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EXOSOME THERAPY: CURRENT PROGRESS OF CARGO-LOADED EXOSOMES IN CLINICAL APPLICATIONS

Nguyen Tat Minh Phuoc[*] and **Phan Phuoc Hien**[†]

[*†] *Research and Application Institute of Science and Technology, Hue Science University, Hue, Vietnam*

[†] *Institute of Applied Science and Technology, Van Lang University, Ho chi Minh city, Vietnam*
e-mail: hien.pp@vlu.edu.vn

Abstract

Exosomes are nano vesicles released from cells that are structurally suitable for loading certain specific therapeutic potential compounds that have specific therapeutic effects in the target disease. Exosomal cargo may vary in response to different physiological or pathological conditions. Depending on the cargo characteristics, we have different ways of cargo loading and enhancing of targeting capacity on exosomes platform for drug deliver purpose. To date, there were a few initial practical applications in clinical practice on several fields, mainly applied in the fields of oncology and neurology. Here, we review the latest progress of exosomal cargo-loading biotechnology for therapeutic purposes. We also present current exosome engineering strategies for optimal clinical safety and efficacy.

I. Introduction

Exosomes are membrane-bound extracellular vesicles (EVs) released from cells into the extracellular space. All cells, prokaryotes and eukaryotes, release EVs as part of their normal physiology and during acquired abnormalities. Exosomes are EVs with a size range of from 40 to 160 nm (average 100 nm) in diameter with an endosomal origin. It is widely accepted that exosomes are

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generated from inward budding of the membrane in endosomes, forming intraluminal vesicles into multivesicular bodies (MVBs) that eventually fuse with the plasma membrane and release exosomes into the extracellular space.

Beside exosome, there are other similar nanoscale carriers have been exploited for drugs delivery in biomedicine, such liposomes [1], dendrimers [2], micelles [3], mesoporous silica nanoparticles [4], metal organic frameworks [5], and many other artificial nanomaterial-based carriers [6]. However, the disadvantages of these carriers are significant considerable such as the low efficiency to cross biological barriers such as blood brain barrier (BBB), short half-life in biological fluids, and the concerns of immunogenicity and toxicity [7, 8]. Because of these disadvantages of the above carries, exosome-based carriers are now receiving growing attention from scientific community [9, 10].

Exosome cargo content can diverse to three diversions. First is the nature contents that already exist inside exosome when it created. Depending on the cell of origin, EVs, including exosomes, can contain many constituents of a cell, including DNA, RNA, lipids, metabolites, and cytosolic and cell-surface proteins. The constituents can be growth factors, cytokines, nucleonic or the endosomal sorting complex required for transport (ESCRT) protein [11] which has been widely accepted as a key mechanism in biogenesis and cargo sorting [12]. Exosomes that only have the first content diversion is called naive exosome which are ready for further engineered to create engineered exosome. Second diversion is the exosome that are only engineered for targeting purpose, the exosomes in this diversion use for transport nature contents in exosome to specific cells type or tissue [13]. The third diversion included exosomes that loaded with either synthetic chemicals such as doxorubicin (DXR) or non-synthetic chemicals that incudes nature primary and secondary metabolites such as curcumin and paclitaxel [14]. It also can be collected from other cells to enhance exosomes clinical ability such as miRNA [13] or enzymes: catalases etc. [15]. Mostly exosomes that loaded with cargo also modified to enhance targeting capacity of exosomes to increase its delivery efficient (Figure 1).

Emerging evidence has implicated exosomes as an essential intercellular communicator in mediating various physiological and pathological processes [16]. Beside their natural biological functions, exosomes have been considered as promising natural carriers for drug loading and delivery due to the following reasons. First, exosomes have the capacity to cross various biological/physical barriers (e.g. BBB) within our body [9]. Second, exosomes are Nano scale non-immunogenic vesicles that are able to minimize drug clearance by mononuclear phagocyte system (MPS) and protect their cargos from enzymatic degradation [17-20]. Third, exosomes are naturally generated vesicles that are less toxic than synthetic nanoparticles [20]. Fourth, exosomes can be intranasal, intravenously, intraperitoneal and intracranial administrated, which indicates the

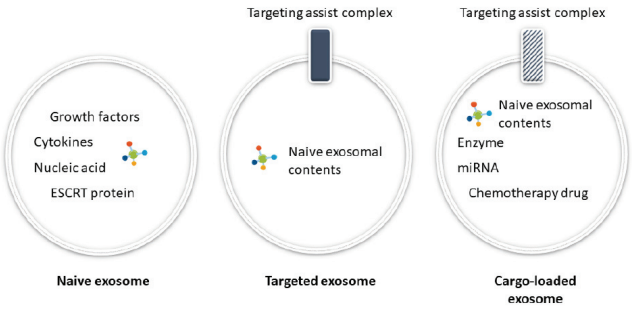

Figure 1: The three diversions of exosome: The naive exosomes are unmodified exosomes that only content basic exosomal molecular. The targeted exosomes with targeting assist complex aim to deliver exosome contents to the specific cells or tissue. The cargo-loaded exosomes contain specular therapeutic potential compounds such as enzyme, miRNA or synthetic chemotherapy drug. The exosomes in the third diversion may or may not have been modified with targeting assist complex for enhance delivery efficient.

high flexibility and compatibility of exosome-based drug delivery [15, 21-23]. Fifth, desired cargos can be selectively loaded into exosomes through physical/chemical/biological approaches to achieve diverse therapeutic effects. Up till now, mounting studies have reported successful loading of a variety of cargos, such as drugs, nucleic acids, proteins, and nanomaterials, into exosomes through incubating cargos with exosomes or exosome-secreting cells, transfection, physical treatments (sonication, electroporation, extrusion, freeze-thaw, surfactant treatment and dialysis) and in situ synthesis. The last but not the least, the surface of exosomes can be modified with homing-molecules (ligands, pH-responsive motifs, magnetic materials) to obtain targeting property for in vivo drug delivery. Therefore, the drug delivery capacity of exosomes has drawn growing attention of scientific community and been foreseen as a promising therapeutic strategy in treating various diseases including cancer and neurodegenerative diseases [9, 17].

In this paper, we review the latest clinical developments of cargo-loading exosome biotechnology for diagnostic and therapeutic purposes. We also present current exosome engineering strategies for optimal clinical safety and efficacy, and assess the technology developed for good manufacturing practice compliant scaling up and storage approaches along with their limitations.

II. Current progress of cargo-loaded exosomes in clinical applications

1) Loading of therapeutic cargos into exosomes

Previous studies that show us exosome is a suitable platform for cargo loading and cargo transfer inside endothelial environment. Cargo can vary from protein, lipid, nucleotide to synthetic secondary compound. Cargo loading method for exosome should be considering depended on physical and chemical characterizations of the cargo substrates.

Here, we provide an overview of two different loading approaches. First approach is based on loading of therapeutics into cells from which the EVs are derived, may result in subsequent EV loading with the drug of interest. The second approach involves loading of EVs after their isolation. An overview of the described loading techniques is depicted by the following diagram (Figure 2).

a) Loading of cells before exosome isolation

Incubation were early used to treat exosome-secretion cell. The method incubates the desired cargos with donor cells, and then collect secreted exosome cells. Luan et al. were treat donor cell with drug by incubation method, and then exosomes loaded with the drug were isolated from cell media [19]. can interact with and be incorporated into exosomes or exosome secreting cells spontaneously.

Transfection is the most stable way for loading nucleic acids, proteins, and peptides into exosomes. Using transfection reagents, specific vector encoding desired DNA, RNA with are transduced into cells to create modified cell lines. The cell lines then ectopically express desired nucleic acids, proteins, or peptides that are packaged into exosomes afterwards.

For example, Luo et al. transfected MSCs with a miR-122-expressing plasmid using Lipofectamine based protocol and generated miR-122-enriched exosomes that exhibit encouraging therapeutic potential in treating hepatocellular carcinoma [24].

b) Loading of exosome after exosome isolation

The basic principle of loading cargo into exosome is to create force that push cargo molecules into exosome all ways though its membrane. Some methods that create nano pores on the exosomes membrane or membrane recombination that promotes cargos' entering into exosomes to achieve cargo-loaded exosomes. These methods divided into incubation, sonication, electroporation, extrusion, freeze-thaw, surfactant treatment and dialysis.

Incubation is a most common and simplest way for post-isolation cargo loading. The method incubates the desired cargos with exosomes to let cargos diffuse into exosomes by concentration gradient. Since most exosomes and plasma membrane are hydrophobic and lipid-enriched, cargos, especially hydrophobic ones such as curcumin and paclitaxel [14]. Moreover, exosome contains a hydrophilic core which can be loaded with hydrophilic cargos [25].

Sonication can be seen used in generate Gemcitabine loaded autologous exosomes by Li et al. to treat pancreatic cancer [26]. Similarly, Haney et al. obtained paclitaxel loaded exosomes by sonication, which possessed higher loading capacity than the simple incubation strategies for treating breast cancer [27].

Both Sonication and Electroporation can create nano pores on exosome surface, but Electroporation can do more than that because it creates electrical field that apply magnetic force on polar molecule and help them effetely migrate into exosome. Tian et al. electrocute the mixture of doxorubicin and immature dendritic cell-derived exosomes with a voltage of 350 V and successfully obtained doxorubicin-loaded exosomes [28]. Similarly, other chemotherapeutic drugs such as paclitaxel, can also be loaded into macrophage derived exosomes by electroporation [29].

Surfactants such as saponin and triton use in surfactant treatment to dissolve exosome membrane molecules (e.g. cholesterol) and create pores on exosomal surface, thus leading to an increase in membrane permeability [30]. Surfactants significantly promote the loading capacity of various types of molecules into exosomes, as compared with the simple incubation method [19]. For instance, the saponin-assisted method obtains an up to 11 folds' higher drug loading of hydrophilic compound compared with passive loading without saponin [31]. Saponin also facilitates the loading of catalase, a natural antioxidant, into exosomes, and the engineered exosomes provided significant neuroprotective effects in PD model post intranasal administration [15]. The disadvantages of this method is the surfactants may degrade/inactivate loaded cargos, potentially influencing the therapeutic effects. Besides, exceeded saponin is hemolytically active in vivo, thus the concentration of saponin used for drug loading should be strictly limited and extra purification process is necessary to remove saponin [30].

Rapid freeze-thaw treatment is a well-recognized reconstitution procedure for liposome formation [32, 33]. Due to the explosion of exosome research, pilot studies have started to apply this method to generate reconstituted exosomes [15, 34]. It is reported that drugs can be loaded into exosomes efficiently by mixing the catalase solution with exosomes and treating the mixture with repetitive freeze-thaw cycles [15]. This strategy is also applicable to fuse lipo-

somes with exosomes [34]. After fusing with various liposomes, these hybrid exosomes are embedded with liposomal functional lipids and membrane proteins that regulate the interactions between the engineered exosomes and cells, therefore modulating exosome uptake. Freeze-thaw approach is simple and effective to load various cargos (e.g. drugs, proteins, and peptides) into exosomes directly. However, its drug loading efficiency is lower than sonication and extrusion, two methods that induce membrane recombination [15]. Moreover, repeated freeze-thaw could inactivate proteins and induce exosome aggregation.

Extrusion is a physical procedure that squeezes exosomes and cargo mixture in an extruder to induce membrane recombination. Exosomes membrane collapses and homogeneously blends with cargos to obtain cargo-loaded exosomes after repeated extrusion with certain parameters [35]. Haney et al. used this strategy to produce catalase-loaded exosomes that can be rapidly taken up by neural cells in vitro and in vivo, which, protect neurons from degeneration in PD models [15]. This strategy is also applicable for the generation of exosome-mimetic nano vesicles [36]. In these studies, extrusion exhibits high cargo loading efficiency but the recombination of exosomal surface structure may alter the immune privileged status of exosomes, making it visible to immune cells like mononuclear phagocytes. Multiple reports also demonstrated that desired cargos could be loaded into exosomes via hypotonic dialysis [31, 37]. Hypotonic dialysis procedure increases the drug loading efficiency into exosomes more than 11 folds versus passive incubation [31]. In addition, the dialysis system significantly promotes the loading efficiency of miRNA and siRNA into exosomes by regulating pH gradient between the inside and outside of exosomal membrane [38]. In contrast, there is a result indicates that dialysis may be harmful for loaded cargos, especially proteins and peptides in exosomes [38].

In situ synthesis is a non-invasive alternative strategy to load nanomaterials onto exosomal surface or into exosomes. Zhang et al. extruded dendritic cells with doxorubicin to get doxorubicin-loaded exosomes, and then incubated these exosomes with chloroauric acid trihydrate for 24 h to achieve the self-growth of gold nanoparticles surrounding exosomes via membrane proteins-mediated reduction reaction [39]. Further, Sancho Albero et al. synthesized Pb nanosheets within exosomes [40].

c) Summary

Overall, to efficiently load desired molecular into exosomes and to minimize exosomal surface damage at the same time we need choose the method that is suitable for the physical and chemical properties of the desired molecular. Loading of cells before exosome isolation methods are most suitable to create stable cells or cell lines that it releases exosomes during its life time. However, this strategy is time- and financial-consuming for generating large batches of

cargo-packaged exosomes and not available for drug loading, together with potential harm or contamination for cells and exosomes due to transfection reagent application. Loading of exosome after exosome isolation methods can provide quick and large quantity of cargo loaded exosome. However, the load cargo exosome sometimes can contaminate by substance that used for the loading method, some methods can cause harmful effect for the loaded cargos such as inactivate proteins. How to utilize the strengths of aforementioned strategies and avoid their disadvantages is the main challenge in front of the modern scientific community, which urgently requires more comprehensive studies to expand our understanding of exosome biogenesis and molecular cargo sorting and packaging mechanisms.

2) Engineering exosomes for targeted drug delivery

Another challenge before applying exosome-based delivery system to scientific research and clinical practice is in order to deliver desired cargos to specific tissues or cells, engineered exosomes with targeting capacity are required.

The native exosome already has capacity in deliver cargo to cells that depended on the cell bio characteristics [41] [42]. It is likely due to the distinct exosome internalization efficiency of different mechanisms that are recruited in exosome including clathrin-mediated endocytosis, caveolin-dependent endocytosis, lipid raft-mediated endocytosis, phagocytosis, and micropinocytosis [16]. However, the majority of reports suggest that native exosomes spread in the extracellular spaces and bio-fluid by free diffusion and are randomly internalized into recipient cells. For example, through intravenous, intraperitoneal, or subcutaneous injection, fluorescence dye/probe-labeled exosomes can be observed in liver, spleen, kidney, pancreas and other organs, suggesting the uncontrolled bio-distribution of exosomes in vivo [43, 44]. Thus, to enhance exosome cargo deliver, enhancement of exosome targeting should be considered.

a) Ligand-receptor interaction.

Currently, the ligand-mediated targeting approach has been considered as a well-established and highly specific method for targeted delivery by presenting ligands that recognize their specific receptors on certain types of cells. There are two common strategies to add ligands on exosomal surface: transfection and chemical modification.

Transfection is most widely used in chemotherapy for cancer, the targeting capacity of exosomes is firstly tested on cancer cells. Ohno et al. fused platelet-derived growth factor receptor with transmembrane domain (PDGFR-TD) to express GE11 peptide on exosomal surface to target and deliver let-7a to EGFR-expressing xenograft breast cancer tissue [45].

The technique can also use to guide exosome to normal cell such as cen-

tral nervous system (CNS). In 2011, Alvarez-Erviti et al. engineered dendritic cells to express Lamp2b, fused to the central nervous system specific rabies viral glycoprotein (RVG) peptide via transfection. RVG is expressed on the surface of exosomes derived from engineered cells and guide these exosomes to the CNS by specifically binding to acetylcholine receptor, delivering glyceraldehyde-3-phosphate dehydrogenase (GAPDH) siRNAs to neurons in the brain, and knocking down GAPDH expression [45]. The technique can also use on another cell type such as immune cells [46].

Unlike transfection, chemical modification is a strategy that directly assembles ligands on the membrane of parent cells or exosomal surface to produce targeted exosomes. In 2017, Wang et al. labeled human umbilical vein endothelial cells chemically with biotin and avidin that can bind to tumor cell-enriched biotin receptor and lectin, respectively, in the phospholipid membrane and induces apoptosis [47]. The other label has been use include folate, antibody or hyaluronic acid [12, 48, 49]. Due to severe toxicity of chemotherapy to normal cells, immunotherapy, which induces rapid and strong immune responses by targeted delivery of antigens and adjuvants to the lymph nodes, has been considered as a promising therapeutic strategy for cancer treatment.

b) pH gradient driven targeting

pH-responsive drug delivery systems a promising platform to target tumor since, tumor microenvironment is acidic compared with normal tissues [50, 51]. Exosomes with pH-sensitive cytosine-rich DNA strand can target tumor cells and release Dox that is intercalated within a double-stranded i-motif/flare duplex [52]. This pH gradient-driven targeting strategy can be combined with ligand-receptor binding strategy to enhance specificity and delivery efficiency. For example, Lee et al. Engineered exosomes containing pH-responsive 3-(diethylamino) propylamine (DEAP) and CD44 receptor-binding HA identify the CD44 receptors of cancer cells and alleviate tumor growth in response to acidic extracellular tumor pH [53]. The technique can also use for targeting efficiency toward desired tissues/cells [54].

c) Magnetism driven targeting

Magnetism force can use to driven exosome to the physicochemical or biological characteristics of specific tissues or cells [55-57]. Exosome with strong response to an external magnetic field, which enables exosomes to be separated from blood and to target cancer cells. It has been use to deliver doxorubicin to cancer cells and inhibit tumor growth [55]. The strategy can combine with other strategy to create more effectual delivery system [57].

d) Summary

Exosomes have to be modified to achieve targeting capacity to be utilized as drug delivery platforms. The targeted exosomes can cross the BBB smoothly,

target glioma specifically, and transfer chemotherapeutic drugs efficiently. Currently, multiple strategies have been designed including ligand-receptor binding, pH gradient guidance, surface charge affinity, and magnetic attraction. Overall, the ligand-receptor binding provides a biological force for targeted delivery. This strategy is characterized by its high specificity, low toxicity, and zero impact on exosome size and shape. Therefore, the latest studies focusing on the development of combined targeting strategies with enhanced exosome homing capacity and drug delivery efficiency are promising to pave the way for the clinical application of exosome-based drug delivery.

3) Clinical use

There is an increasing number of trials are investigating exosomes as therapeutic agents in a wide range of diseases including cancer, immunomodulation, neurodegeneration and infectious diseases. Mesenchymal stem cells (MSCs), dendritic cells (DCs), autologous tumor cells and even plant cell are playing the role of carrier for cargo loaded exosome therapy. There is a few clinical trial conducted in the last decade, mainly applied in the fields of oncology and neurology (Figure 3).

a) Cancer

Recently, cargo loading exosome have been used to treat cancer and have yielded many remarkable results and achievements, proving it to be an effective drug carrier for cancer treatment.

Exosome can deliver chemotherapeutic agents, with the aim to enhance their efficacy and reduce side effects. For example, Tian et al. observed significantly improved suppression of breast tumor growth after injection of integrin targeted, dendritic cell-derived exosome loaded with the chemotherapeutic drug doxorubicin in mice, com-pared to free drug [28]. Moreover, doxorubicin was shown to cause less cardiac damage, the most important dose-limiting side effect of the drug, when packaged in EVs. Furthermore, paclitaxel loaded EVs were shown to be more effective for inhibiting growth of Lewis lung carcinoma metastases than Taxol, a commercially available formulation of paclitaxel [58]. Molecular signatures loaded into HCC-derived exosomes may be used to for diagnosis. Exosomal miR-21 was found to suppress the apoptosis of HCC cells and be upregulated in HCC patients [59].

Exosome can effetely be used to treat cancers that resistance to chemotherapy. For example, Hepatocellular carcinoma (HCC) displays high resistance to conventional chemotherapy. Considering that microRNA-122 (miR-122) performs an essential function to promote chemo sensitivity of HCC cells, an effective vehicle-mediated miR-122 delivery may represent a promising strategy for HCC chemotherapy. Luo et al. transfected MSCs with a miR-122-expressing plasmid and create miR-122-enriched exosomes that capable in treating hep-

atocellular carcinoma [24]. Macrophage-derived exosomes loaded with PTX (EV-PTX) and Dox (EV-Dox) shows capable of target cancer cells and exhibited high anticancer efficacy in a mouse model of pulmonary metastases [27].

b) Neurology

Exosomes have the advantages on neurology therapy since it is able to cross the blood-brain barrier. This property has been exploited in diseases such as Parkinson [15] or ischemic stroke. Dong et al. suggested the use of neutrophil membrane-derived vesicles loaded with resolvin D2, acting as anti-inflammatory agent and specifically delivered to the brain and in particular to a stroke lesion [60].

Targeting modified exosomes have been used to enhance effective targeting and reduce impact of chemotherapy on other area of the brain. For example, Alvarez-Erviti et al. delivering glyceraldehyde-3-phosphate dehydrogenase (GAPDH) siRNAs to neurons in the brain with RVG modified exosomes.

c) Inflammation

Exosome is a great platform that is low immunogenicity and capable of carrying anti - inflammation agent to the target tissue and induce the anti - inflammatory process. It has been used as a drug delivery approach for the treatment of inflammatory bowel disease [61]. Sun et al. showed that EVs as vehicles for curcumin increase its solubility, stability, and bioavailability [62]. Furthermore, curcumin-loaded EVs protected mice from lipopolysaccharide (LPS)- induced septic shock. In a follow-up study, daily intranasal administrations of curcumin packaged in EVs delayed and attenuated experimental autoimmune encephalomyelitis, an effect not observed after administration of curcumin alone. Mechanistically, treatment success was likely caused by increased induction of apoptosis in microglial cell [63].

d) Infection Exosome are also used to create vaccine for infectious diseases. Exosome-based technologies to generate vaccines have been exploited for years since it is a suitable vaccines loading and delivering platform [63]. Exosome loaded with the virus spike S protein also used in vitro against the SARS-CoV-2 coronavirus [64]. This technique promises a new approach in the vaccination research for the common diseases prevention and treatment solutions.

e) Summary

Current therapeutic cargo-loaded exosomes are mainly applied in the fields of oncology and neurology. Studies show one of the most important properties of exosomes are that they can pass through some biological barriers (e.g. BBB) to reach the target tissues. This property has been exploited in diseases such as Parkinson [15] or ischemic stroke. When loaded with anti-cancer drugs, exosome not only enhance drug efficacy but also reduce side effects on normal

cells and tissues. Exosome loaded with chemotherapy drug can treat cancers that resistance to chemotherapy which is a very important feat, opening new paths on approaching of cancer treatment.

The application cargo-loaded exosomes in the other clinical fields such as inflammation and infection are still limited. Exosome protect anti - inflammatory and anti-infective agent from digestive system and immune system such as acid and digestive juice in stomach. Modified exosome also help carry drug to damaged tissue which increase effect of the treatment agent on target disease. Therefore, Exosome-based drug delivery is a perfect drug deliver platform is getting a lot of attention from the scientists, that's why it's been used in research to find effective treatment for SARS-CoV-2 - the world pandemic from the year of 2019.

III. Challenges and future prospects

Exosomes have been considered as an excellent delivery platform in biomedicine because of the low toxicity, minimal immune reaction risk, long in vivo circulation, nanoscale size for deep tissue permeation, multiple cargo loading ability, and surface molecule editing potential. Despite of their amazing clinical potential, our knowledge and clinical applications of exosomes as drug deliver platforms is still limited.

To date, many methods have been developed for loading these cargos into exosomes, such as incubation, transfection, electroporation, sonication, and in situ synthesis, etc. However, concerns for current exosomal cargo loading strategies also restrict further utilization of exosome-based carriers in clinical practices as we mentioned above. Particularly, loading cargos into exosomes by incubation is in thrall to the limited loading efficiency. Several model studies on loading cargos into liposome are great references for better understanding of the interaction between cargos and exosomes, benefiting the upgrading of incubation strategy. Transfection can be utilized to stably express desired molecules for therapeutic or targeting purposes, but the transfection process needs to be simplified and the cost needs to be reduced for bulk production. Furthermore, the influence of transfection and gene-editing on exosome secreting and other cellular processes has to be investigated carefully. The physical treatments enhance loading efficiency but bring potential damage and contamination to exosomes, which, also requires more exploration of experimental conditions to precisely control formation of micro-pores or the process of membrane recombination. For in situ assembly & synthesis, it is stringently demanding to broaden the types of loaded cargos.

Exosomes containing therapeutic cargo could be generated by loading ex-

ogenous cargo into cells or by directly loading cargo into exosomes. However, the direct loading of nucleic acids into exosomes may not deliver functionally active cargo into recipient cells efficiently. This phenomenon may result from the low efficiency of transfection by exosomes [19, 60, 61] or the aggregation and degradation of nucleic acids during loading [61]. In addition, cells secrete a limited number of exosomes, which significantly hampers the development of basic research and clinical trials using exosomes. Thus, RNAs or proteins passively loaded into exosomes by lipofection or electroporation without cellular cargo sorting regulation might be less favorable than RNAs or proteins loaded into naturally occurring or preconditioned cell-derived naive exosomes.

Furthermore, the development of drug-targeting methodologies provides effective methods to assemble homing-molecules on exosomal surface for targeted delivery. Molecules that guide exosomes to their targets can be by ligand receptor binding, pH/charge sensitive binding, magnetic attraction strategies, or a combination of different methods for maximum efficiency. By equipping with ligands for specifically expressed receptors, exosomes can target any types of cells/tissues/organs theoretically. However, due to the shortage of ultra-high precision screening technologies and comprehensive investigations, only limited cell-, tissue-, and organ-specific receptors have been identified. To enhance the specificity and applicability of this strategy, more comprehensive systematic screening studies are urgently required. PH/charge sensitivity application strategies are simple-achieved, accompanied with concerns about applicability, bio distribution, and cell/tissue accumulation. In magnetic attraction strategies also has problems including low specificity, poor biocompatible and short half-life of magnetic materials, unwanted influence of magnetic nanoparticles on the morphology and functions of exosomes [64] which need to be solved and improved with more extensive in vivo studies.

Hence, although exosome drug deliver platform has been studied for many years, there are only a few initial practical applications in clinical practice. The limited knowledge of exosomes holds both challenges and opportunities for scientists. Given the predictable future success of the cargo-loaded exosome in clinical application, it will also find application across many other related fields such as beauty and cosmetics. Thus, from now, a significant amount of efforts is desperately needed to push exosome-based cargo delivery from scientific theory to clinical application.

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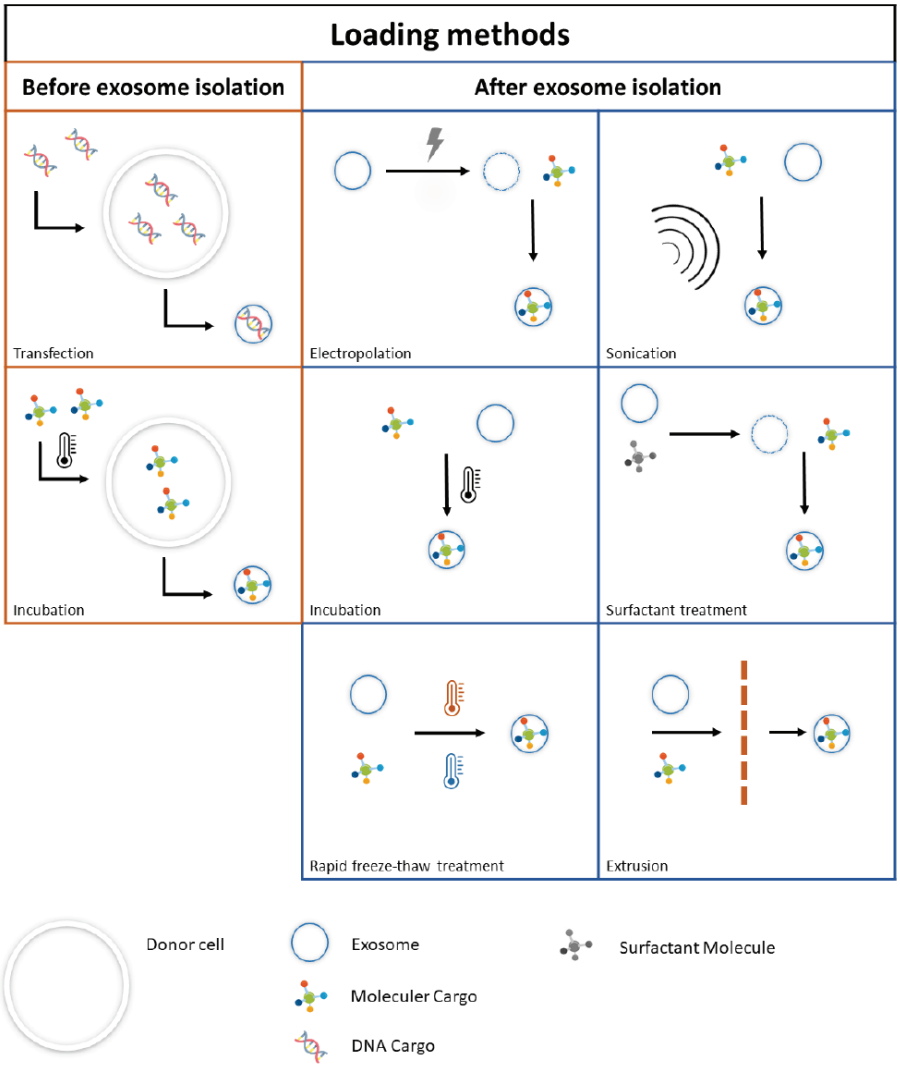

Figure 2: Animated overview of extracellular vesicle loading strategies. Before exosome isolation loading methods: Loading of EVs via transfection of the donor cell with a vector encoding desired DNA, RNA or via incubation of the donor cell with desired molecules. After exosome isolation loading methods: application electrical pulse to create temporary pores, application of ultrasonic frequencies, incubation, treatment with a detergent-like molecule (e.g. saponin), dialysis, repeated freeze-thaw cycles or extrusion.

Medical specialty	Condition	Phase	Year of initiation	Origin	Therapeutic cargo	Outcome	Clinical trial number	Reference
Cancer	Vaccination against non-small cell lung tumors	II	2010	DCs	mCTX, tumor antigen	Significant NK cell activation, no specific T cell response against tumor cells	NCT01159288	[65]
	Recurrent malignant glioma	I	2012	Tumor cells	IGF1R anti-sense molecule	IGFIR downregulation ≤10%	NCT01550523	[66]
	Metastatic pancreas cancer with KrasG12D mutation	I	2020	MSCs	KrasG12D siRNA	IExosomes treatment suppressed cancer in multiple mouse models of pancreatic cancer and significantly increased their overall survival	NCT03608631	[67]
	Colon Cancer	I	2011	Plant	Curcumin	Ongoing	NCT01294072	
Neurology	Acute ischemic stroke	I/II	2019	MSCs	miR-124	miR-124-Loaded Exosomes Ameliorate the Brain Injury by Promoting Neurogenesis	NCT03384433	[13]
					resolvin D2			[60]
Miscellaneous	Dystrophic epidermolysis bullosa	I/II	2020	MSCs	COL7 and COL7A1 mRNA	Ongoing	NCT04173650	
	Irritable Bowel Disease	N/A	2018	Plant	Curcumin	Ongoing	NCT04879810	
	Homozygous Familial hypercholesterolemia	I	2021	MSCs	mRNA	Ongoing	NCT05043181	

Figure 3: Clinical trials investigating cargo-loaded exosomes as therapeutics (from https://clinicaltrials.gov)

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