin, miR-21, miR-126 [115], and miR-210, resulting in reduced infarct size, hypertrophy, apoptosis, and stimulation of angiogenesis [116]. The exosomes isolated from MSCs of different tissues have been extensively used for

regeneration of tissues and wound healing. This approach is a safe and non-immunogenic cell-free alternative to cell-based therapies [117]. However, the lack of targeted therapy and the complexity of exosomal contents are still the main challenges for tissue repairing by exosomes (Fig. 4). Wang et al. [119] developed an efficient method eliminating the risk of the accumulation of exosomes in non-specific organs. Similar to the previously described methods involving targeted designer exosomes, they reconstructed exosomal Lamp2b protein to be fused to ligands or homing peptides to target a specific organ. Fusing ischemic myocardium-targeting peptide (IMTP) to Lamp2b (IMPT-Lamp2b) in the treatment of acute myocardial infarction (AMI) resulted in accumulation of engineered exosomes in the myocardium. In this study, by using IMTP, and in another study, by attaching glycosylation sequences to a cardiac-targeting peptide (CTP-Lamp2b [118]), an increase was recorded in the retention of engineered exosomes in the myocardium of mice in a mouse myocardial infarction (MI) model ex vivo and in vitro. Furthermore, high specificity and efficiency of targeting myocardium using the engineered exosomes resulted in the reduction of inflammation and apoptosis, induced angiogenesis, decreased infarct size, and refined cardiac function [109, 119].

A real challenge for targeting different tissue injuries is the development of tissue-specific targeting moieties. For instance, cardiomyocyte-specific cTnI, is involved in the regulation of cardiac muscle contraction and exists in higher concentrations in the microenvironment of the infarct area. In this regard, cTnI is a potential ligand for targeted delivery of functional regenerative molecules to the MI site. When a targeting peptide that specifically targets cTnI is fused with a regenerative protein, this protein is delivered to the MI site. For targeting exosomes to the MI site, a cTnI-targeting small peptide "STSMLKA" was fused to the N-terminal of Lamp2b and expressed in MSCs. P1–8 BM-MSC was used as the source of designer exosomes. In addition, hsa-miR-590-3p, which downregulates the expression of cell proliferation inhibitor genes, was loaded into the lumen of exosomes after isolation. Treatment of MI models of rats showed that after systemic administration, designer exosomes are localized to the MI site through the concentration gradient of cTnI. As a result, these designer exosomes enhanced cardiomyocyte proliferation in the peri-infarct area and improved cardiac function [4].

For cerebral ischemia treatment, the main obstacle is crossing the BBB. Mesenchymal stromal cell-derived exosomes fused to a cyclo (Arg-Gly-Asp-D-Tyr-Lys) peptide [c(RGDyK)] showed high affinity for integrin αvβ3 in reactive cerebral vascular endothelial cells. The c(RGDyK)-conjugated exosomes (cRGD-Exo) loaded with various drugs, such as curcumin, suppressed the inflammation and

Table 2 Developed targeted designer exosomes using different methods and targeting molecules

Targeting molecule	Target to	Target cell	Display method	Cargo	Loading method	EVs source	Aim	References
RVG	Acetylcholine receptor	Neurons	Fusion to lamp-2	GAPDH siRNA	Electroporation	DCs	Knockdown of BACE1 in Alzheimer's disease	[43]
Pullulan	Asialoglycoprotein-R	Hepatocytes	Incubation	–	–	MSCs	Targeting regenerative exosomes to injured liver	[91]
4F-LDL peptide	LDL-R	Glioma cell line U87	Decoration	Methotrexate	Incubation	L929 cells	Crossing BBB and distribution in glioma site	[92]
FA	Folate-R	TNBC	Coating with DSPE-PEG2000-FA	Erastin	Sonication	HFL-1 cells	Suppression of GPX4/upregulation of CDO1	[76]
GEa1	EGFR	Neuro2A	Bioconjugation	–	-	Neuro2A	Targeting tumor cells	[83]
cRGD	$\alpha v\beta 3$ integrin	Neurons	Bio-orthogonal copper-free azide alkyne cyclo-addition	Curcumin	Incubation	MSCs	Suppression of the inflammatory response and apoptosis	[93]
c-Met binding peptide	c-Met	TNBC/MDA-MB-231	Decoration	DOX	Incubation	Macrophage	Release of the encapsulated DOX, which will accumulate in the nuclei of cancer cells	[94]
IL-3	IL-3-R	CML	Fusion to lamp-2	Imatinib and BCR-ABL siRNA	Transfection	HEK293T	Targeting and inhibition of tumor growth	[95]
iRGD	av integrin	MDA-MB-231 and MCF-7	Fusion to lamp-2	DOX	Electroporation	imDCs	Targeting and inhibition of tumor growth	[96]
Tf	Tf-R	Neurons	Natural	Dopamine	Incubation	HeLa and DCs	Binding to Tf-R, receptor on cells	[97]
cRGD	$\alpha v\beta 3$ integrin	Neurons	DBCO-NHS	miR-210	Incubation	MSCs	Treat ischemic stroke	[98]
AS1411 aptamer	Nucleolin	MDA-MB-231	Conjugating polypeptides with cholesterol	let-7 and VEGF siRNA	Electroporation	DCs	Targeting tumor tissues	[99]
RVG	Acetylcholine receptor	Neurons	Fusion to Lamp2b	AuNPs	Extrusion	HEK293T	Targeting to the brain	[100]
Darpins	Her-2	SK-BR-3 and BT-474	Fusion to Lamp2b	DOX	Electroporation	MSCs	Targeted delivery of DOX to Her-2$^+$ cells	[101]

Table 2 (continued)

Targeting molecule	Target to	Target cell	Display method	Cargo	Loading method	EVs source	Aim	References
Anti-HIV scFvs	Env	Env+ CHO cells	10E8-pDisplay fusion protein	Curcumin and miR-143	Saponin	HEK293T	Targeting Env+ cells	[102]
Anti-Her2 scFvs	Her-2	HeLa, SK-BR-3	Fusion with C1C2 domain of lactadherin	CFSE	Incubation	HEK293T	Targeting to cells	[103]
RGD	αvβ3 integrin	MCF-7 and HUVECs	DSPE-PEG-RGD assemble	Ac4ManNAz	Reversible permeabilization with streptolysin O	K562 cells	Angiogenesis therapy and imaging	[104]
Darpins	Her-2	SK-BR-3 and BT-474	Fusion to Lamp2b	TPD52 siRNA	Electroporation	HEK293T	Delivering siRNA and downregulating of TPD52	[105]
Apo-A1´	Scavenger receptor class B	HepG2 cells	Fusion to CD63	miR-26a	Electroporation	HEK293T	Upregulation of miR-26a and decrease the cell migration and proliferation	[106]
AA-PEG	Sigma receptor	Lung cancer cells	DSPE-PEG-AA assemble	PTX	Sonication	RAW264.7 and BMM	Targeting cancer cells upon systemic administration	[80]
FA	FA-R	MDA-MB-231	DSPE-PEG-FA assemble	Bcl-2 siRNA and PTX	Electroporation	Cal-27	Targeting tumor cells with the aid of FA-R binding	[107]
FA	FA-R	Tumors MCF-7 and HeLa	DSPE-PEG assemble	DOX	Electroporation	Macrophage	Targeting FA-R expressing cells	[21]
Anti-HPV16-E7 scFv	HPV16-E7	HPV16-E7+ cells	Fusion to Nef^mut	–	–	HEK293T	Binding to HPV16-E7+ cells, and inhibition of the proliferation	[108]
CHP	–	Cardiomyocytes	Incubation with DOPE-peptide	–	–	CDCs	Targeted delivery to myocardial infarction	[109]
RVG	Acetylcholine receptor	Neurons	Fusion to Lamp2b	Catalase Mrna	Packaging device	HEK293T	Attenuation of neurotoxicity Parkinson's disease	[68]
RVG	MOR	Neurons	Fusion to Lamp2b	MOR siRNA	Transfection	HEK293T	Downregulating the expression of MOR	[110]
GE11	EGFR	Hcc70	Fusion to PDGFR	Let7 miRNA	Electroporation	Hek293	Binding to EGFR in Rag2-/- mice model of breast cancer	[56]

Table 2 (continued)

Targeting molecule	Target to	Target cell	Display method	Cargo	Loading method	EVs source	Aim	References
RGE	NRP-1	U251	Phosphatidylethanolamine	SPIONs and curcumin	Electroporation	Raw264.7	Crossing the BBB, targeted imaging	[111].

Apo-A1 apolipoprotein A1, *BACE1* β-secretase 1, *BBB* blood–brain barrier, *BMM* bone marrow-derived macrophages, *BT-474* human breast tumor cell line, *Cal-27* oral adenosquamous cell carcinoma, *CDC* cardiosphere-derived cell, *CFSE* carboxyfluorescein succinimidyl ester, *CHO* Chinese hamster ovary, *CHP* cardiac homing peptide, *DC* dendritic cell, *DOX* doxorubicin, *EGFR* epidermal growth factor receptor, *EV* extracellular vesicle, *FA* folic acid, *FA-R* folic acid receptor, *HEK293T* human embryonic kidney cell line, *HeLa* immortalized human adenocarcinoma of the cervix cells, *HepG2* human hepatoma cell line, *Her-2* human epidermal growth factor receptor 2, *HFL-1* human fetal lung fibroblast cells, *HPV* human papilloma virus, *HUVEC* human umbilical vein endothelial cell, *K562* human leukemia cell line, *L929* mouse fibroblast cells, *Lamp2* lysosome-associated membrane protein 2, *LDL-R* low-density lipoprotein receptor, *MCF-7* breast cancer cell line, *MOR* mu-opioid receptor, *MSC* mesenchymal stem cell, *Nef^mut^* mutant of Nef, *Neuro2A* murine neuroblastoma cell line, *NRP-1* neuropilin-1, *PDGFR* platelet-derived growth factor receptor, *PEG* polyethylene glycol, *PTX* paclitaxel, *RAW264.7* murine macrophage tumor cell lines, *RVG* rabies virus glycoprotein, *scFv* single-strain variable fragment, *siRNA* short interfering RNA, *SK-BR-3* human breast cancer cell line, *SPION* superparamagnetic iron oxide nanoparticle, *Tf* transferrin, *TPD52* tumor protein D52, *U251* human glioma cell line, *VEGF* vascular endothelial growth factor

cellular apoptosis in the lesion region in the mouse model of transient middle cerebral artery occlusion (MCAO) [93].

In another study, using cRGD peptide, the RGD-displaying exosomes loaded with miR-210 were systemically administered to localize in the injury site of the ischemic brain. Following intravenous administration, the miR-210 level significantly increased at the site of lesion in a mouse model of transient MCAO. Intravenous administration of these designer exosomes once per day for 14 days resulted in upregulation of integrin β3, vascular endothelial growth factor (VEGF), and CD34 and enhanced the survival rate of animals (5). RGD surface functionalized regenerative exosomes also can be used for angiogenesis therapy (3).

In a study using designer exosomes for renal regeneration, MSCs were transfected with multiple miRNA mimics (miR-10a-5p, miR-127-3p, miR-29a-3p, let-7a, miR-30a-5p, and miR-486-5p) and their significant enrichment in both MSCs and their EVs confirmed. These miRNAs were selected based on their involvement in tissue regeneration and cell proliferation pathways, and were enriched in MSC-derived EVs to promote renal regeneration. Results demonstrated that EVs loaded with these miRNAs maintain their pro-regenerative effects, and verified the potential application of a low dose of the engineered EVs (82.5×10^6) for the promotion of renal regeneration [9].

Polyethylenimine (PEI) is a polymer capable of forming non-covalent complexes with DNA, making it a suitable carrier for DNA and RNA delivery. In order to promote bone regeneration, EVs derived from human periodontal ligament stem cells were engineered with PEI polymer. The PEI-EVs may act through enhancement and promotion of EVs' content delivery to the target cells. The in vivo analysis of PEI-EVs in rat models of calvarial defect showed significant expression of osteogenic markers, increased VEGF and VEGF receptor 2 (VEGFR2), increased vascularization, and consequently enhanced bone regeneration [120].

Finally, using previously described parental cell-based and direct methods, therapeutic molecules including drugs, mRNAs, miRNAs, siRNAs, peptides, growth factors, anti-scarring factors (transforming growth factor [TGF]β1 inhibitors such as decorin and TGFβ3 [121, 122]), and wound healing factors (SDF-1, which has a chemotactic function for bone marrow MSCs [BMSCs] and endothelial progenitor cells [EPCs] in vascularization and healing [123]) can be efficiently loaded (as fully described in Sect. 3.1) into the regenerative engineered exosomes. This kind of regenerative exosome loaded with effective wound healing, regenerative, and anti-scarring factors can be targeted to the wound and scar tissues for regenerative purposes, using targeting peptides such as cardiac homing peptide (CHP) [109] and SDF-1 (with CXCR4 receptor on BMSCs). The recently reported targeting peptide, CAR (CARSKNKDC), showed a potential capacity for homing and penetration into injured

and diseased tissues, and can be expressed on the surface of regenerative exosomes for targeted regeneration [124].

5 Companies Developing Engineered Exosome-Based Therapeutics

Like other areas of basic science, exosomes have been favored by many biotechnology companies because of their predicted lucrative future, which has encouraged companies to invest in exosomes research and development. Consequently, various companies have been funded to promote the commercialization of a particular exosomal product. One of these companies is Codiak Bioscience, founded in 2015, which is arguably the largest developer of exosome-based therapeutics. This company seeks to produce engineered exosomes for the development of advanced targeted drug delivery platforms for neuronal cells. In addition, developing the large-scale production systems of exosomes is one of the main goals of this company (http://www.codiakbio.com/). Evox Therapeutics is pursuing a completely biotechnological approach to produce new platforms for engineered exosomes with novel features in the loading of drugs and targeting

them to specific cancers and tissues such as the brain and the central nervous system (https://www.evoxtherapeutics.com/). Exopharm works on the commercialization of exosome-based products for regenerative medicine, currently including a platform for downstream analysis and two products. The downstream technology developed by Exopharm is called LEAP technology, which is based on chromatography for the separation of ultrapure exosomes for pharmaceutical use (https://exopharm.com/). With a long history in stem cell production, Aruna Bio Company has commercialized the first neuronal stem cell or neuronal precursor from human pluripotent stem cells. Recently, the company decided to commercialize pharmaceutical exosomes for drug delivery to the brain and neurons. This company is also seeking to provide a solution for the large-scale production of exosomes (https://arunabio.com/). In 2018, at the Gordon Scientific Conference, Capricor Therapeutics reported successful development of an exosome-based product called Cap-2003 from cardiosphere-derived EVs that can be used for immunomodulation in immune-related diseases (http://capricor.com/). Many other companies are also active in the development of exosome therapeutics, including Exogenus (www.
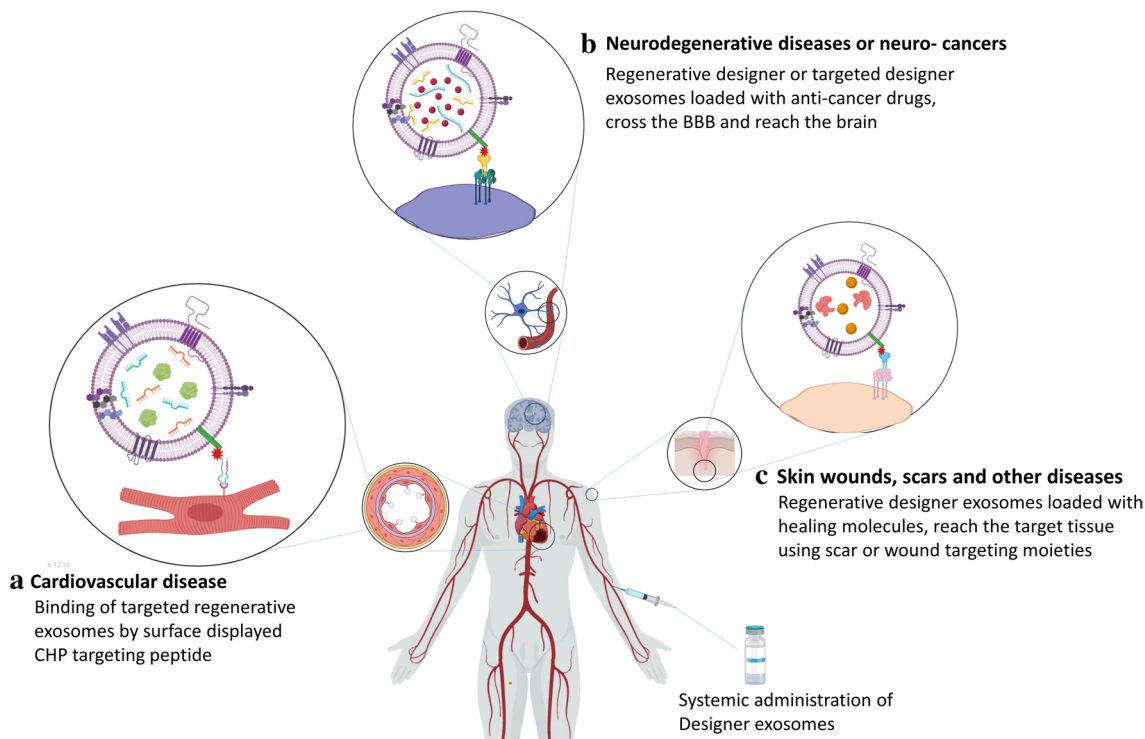
Fig. 4 Application of regenerative designer exosomes for cardiovascular and neurodegenerative disorders and malignancies. **a** Targeted designer exosomes loaded with healing molecules reach the injury site of MI by injury-targeting specific peptides after intravenous injection. **b** Use of neuron- and brain-targeting moieties on exosomes allows the exosomes to cross the BBB and reach the injury site or tumor tissue in the brain and deliver healing molecules or anti-cancer drugs and **c** regenerative designer exosomes containing wound healing factors and anti-scarring and anti-inflammatory factors targeting the scar tissue for treatment of wounds and dermal diseases. *BBB* blood–blood barrier, *CHP* cardiac homing peptide, *MI* myocardial infarction

Exogenus-t.com), Versatope Therapeutics (www.versatope.com), and VivaZome Therapeutics (www.vivazome.com).

6 Future Perspectives and Conclusions

Exosomes are fascinating biological materials. Promising recently published research and developments indicate that most of the current cell therapies could be replaced by their exosomes. The exosomes produced by MSCs, which were widely noticed after the publication of an article by Lai et al. [125], encouraged thousands of other studies on exosomes in the field of regenerative medicine. Interestingly, these studies showed that exosomes could exhibit MSCs' potentials. Therefore, exosomes have been introduced as an advantageous alternative for whole MSCs in cell therapies and regeneration medicine.

Exosome engineering and the production of designer exosomes are entering a new phase. The previous phase of exosome engineering was based on the curiosity of the researchers about the engineering of exosomes. In recent years, development of biological therapeutics has been steadily pursued, and some companies have been founded to manufacture the engineered exosome therapeutics.

As discussed in various sections of this review article, some of the undesirable features of natural exosomes seem to prevent their extension to clinical applications. In this regard, the problems associated with the production and manufacture of exosomes on a large scale and their purification and heterogeneity need to be addressed. It is expected that with the development of large-scale production methods for exosomes as well as engineered cell lines for the production of improved engineered exosomes, the establishment of therapeutic and diagnostic platforms associated with EVs may be possible in the near future. Designer exosomes have been shown to have a high potential, while methods of exosome engineering for improving the effectiveness of natural exosomes expand rapidly. For example, chemotherapeutic drugs [126, 127] commonly used for cancer treatment are associated with significant adverse effects due to undifferentiated cytotoxicity for tumor and healthy cells. In this regard, specifically targeted designer exosomes displaying specific targeting moieties on their surface and loaded with anti-cancer drugs in their lumen are a versatile platform that meet several challenges together [80].

Finally, another highly promising application of designer exosomes is in vaccine development. Studies on vaccine development showed that host cells infected with parasites, viruses, or bacterial pathogens secrete exosomes filled with several antigens of those pathogens [128]. Experiments in vaccine development showed that cocktail and multi-epitopic vaccines (MEVs) could overcome antigenic escape of pathogens from the immune system [129, 130]. Hopefully, using exosome engineering methods, exosomes could be recruited to present multiple antigens as designer vaccine exosomes.

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