REVIEW

Cell membrane-derived vesicles for delivery of therapeutic agents

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Abstract   Cell membranes have recently emerged as a new source of materials for molecular delivery systems. Cell membranes have been extruded or sonicated to make nanoscale vesicles. Unlike synthetic lipid or polymeric nanoparticles, cell membrane-derived vesicles have a unique multicomponent feature, comprising lipids, proteins, and carbohydrates. Because cell membrane-derived vesicles contain the intrinsic functionalities and signaling networks of their parent cells, they can overcome various obstacles encountered *in vivo*. Moreover, the different natural combinations of membranes from various cell sources expand the range of cell membrane-derived vesicles, creating an entirely new category of drug-delivery systems. Cell membrane-derived vesicles can carry therapeutic agents within their interior or can coat the surfaces of drug-loaded core nanoparticles. Cell membranes typically come from single cell sources, including red blood cells, platelets, immune cells, stem cells, and cancer cells. However, recent studies have reported hybrid sources from two different types of cells. This review will summarize approaches for manufacturing cell membrane-derived vesicles and treatment applications of various types of cell membrane-derived drug-delivery systems, and discuss challenges and future directions.

1. Introduction

The technology for engineering drug-delivery systems continues to evolve, bringing improvements in therapeutic index[1,2]. The ability of therapeutic agents to survive intact in a harsh extracellular environment is instrumental to the success of drug development efforts[3]. With this in mind, modifications of biopharmaceuticals that increase stability and reduce immunogenicity have been an increasingly active focus of such efforts[4−6]. Since the first introduction of the hydrophilic polymer, polyethylene glycol (PEG), into a protein medicine (Adagen; PEGylated adenosine deaminase)[7], PEGylation has remained the most widely used modification technology in drug-delivery systems[8,9]. However, because of reports of the unexpected clearance of PEGylated materials *in vivo* after repeated administration, the immunogenicity of PEG has come to be considered a limitation that needs to be overcome[10,11].

Biomimetic technology, an emergent alternative to PEGylation, meets these needs and is actively being used in drug-delivery systems[12,13]. This technology seeks to overcome the limitations of drug delivery systems by taking its inspirations from biological elements that make up living matter. A representative biomimetic delivery technology utilizes immune evasion and intracellular uptake strategies of pathogens such as viruses and bacteria[14]. Viral vectors have been used in cell and gene therapy products that recently have been approved by the US Food and Drug Administration (FDA), including Imlygic, Kymriah, Zolgensma and Luxturna[15,16]. However, pathogen-derived delivery systems are still not free from safety concerns, including virulence and immunogenicity[17]. In addition, because viral vectors are not inherently targetable, their use in a wider range of drug delivery systems is limited[14].

Cell membrane components are a newly emerging class of biomaterials and delivery systems for therapeutic cargoes[18−20]. Red blood cells (RBCs) have long been studied as delivery systems capable of entrapping various cargoes, such as nucleic acids or chemical drugs[21−23], but the range of cell types used as drug-delivery systems is rapidly expanding[24,25]. Compared with synthetic delivery systems, cell membrane-derived vesicles offer advantages of natural cell-to-cell interactions and functional membrane proteins on their surface. In this review, we will cover manufacturing methods, modification strategies, and therapeutic applications of cell membrane-derived vesicles for the delivery of therapeutic cargoes.

Some published reviews have focused on cell membrane-derived vesicles[18,26,27], but they mainly introduced new concepts using a few types of cell membranes. As this field has progressed rapidly, we herein comprehensively review various aspects of cell membrane-derived vesicles, including manufacturing methods and surface modification strategies. Moreover, this review highlights the feasible biomedical applications of vesicles from different source cell types. Finally, we provide an in-depth discussion on the current challenges and future directions in relation to methodologies, evaluation, manufacturing, and regulations.

2. Technologies for engineering cell membrane-derived vesicles

2.1. Preparation of cell membrane-derived vesicles

Cell membrane-derived vesicles are prepared through a multistep process that includes digestion of parent cells, purification of cell membranes, and formation of vesicles. A typical protocol for cell membrane vesicle preparation includes three basic steps (Fig. 1). First, the parental cells are broken down by lysing with a hypotonic buffer. Second, the mixture of cell membranes and other cellular components, such as cell nucleus and cytoplasmic organelles, are separated by centrifugation[19]. The centrifugation method may differ depending on the cell type. For instance, preparation of eukaryote cell membranes requires discontinuous sucrose gradient centrifugation to separate the membrane from other cell components and nuclei, whereas this gradient centrifugation step is dispensable for preparation of membranes from nucleus-free cells, such as RBCs. Third, the collected cell membrane is physically broken to yield cell membrane nanovesicles of the size of interest. Various strategies have been reported for cell membrane disruption to form vesicles, including homogenization, sonication, extrusion, and nitrogen cavitation[28]. The choice of disruption method depends on the cell source, the scale of the preparation, and the purpose of the cell membrane. Vesicles can be derived from single or multiple cell sources.

2.2. Modification of cell membrane-derived vesicles

With continued progress in cell biology, our understanding of the components and functions of cell membranes has steadily increased[29,30]. Cell membranes are composed of three main components: the lipid bilayer, comprising phospholipids and cholesterol; protein molecules anchored on the outer region of the lipid layer or embedded in the hydrophobic region of the lipid layer; and carbohydrates, in the form of glycolipids or glycoproteins[31]. Cell membranes function as a biolayer to protect intracellular organelles, regulate metabolism, transport nutrients and waste, and mediate cell contact-dependent signaling.

The functions of cell membrane-derived vesicles can be modified using two basic strategies: pre-modification and post-modification, with pre-modification referring to changes made before disruption of parent cells, and post-modification corresponding to introduction of new components into membranes after isolation.

2.2.1. Pre-modification of cell membrane-derived vesicles
Pre-modification is the method for modifying cell membrane properties based on manipulating parental cells at a genetic or metabolomic level. In this approach, parent cells are pre-treated so as to modulate expression levels of specific proteins or ratios of lipid components, or engineered to alter the structure of hydrocarbon chains in membranes. Vesicles obtained using this method possess cell membrane properties similar to those of parental cells[32,33]. This is exemplified by a recent study showing similar membrane expression of C-X-C chemokine receptor type 4 (CXCR4) protein between parental cells and vesicles[32]. In this study, adipose-derived stem cells were induced to express CXCR4 using a retroviral vector encoding the *CXCR4* gene. Nanovesicles prepared from these cells were found to contain CXCR4 on their surfaces, a modification that conferred on them the ability to penetrate the endothelial barrier. In another study investigating therapeutic strategies against rheumatoid arthritis, human umbilical vein endothelial cells were transfected with a lentiviral vector encoding tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)[33]. Nanovesicles prepared from transfected cells expressed TRAIL, which was able to target inflammatory macrophages and induce their apoptosis.
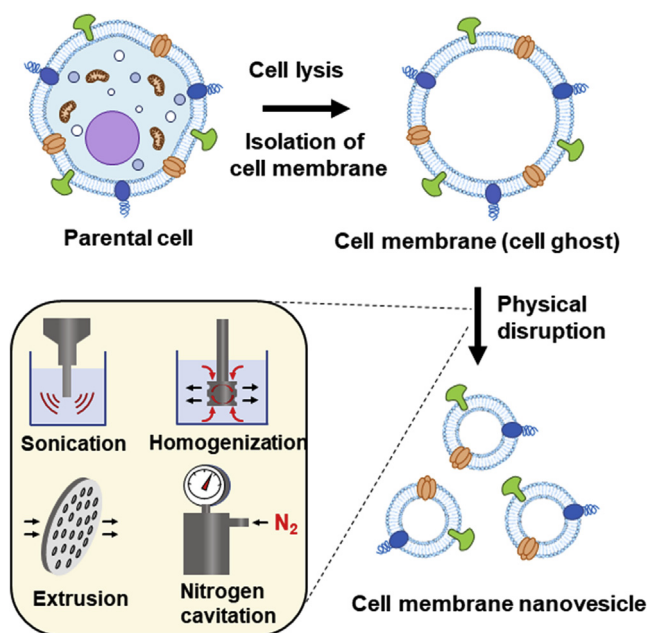
Figure 1 Multistep process for preparation of cell membrane-derived vesicles. Cell membrane-derived vesicles are generally produced through a three-step process of cell lysis, separation of cell membranes, and disruption to obtain nanosize cell membrane vesicles.

A strategy for modifying the carbon hydrate chains of cell membrane nanovesicles through metabolic glycol-engineering of parental cells has also been reported[34]. Metabolic glycol-engineering, a powerful tool for manipulating glycosylation, is not only used for controlling expression levels of natural glycans, but also, and more importantly, introduces artificial mono-saccharides into glycol-conjugates. In this study, T cells were metabolically modified to contain the unnatural azido sugar moiety, *N*-azidoacetylgalactosamine tetraacylated. Vesicles derived from these metabolically modified T cells were meta-bolically labeled with azide groups and attached to tumor cell surfaces *via* a 'click' reaction.

Cells genetically edited *in vivo* can also be collected as parental cells[35]. RBCs are among the most popular parental cell sources for use in generating cell membrane-derived vesicles. However, because mature RBCs lack nuclei, genetic modification of RBCs is impossible. To circumvent this limitation, Lv et al.[35] engineered RBC membrane vesicles to express the tri-peptide, Asn-Gly-Arg (NGR) using the clustered regularly interspaced short palindromic repeats (CRISPR) gene-editing technique. In this study, the transgenic mice were generated by knocking-in a gene encoding NGR peptide during the pre-embryo stage. Fertilized eggs of C57BL/6 mice were injected with Cas9-encoding mRNA, gRNA, and a donor vector containing NGR cDNA. The *Gypa* gene locus (mouse chromosome 8) was targeted for insertion of the NGR-encoding cDNA. Expression of NGR was validated by genotyping of newborn mice. RBCs collected from these mice were exploited to generate RBC membrane vesicles as a tumor-targeting delivery system for an oncolytic virus.

It should be noted that, in this study, the expression of a specific protein on the cell membrane was programmed at the genetic level. Moreover, genetic engineering of parent cells may allow the biosynthesis and integration of specific proteins onto the cell membrane using the existing protein sorting and trafficking machinery, eliminating concerns about alterations in protein function by exogenous chemical or physical coupling onto vesicles. However, this method may be limited by difficulties in controlling the density of specific proteins on the surfaces of vesicles. Thus, the topology of expressed proteins on parent cells needs to be carefully checked. One future research direction regarding pre-modification processing would be to investigate strategies for controlling the density and topology of expressed proteins on parent cell membranes.

2.2.2.   Post-modification of cell membrane-derived vesicles

Although pre-modification of parental cells would be advantageous in providing a homogenous and stable source of vesicles, the types of components and ligand options are limited compared with the post-modification method. With post-modification, functional molecules are introduced after cell membrane vesicles have been collected. Because of the convenience and diversity of modified materials to select from, a number of post-modification strategies have been investigated. Modification materials range from the basic components of cell membrane such as lipids[36], proteins[37] and nucleic acids[38], to synthetic components[39].

One lipid used for post-modification of cell membrane-derived vesicles is cholesterol (Fig. 2A). Cholesterol is an important molecule in the construction of the lipid bilayer structure of the cell membrane. Alterations in cholesterol ratios can change membrane fluidity and rigidity[40]. For example, insertion of cholesterol improves the stability of cell membrane-derived vesicles in terms of their resistance to pH changes in the environment[36]. In the case of RBC membrane-derived vesicles, addition of cholesterol to RBC ghosts at a 5% input ratio, aided by slightly increasing the temperature (37 °C) for 10 min, enhanced the rigidity of RBC membrane-derived vesicles, thereby significantly improving the efficacy of drug loading using a pH gradient-based remote loading method.

Proteins have been tethered to cell membrane-derived vesicles through conjugation[37] or insertion[39] (Fig. 2B). For example, recombinant human hyaluronidase was grafted onto RBC membrane vesicles *via* a bifunctional linker[37]. The linker used contained a maleimide terminal for attaching to recombinant human hyaluronidase *via* cysteine residues, and the other end was functionalized with *N*-hydroxysuccinimide for anchoring *via* amine groups to the membrane surface. This method allows convenient optimization of linker length, a critical factor that affects enzymatic activity. Another study exploited an amphiphilic lipid as a linker for anchoring protein to a membrane vesicle surface[39]. In this application, streptavidin was conjugated to the maleimide terminal of 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[maleimide (polyethylene glycol)-2000] and attached to the cell membrane through insertion of a lipid tail; the latter step was performed at 37 °C for 30 min.

Insertion of a lipid derivative of a protein into lipid bilayers of vesicles allows for protein anchoring without affecting membrane surface proteins, increasing the likelihood of preserving the intact structure of membrane proteins in the vesicles. However, it is possible that chemical modification of a protein with lipid moieties could affect the configuration of attached proteins. Strategies for site-specific conjugation of proteins with lipid moieties need to be carefully designed to minimize possible alterations in the configuration.

Nucleic acids are another type of material used to functionalize cell membrane-derived vesicles (Fig. 2C). Aptamers—short, single-strand oligonucleotides that can specifically bind to a target
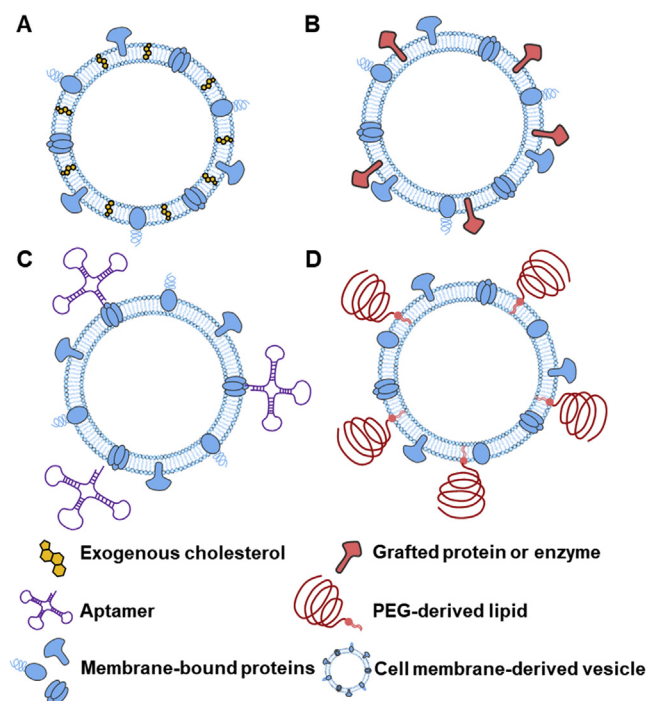
Figure 2 Post modification of cell membrane-derived vesicles. Cell membrane-derived vesicles were modified with various materials to modulate their chemical and biological behaviors. (A) The lipid compositions of membranes were modified to increase the stability of vesicles. (B) Enzymes or other proteins were grafted onto the surface of cell membrane vesicles to provide functionality. (C) Nucleic acids, such as aptamers, were conjugated to cell membrane vesicles for targeted delivery. (D) Synthetic polymers (such as PEG) were grafted into cell membrane vesicles to prolong their circulation time in blood.

substrate—are an important example of this class of material. Peng and colleagues[38] modified a cancer cell membrane with the aptamer, AS1411, a 26-mer G-quadruplex oligonucleotide that binds to nucleolin. The AS1411 aptamer was synthesized by aldehyde capping, a conjugation method involving the reaction of aldehydes and amines of membrane proteins. Equipping cancer cells with AS1411 conferred a tumor-targeting feature on membrane-derived nanovesicles owing to high expression of nucleolin in tumor tissue. Cancer cell membrane-derived vesicles encapsulating the anticancer drug doxorubicin and contrast agent indocyanine green were found to provide enhanced tumor accumulation and tumor-killing effects compared with the free drug.

Synthetic polymers have attracted considerable attention for manipulation of cell membrane-derived vesicles (Fig. 2D). The hydrophilic polymer, PEG, in particular has been exploited for modification of cell membrane-derived vesicles[41−44]. PEG conjugation onto nanoparticles has been reported to increase the colloidal stability of nanoparticles and protect them from phagocytosis, thus prolonging their circulation half-life *in vivo*. PEGylation is applicable to cell membrane-derived vesicles, where it may prevent serum protein opsonization and thus increase retention time in blood. PEGylation can be achieved simply by incubating cell membrane-derived vesicles at 37 °C. Under these conditions, lipid tails in lipid derivatives of PEG are readily inserted into the membrane layers of vesicles.

PEGylation may provide physicochemical and biological advantages to cell membrane-derived vesicles. From the physicochemical viewpoint, the presence of hydrophilic PEG layers on the vesicles may increase the dispersion stability in aqueous conditions and prevent aggregation and size changes of membrane-derived vesicles during storage. Biologically, PEGylation can reduce the interaction of membrane-derived vesicles with macrophages and increase the blood circulation time. In addition to modifying the pharmacokinetic profiles, PEGylation may reduce immune responses to cell membrane components from allogeneic donors.

One concern regarding the current strategies for PEGylation of membrane-derived vesicles is the lack of an exact quantitation method. The effect of PEGylation may depend on the density of PEG on the cell membrane-derived vesicles. Unlike synthetic liposomes, which are designed to have exact amounts of PEGylated lipids, the PEGylation of membrane-derived vesicles is based on the insertion of lipid tails. Going forward, researchers need to develop methods to quantify the amount of PEG on the surfaces of cell membrane-derived vesicles and establish standard procedures that generate reproducible amounts of PEGylation.

2.3. Cell membrane hybridization

Hybrid cell membrane-derived vesicles can be prepared by fusing two different original parent cell membranes (Fig. 3). These vesicles inherit the properties of both parental cell membranes. Equipped with the functionality of each original cell type, hybrid cell membrane-derived vesicles can synergistically perform complex activities.

Several studies have employed a strategy for preparing hybrid cell membrane-derived vesicles in which different combinations of cell types are used to coat synthetic nanoparticles[45,46]. Hybrid RBC-platelet membrane-derived vesicles have been used to coat polymeric nanoparticles[45]. Because RBCs express the immuno-regulatory marker CD47, which acts as a "don't eat me" signal, RBC membrane-coated nanoparticle can avoid clearance by the reticuloendothelial system. On the other hand, platelet membranes highly express P-selectin, a natural ligand of the CD44 receptor[47], thereby allowing targeting of CD44 receptors on cancer cells. Thus, hybrid RBC-platelet membranes are an ideal biomaterial for coating nanoparticles to enhance drug delivery efficacy. Hybrid RBC-platelet membrane-coated poly (lactic-*co*-glycolic acid) (PLGA) nanoparticles were shown to exhibit prolonged circulation time in blood and enhanced binding to MDA-MB231 breast cancer cells compared with uncoated, plain PLGA nanoparticles.

In another study, researchers fused RBC membranes with cancer cell membranes to take advantage of homotypic targeting[48]. The hybridized RBC-MCF7 membrane-coated melanin nanoparticle was found to out-perform each membrane-coated nanoparticle counterpart in terms of tumor accumulation and photothermal effect on MCF7 tumor-bearing mice. Notably, this study indicated that the protein ratio of dual membranes was a critical determinant of the blood retention and homotypic effect. The optimal ratio of the two membranes needs to be empirically determined to maximize performance. Hybrid membrane vesicles can be produced not only using two cell membranes, but also by fusing a cell membrane with a liposome. Pitchaimani and colleagues[49] introduced a hybrid natural killer cell-liposome membrane nanoparticle. The use of liposome membranes in this hybridization technique allows facile simultaneous integration of various lipid components from liposomes into cell membrane vesicles.
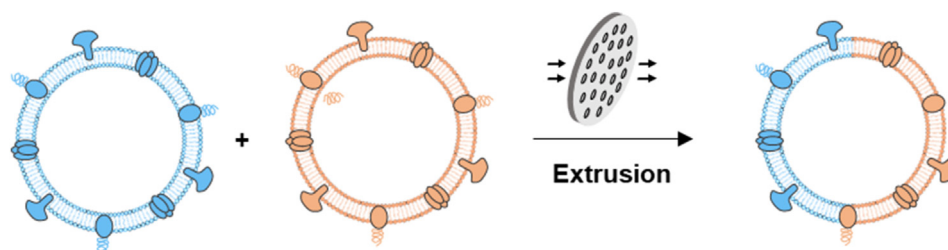
Figure 3 Hybrid cell membrane-derived vesicles. Membranes of two or more cells can be fused to form hybrid membrane-derived vesicles using a co-extrusion technique. Hybridized membrane vesicles can be generated by fusion of cell membrane vesicles with synthetic lipid vesicles, such as liposomes. The hybrid cell membrane vesicles inherit the compositions and characteristics of the parent cells, which can provide synergistic effects depending on the type and source of the parent cells.

Several studies have investigated using cell membranes to coat synthetic nanoparticles. The mechanism by which cell membranes coat nanoparticles depends on the source materials used to prepare the nanoparticles, but our current understanding of these mechanisms is limited. Biophysical studies elucidating the predominant interactions that allow anchoring of cell membranes onto each type of synthetic nanoparticle surface would be informative.

2.4. Coating of nanoparticles with cell membranes

Because cell membrane-derived vesicles have a hollow core structure, they are ideal for use as a coating material for various therapeutic cargo-loaded nanoparticles. In addition to encapsulating small molecules within their interior, cell membrane-derived vesicles have been used to coat a wide range of therapeutic nanoparticles composed of different material types and with different shapes.

Core nanoparticles entrapped in cell membranes are designed to act as drug carriers or as an intrinsically therapeutic entity[50]. By coating a nanoparticle with cell membrane, developers take advantage of the combinational features provided by the cell membrane and the core materials. For example, the lipid bilayer structure of cell membrane-derived vesicles may serve as an additional physical barrier, and such barriers may prevent the burst release of the loaded drug(s) from core nanoparticles[51]. Indeed, sustained release has been observed for drugs that were encapsulated in polymeric core nanoparticles and sequentially coated with cell membranes. In one study, RBC vesicles were found to release more than 50% of encapsulated doxorubicin in the first 16 h[52]. However, the encapsulation of doxorubicin in a PLGA core and subsequent RBC membrane coating was shown to delay the release, with 50% release seen at 36 h[53]. This difference in release kinetics was attributed to the ability of the cell membrane to act as a diffusion barrier.

Encasing nanoparticles within cell membrane-derived vesicles allows increased drug loading. For example, enwrapping PLGA nanoparticles with cell membrane-derived vesicles was reported to increase doxorubicin-loading content to 21%[50] compared with a maximum loading content of 10% in cell membrane-derived vesicles without a nanoparticle core[36]. Various therapeutic drugs have been encapsulated in core nanoparticle, ranging from small molecules including doxorubicin[39], indocyanine green[41] and clarithromycin[54], to macromolecules, such as glucose oxidase[55] and growth factors[56].

Another advantage of a cell membrane coating is that it increases the biocompatibility of the core material. Because cell membranes are constructed from lipids, proteins and carbohydrates, which are biodegradable and found naturally in the body, cell membrane coatings may reduce the cellular toxicity of the core material. Among the nanoparticles that have been coated with cell membranes are metal[57,58], carbon[59], and gold[60] nanoparticles. Cell membrane coating has been reported to reduce plasma protein opsonization and phagocytosis by immune cells, thereby prolonging the circulation time of the core material[61].

The fluidity of the cell membrane is important for the ability of cell membrane-derived vesicles to adopt different morphologies. It has been shown that cell membrane-derived vesicles are capable of coating core materials in a variety of shapes, including spherical, nano cube[62], and nanorod shaped[63] (Fig. 4). In one specific application, iron oxide/manganese oxide nano cubes were coated with U-251MG cancer cell membranes to increase delivery to tumor tissues[62].

Several methods for coating nanoparticles with cell membranes have been reported, including extrusion[64,65], sonication[66], and electroporation[67]. Co-extrusion of cell membranes and core nanoparticles is the most popular method for membrane coating. One advantage of co-extrusion is its use of a polycarbonate membrane with a determined pore size, which allows proper size
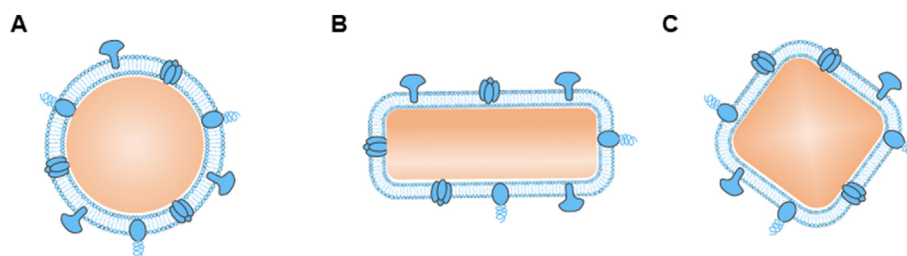

Figure 4 Shapes of cell membrane-coated vesicles. Due to the fluidity and flexibility of cell membranes, core materials of various shapes may be coated with cell membranes. The coated core materials may be shaped as nanoparticles (A), nanorods (B), and nanocubes (C).

control of the final particles. However, the extrusion method may be difficult to adapt to large-scale preparation and may cause loss of material due to sticking to the membrane during extrusion.

A simple alternative method is ultrasonication, which takes advantage of ultrasound wave vibration to fuse membranes and nanoparticles. The efficiency of this coating method may depend on the input power and sonication time. Although ultrasound may be amenable to large-scale production, it requires optimization of parameters (*e.g.*, power and time) to prevent protein denaturation.

Electroporation is another method that has been used for membrane coating. In this approach, an electrical voltage is applied to a mixture of cell membrane vesicles and nanoparticles; this creates multiple, transient pores in the membrane, allowing nanoparticles to enter the membrane vesicles[67]. The advantage of this method compared with the co-extrusion method is its preservation of membrane integrity. However, additional studies may be required to confirm the coating efficiency of the electroporation method.

Several studies investigated the coating of synthetic nanoparticles with cell membranes. The strategies used to realize the cell membrane coating of synthetic nanoparticles may depend on their source materials, and the precise underlying mechanisms are not fully understood. Going forward, biophysical studies are needed to further elucidate the predominant interactions that allow cell membranes to be anchored on each type of synthetic nanoparticle surface.

3. Cell membrane-derived vesicles as delivery systems

3.1. RBC membrane-derived vesicles

RBC membranes have received considerable attention as a nanoparticle-coating biomaterial[19]. RBCs are known to have a long lifespan—up to 120 days in humans. Because of this property, RBC membrane-coated nanoparticles are an attractive option for prolonging the systemic circulation time of drug cargoes. RBC membranes are capable of coating diverse cargo-containing nanoparticles. For example, RBC membranes can be used to coat nanoparticles encapsulating effective photothermal or photodynamic agents or anticancer drugs, the latter of which have been shown to exhibit greater tumor accumulation compared with uncoated nanoparticles[68].

Coating nanoparticles encapsulating photothermal or photodynamic features with RBC membranes has been used to address the issue of short blood retention time, a challenge for using nanoparticles for phototherapy. One recent study used RBC membrane-coated melanin nanoparticles for enhanced photothermal therapy[69]. Because of their enhanced blood retention and improved accumulation at tumor sites, RBC membrane-coated melanin nanoparticles showed significantly higher photothermal efficacy *in vivo* compared with bare melanin nanoparticles. Iron oxide nanomaterials, which are capable of photothermal conversion, have also been coated with RBC membranes[70]. The resulting RBC membrane-coated iron oxide magnetic clusters were found to maintain the photothermal feature of their iron oxide nanocluster core while showing reduced uptake by macrophages. With their prolonged blood circulation pharmacokinetics, RBC membrane-coated iron oxide magnetic clusters showed a lower distribution to the liver and greater tumor accumulation upon intravenous injection in mice.

Nanoparticles loaded with chemotherapeutic anticancer drugs have been coated with RBC membranes. For example, doxorubicin-loaded mesoporous Prussian blue nanoparticles have been coated with RBC membranes for photo-chemotherapy applications[71]. Plain mesoporous Prussian blue nanoparticles suffer from physical instability, short half-life, and nonspecific uptake by macrophages. The RBC membrane coating improves these pharmacokinetic properties, increasing blood circulation time and decreasing non-specific uptake; it also provides synergistic anticancer effects through combined chemotherapeutic and phototherapeutic actions. Co-assembled hydroxycamptothecin and indocyanine green small-molecule drugs have also been coated with RBC membranes[72]. Indocyanine green was used for molecular imaging and as a photothermal agent in conjunction with near-infrared (NIR) irradiation. Coating of the two-drug molecular co-assembly with RBC membranes was shown to provide more effective ablation of tumors compared with treatment with each agent alone.

RBC membranes have been modified to display ligands that enhance delivery to target tissues. This includes peptides, such as the tumor-targeting peptide RGDyK, which in one application was inserted into RBC membranes using a strategy based on avidin-biotin interactions (Fig. 5B)[39]. The peptide-modified RBC membrane was used to coat a drug nanocrystal, and the resulting RGDyK peptide-modified RBC membrane-coated nanocrystal showed greater distribution to the tumor and enhanced antitumor efficacy compared with plain nanocrystals and unmodified RBC-coated nanocrystals. In another application, the neurotoxin-derived peptide CDX was used to modify RBC membranes for brain-targeted delivery[61]. Streptavidin-biotin interactions were again used to tether the CDX peptide to RBC membranes. This was accomplished by preinserting streptavidin into the RBC membrane and then allowing it to interact with biotinylated CDX peptide. Modification of RBC membranes with CDX peptide was found to increase delivery to the brain in a mouse model of glioma.

To enhance tissue penetration, Zhou et al.[37] chemically conjugated human recombinant hyaluronidase to RBC membranes, modifying RBC membranes with the bifunctional linker, succinimidyl-[(*N*-maleimidopropionamido)-polyethyleneglycol] ester, and then fabricating the modified RBC membranes into vesicles. These researchers showed that hyaluronidase maintained its activity after conjugation on RBC membranes using a linker with a molecular weight of 3400, and further demonstrated that the hyaluronidase modification did not change the pharmacokinetics of RBC membrane-derived vesicles *in vivo*.

Cholesterol-enriched RBC membrane-derived vesicles have been studied for their potential to increase encapsulation of drugs[36]. In this application, cholesterol-enriched RBC membrane-derived vesicles were formed by extrusion after hypotonic lysis of RBCs in the presence of free cholesterol. Cholesterol enrichment was shown to stabilize vesicles and increase the efficiency of doxorubicin loading, and the resulting cholesterol-enriched doxorubicin-loaded vesicles exerted higher antitumor efficacy compared with free drug.

Additional concerns relating to temperature and NIR irradiation conditions arise in considering combined use of phototherapy and cell membrane-derived vesicles[71]. Phototherapy provides the unique feature of allowing remote, spatial control of treatment. In general, a high temperature (50−60 °C) within tumor tissue is required for phototherapy to exert an antitumor effect. However, because overheating could result in denaturation of the cell
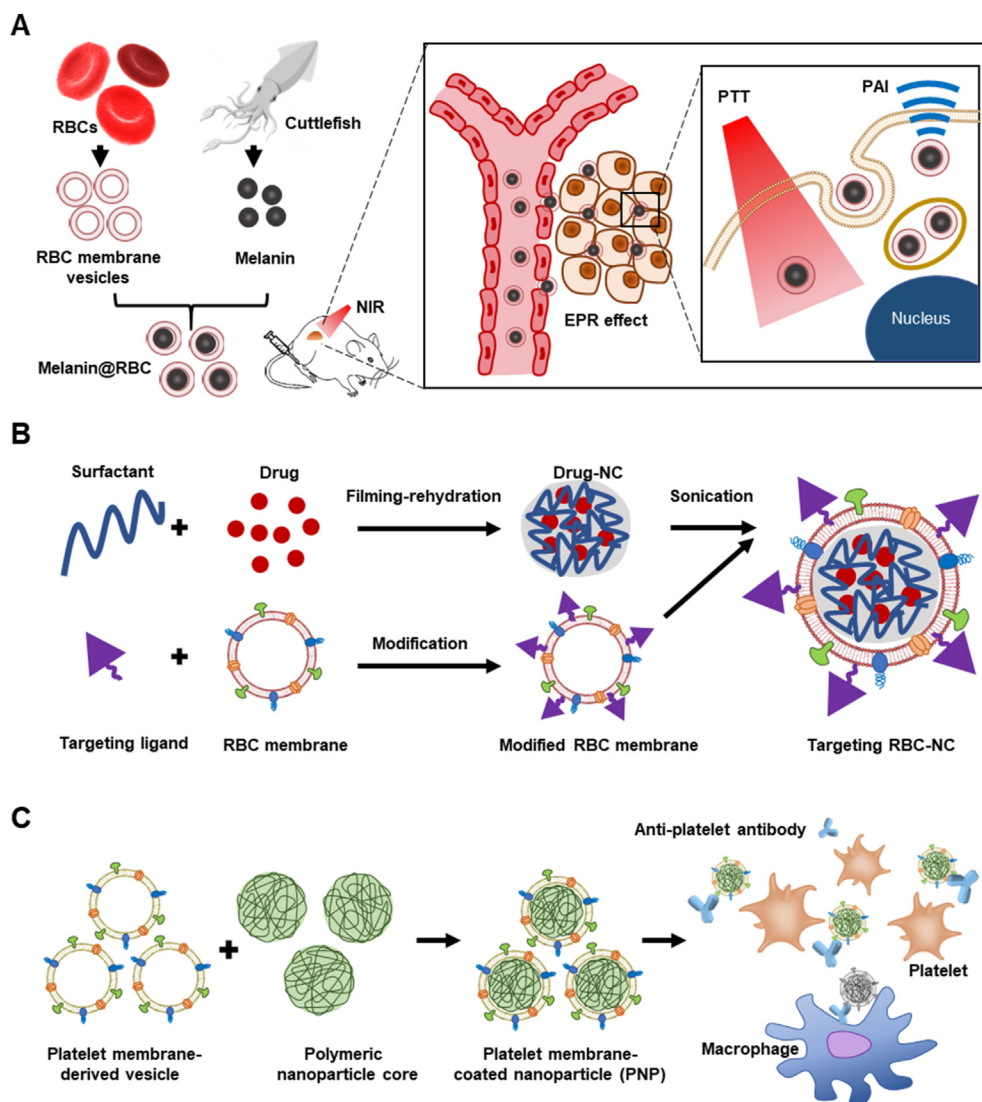
Figure 5 RBC and platelet membrane-derived vesicles entrapping drug-loaded nanoparticles. (A) RBC membrane vesicles enwrapping melanin nanoparticles for photothermal therapy. Adapted from Ref. 69. Copyright © 2017 Elsevier Ltd. (B) Targeting ligand-modified RBC membrane-derived vesicle entrapping nanocrystals. Adapted from Ref. 39. Copyright © 2019 American Chemical Society. (C) Platelet membrane-derived vesicles enwrapping polymeric nanoparticles for treatment of immune thrombocytopenia purpura. Adpated from Ref. 85. Copyright © 2016 Elsevier Ltd.

membrane and associated proteins and cause a corresponding loss in bioactivity, photostimulation conditions need to be carefully optimized. Heating increases cell membrane fluidity, creating a risk of the membrane being stripped from the core material. Hence, the masking effect of the membrane coating, which provides biocompatibility and a targeting feature for core material, may be lost. This underscores the importance of carefully optimizing temperature and laser irradiation schedule in ensuring that cell membrane proteins maintain their functions within the target tissue. Examples of core particles cloaked with RBC membrane-derived vesicles are listed in Table 1.

3.2. Platelet membrane-derived vesicles

Researchers in the drug-delivery field have developed an interest in platelets because of their ability to target specific sites and evade the immune system. Platelets—anucleate blood cells

formed by fragmentation of megakaryocytes—are involved in various physiological phenomena, including blood coagulation, thrombosis, and tumor metastasis[78].

One advantage of platelet membrane is its capacity to evade phagocytosis while in blood circulation. Similar to RBC, platelets have CD47 receptors on their surfaces. CD47 receptors are known to interact with the inhibitory macrophage receptor signal regulatory protein α[79]. The presence of CD47 in platelet membrane can modulate the pharmacokinetics of entrapped drug molecules. In addition, glycoproteins on platelets have been reported to interact with collagen-rich plaque[80], potentially helping localize platelet membrane-derived nanomaterials to atherosclerotic sites.

When using platelet membrane-derived nanomaterials, researchers should emphasize quality control regarding the integrity of CD47 receptors. Any functional alteration of the CD47 receptors on a platelet membrane-derived nanomaterial may change the pharmacokinetics and biodistribution features of the nanomaterial.

Table 1 Core particles entrapped in RBC membrane-derived vesicles.

Core particle	Purpose	Disease	Ref.
Hydroxycamptothecin/indocyanine green	Enhance blood retention Improving tumor accumulation	Human cervical cancer (HeLa)	72
Ag_2S quantum dot	Enhance blood retention Biocompatibility	Mouse colon cancer (C26)	73
Gold nanowire motor	Absorbing membrane damaging toxins	Toxin-mediated	74
Oncolytic adenovirus	Tumor-targeting	Human liver cancer (HepG)	35
Dimeric prodrug	Enhance blood retention Improving tumor accumulation	Human cervical cancer (HeLa)	64
Drug nano-crystal	Tumor-targeting, Improving tumor accumulation	Human glioblastoma (U87)	39
Iron oxide	Avoid immune clearance	Human breast cancer (MCF-7)	70
Magnetic mesoporous silica	Avoid immune clearance Improving tumor accumulation	Human breast cancer (4T1)	75
Melanin	Enhance blood retention Improving tumor accumulation	Human lung cancer (A549)	69
Oil nanodroplet	Absorbing membrane damaging toxins	Toxin-mediated	76
PLGA	Absorbing membrane damaging toxins	Toxin-mediated	77
	Tumor-targeting Improving tumor accumulation	Human lung cancer (A549)	61
Prussian blue	Immune evasion Chemotherapy + photothermal therapy	Human breast cancer (4T1)	71

In addition, platelet membrane-derived vesicles should not be used in patients suffering from autoimmune diseases, such as immune thrombocytopenic purpura. This is because such patients may have autoantibodies against platelets that could form immune complexes with platelet membrane-derived nanomaterials.

Platelet membrane-derived vesicles have been investigated for their potential to cloak various core particles. One example of this is the use of platelet membranes to coat PLGA nanoparticles containing the anticancer drug bufalin[23]. Because platelet membranes are known to express P-selectin, a cell adhesion protein that can bind to CD44 receptors overexpressed in cancer cells[23], these platelet membrane-coated nanoparticles showed greater uptake by H22 hepatoma cells *in vitro* than plain nanoparticles. *In vivo*, intravenously administered platelet membrane-coated nanoparticles exhibited a greater distribution to tumor sites and exerted enhanced antitumor efficacy.

A platelet membrane coating was recently reported for tumor-targeted delivery of photoresponsive nanoparticles encapsulating anticancer drugs[81]. In this application, polypyrrole nanoparticles were mixed with doxorubicin and platelet membranes and subsequently co-extruded. Upon NIR irradiation, platelet membrane-coated polypyrrole nanoparticles showed higher photothermal antitumor efficacy and greater inhibition of tumor metastasis than uncoated nanoparticles.

Platelet membranes have also been used to coat magnetic nanoparticles for ferroptosis-enhanced cancer immunotherapy[82]. Specifically, Jiang and colleagues[82] loaded mesoporous magnetic nanoparticles with sulfasalazine, a drug that suppresses tumor growth and induces ferroptosis by inhibiting uptake of cysteine, and then coated the particles with platelet membranes. The resulting magnetic nanoparticles exerted cytotoxicity through ferroptosis. Intravenously administered platelet membrane-coated nanoparticles were shown to trigger immune responses, and when combined with an anti-PD-1 antibody, they were able to eradicate the tumor and suppress metastasis.

Atherosclerosis is another disease model that may be amenable to treatment with platelet membrane-derived vesicles. Atherosclerosis is characterized by the formation of fibrofatty lesions in the artery wall[83], and platelets can attach to these plaques and activate the endothelium near lesions. One recent study took advantage of the inherent affinity of platelets for plaques by coating immunosuppressant rapamycin-loaded PLGA nanoparticles with PEGylated platelet membranes[44]. Intravenously administered platelet membrane-coated nanoparticles were shown to accumulate at plaques and promote regression of atherosclerosis in an $ApoE^{-/-}$ mouse model of atherosclerosis.

In another study, platelet membranes were used to coat PLGA nanoparticles with imaging agents[84]. For diagnostic imaging, a fluorescent dye was loaded into PLGA nanoparticles, with concurrent incorporation of lipid-chelated gadolinium into the lipid bilayer of the platelet membrane. The resulting membrane-coated nanoparticles provided magnetic resonance imaging capability that was localized to regions of arteries that are prone to plaque formation.

Platelet membrane-derived vesicles have been studied for the treatment of immune thrombocytopenia purpura[85], a disease characterized by low levels of platelets caused by the presence of anti-platelet autoantibodies. In this application, platelet membrane-coated PLGA nanoparticles, acting as decoys, were used to neutralize pathological anti-platelet antibodies. These platelet membrane-coated nanoparticles were found to significantly decrease the levels of anti-platelet antibodies *in vitro* and to restore platelet numbers and hemostatic capacity *in vivo*. Table 2 shows examples of core particles in platelet cell membrane-derived vesicles. RBC and platelet membrane-derived vesicles are depicted in Fig. 5 [39,69,85].

3.3. Stem cell-derived vesicles

Stem cells have been widely studied for a variety of therapeutic purposes, especially in regenerative medicine. Stem cell membranes have also been used to coat drug-loaded nanoparticles. In particular, stem cell membrane-coated nanoparticles have been studied for their tumor-targeting functionality, reflecting the tumor-distribution feature of stem cells. In one such application, polymeric nanoparticles coated with membranes of umbilical

Table 2 Core particles entrapped in platelet cell membrane-derived vesicles.

Core particle	Purpose	Disease	Ref.
PLGA	Immune evasion Tumor-targeting	Mouse liver cancer (H22)	23
	Immune evasion Subendothelium binding Pathogen adhesion	Coronary restenosis Systemic bacterial infection	59
Magnetic nanoparticles	Homing to atherosclerotic sites	Atherosclerosis	44,84
	Specific clearance of anti-platelet antibodies	Immune thrombocytopenia purpura	85
	Immune evasion Tumor-targeting	Mouse breast cancer (4T1)	82
Polypyrrole	Immune evasion Tumor-targeting	Human liver cancer (Huh 7)	81
Mesoporous silica	Enhance blood retention Improving target accumulation	Carotid thrombosis	59

cord-derived mesenchymal stem cells, which have been reported to exhibit tropism towards malignant legions, were studied for tumor-targeted therapy[86]. Mesenchymal stem cell membrane-coated PLGA nanoparticles loaded with doxorubicin showed enhanced tumor distribution and a greater anticancer effect than plain doxorubicin-loaded nanoparticles.

Bioengineered stem cell membranes have also been coated onto nanoparticles for targeted delivery[32]. In this example, bioengineered stem cell membrane-functionalized nanocarriers harboring CXCR4 were fabricated to promote targeting to and retention in ischemic tissue. Specifically, human adipose-derived stem cells were engineered to overexpress CXCR4, and the resulting CXCR4-engineered stem cell membrane-coated nanocarriers were shown to exhibit significantly enhanced accumulation in ischemic tissues after administration in ischemic mice.

PLGA nanoparticles have also been coated with cardiac stem cell membranes for use in tissue-repair applications[56]. In this application, direct intramuscular injection of cardiac stem cell membrane-coated nanoparticles carrying stem cell-secreted proteins was found to alleviate symptoms in a mouse model of myocardial infarction. Although stem cell therapy has received attention as a promising regenerative medicine strategy, careful processing and preservation of these cells is essential for limiting immunogenicity and tumorigenicity risks.

3.4. Immune cell membranes as delivery systems

Immune cell-derived membranes have been gaining attention by virtue of their expression of immune-related receptors and immune-modulating proteins. Among immune cells used as a membrane source are neutrophils, T cells, macrophages, dendritic cells (DCs), and natural killer cells. With further processing, the resulting immune cell membrane-derived vesicles have been used to cloak core particles, including silica particles, iron oxide particles, liposomes, and polymeric nanoparticles.

3.4.1. Neutrophil membrane-derived vesicles

Neutrophils, the most abundant circulating polymorphonuclear leukocytes[87], are able to move out of blood vessels through extravasation and infiltrate tissue to reach inflammatory sites by following gradients of chemical signals in a process called chemotaxis[88]. In the first step in this migration process, neutrophils adhere to the surface of endothelial cells by virtue of their

expression of a number of selectins and integrin molecules, such as lymphocyte function-associated antigen-1, integrin $\alpha4\beta1$ and macrophage antigen-1[89]. Because of their high binding affinity for the inflammatory site, neutrophil membranes have been studied for drug delivery in cancer and inflammatory diseases[28,90].

Neutrophil membrane vesicles have been studied to deliver an inhibitor of nuclear factor kappa B (NF-κB) for the treatment of acute lung inflammation[28]. Neutrophils are recruited to inflammation sites by intercellular adhesion molecule 1, which is upregulated on the surface of endothelial cells. Because neutrophils highly express integrin $\beta2$, membranes derived from them responded rapidly to inflammation by binding to the endothelium. In this study, neutrophil membrane vesicles derived from activated HL-60 human promyelocytic leukemia cells, which express integrin $\beta2$, were loaded with an NF-κB inhibitor. The resulting vesicles were shown to accumulate in lung vessels after intravenous administration. Moreover, they reduced neutrophil infiltration and cytokine levels to a greater extent than free drug, thereby alleviating lung inflammation.

Taking advantage of the pivotal role neutrophils play in the pathogenesis of rheumatoid arthritis, Zhang et al.[90] used neutrophil membrane-derived vesicles as a nanoparticle coating material to enhance delivery to the joint in inflammatory arthritis. These researchers found that neutrophil membrane-coated nanoparticles reduced the levels of proinflammatory cytokines and suppressed synovial inflammation. Compared with RBC membrane-coated nanoparticles, neutrophil membrane-coated nanoparticles penetrated cartilage more efficiently and conferred chondroprotection.

Cao et al.[91] recently used neutrophil membranes to enhance drug delivery *via* polymeric nanoparticles in pancreatic cancer. Drug delivery in pancreatic cancer still faces many challenges owing to the aggressive nature of this cancer and the harsh microenvironment it produces. The pancreatic tumor environment, in turn, secretes pro-inflammatory cytokines, which recruit neutrophils to assist tumor progression and metastasis[92,93]. The resulting chronic inflammation has been linked to the pathogenesis of pancreatic cancers, with NF-κB receiving considerable attention as an attractive target for treatment. In the study by Cao et al.[91], celastrol was encapsulated in neutrophil membrane-coated PLGA nanoparticles. Following intravenous injection, the membrane-coated nanoparticles accumulated in pancreatic tumor tissue and significantly inhibited tumor growth, ultimately prolonging the survival of treated mice by ~3-fold compared with controls.
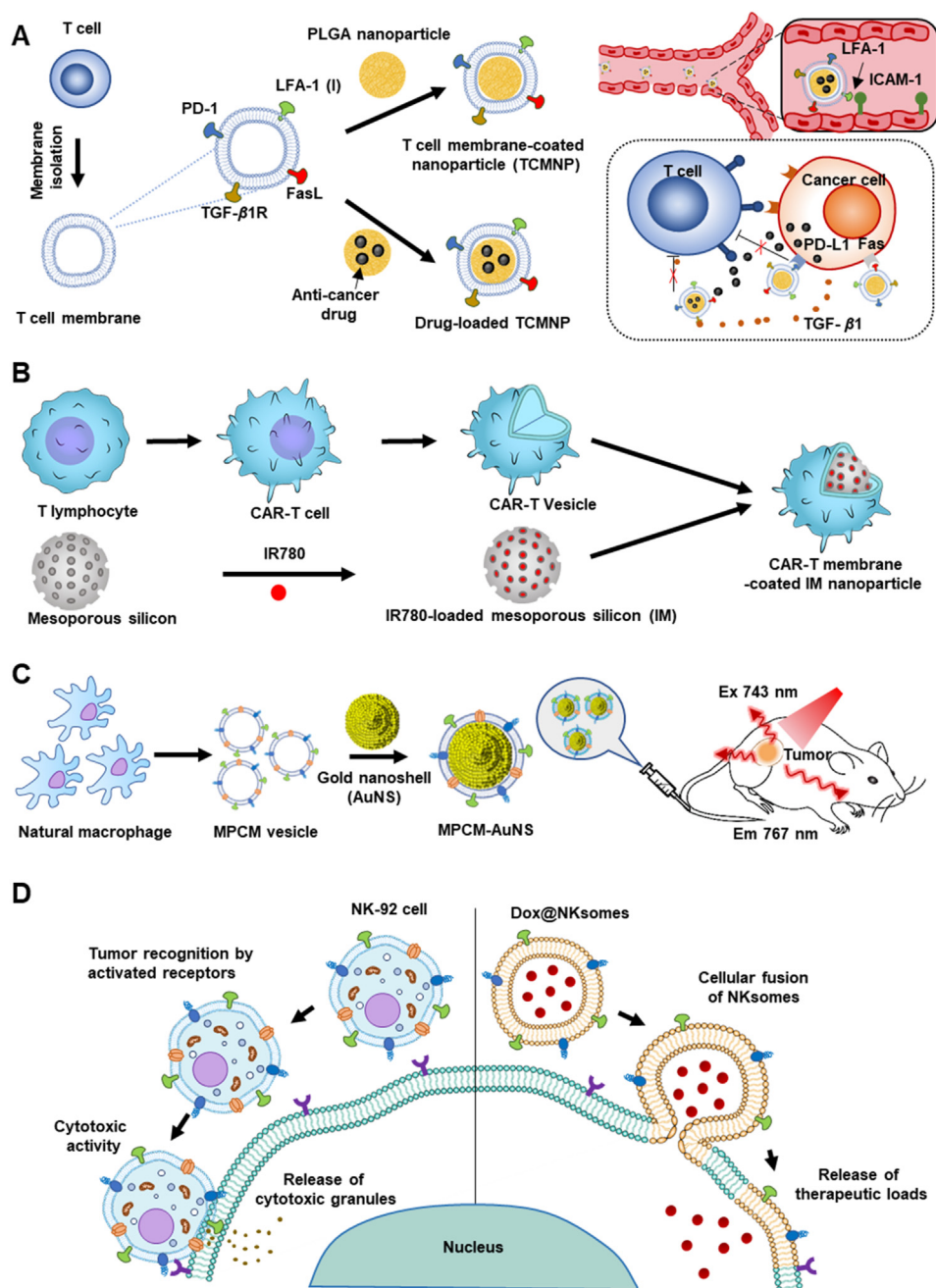
Figure 6 Immune cell membrane-derived vesicles cloaking core particles. (A) Preparation process and therapeutic mechanism of PLGA nanoparticles coated with T cell membrane-derived vesicles. Adapted with permission from Ref. 95. Copyright © 2020 Wiley-VCH GmbH. (B) CAR-T cell membrane-derived vesicles were exploited for cloaking IR780-loaded mesoporous silica nanoparticles for photothermal therapy. Adapted with permission from Ref. 96. Copyright © 2020 Ivyspring International Publisher. (C) Macrophage cell membrane-camouflaged gold nanoshells. Adapted with permission from Ref. 98. Copyright © 2016 American Chemical Society. (D) NK cell membranes were fused with doxorubicin-loaded liposomes, yielding hybrid membrane-derived vesicles. Adapted with permission from Ref. 49. Copyright © 2018 Elsevier Ltd.

3.4.2. T cell membrane-derived vesicles

The T cell is an important lymphocyte contributor to adaptive immune responses[94]. Unlike NK cells, T cells require antigen priming *via* a distinct T-cell receptor (TCR). Engagement of the TCR by the MHC-antigen complex, presented by DCs with the assistance of costimulatory signals, induces T cell activation. Following activation, naïve T cells are transformed to effector T cells or regulatory T cells, depending on the context of the DC-T cell immune synapse. Effector T cells, especially cytotoxic T cells, circulate in the bloodstream and scavenge and kill virus-infected cells or cancer cells. On the other hand, T cells also differentiate into memory T cells, which provide long-term protection from the pathogens that evoked their activation.

T cell membranes have been used to coat dacarbazine-loaded PLGA nanoparticles (Fig. 6A)[95]. In this application, the mouse lymphoma cell line EL4 was used as a source of T cell membranes. Unlike plain nanoparticles, T cell membrane-coated nanoparticles were able to escape immune suppression by

Table 3   Immune cell membrane-derived vesicles with core particles.

Parent cell	Core particle	Purpose	Disease	Ref.
Leucocyte	Silica NPs	Cancer cell targeting	Human cervical cancer (HeLa)	101
	(Alginate/chitosan) 8 capsules	Immune evasion, tumor accumulation	Inflammation	28
Macrophage	Silica NPs	Cytocompatibility	Rheumatoid arthritis	99
	Au nanoshells	Tumor-targeting	Mouse breast cancer (4T1)	98
Myeloid-derived suppressor cells	Iron oxide	Immune evasion, tumor-targeting	Mouse melanoma (B16−F10)	57
Natural killer cell	Liposome	Tumor-targeting	Human breast cancer (MCF-7)	49
	PLGA	Tumor-targeting	Human breast cancer (4T1)	65
T cell	PLGA	Tumor-targeting	Human lymphoma (Raji)	34
Monocyte	PLGA	Tumor-targeting	Human breast cancer (MCF-7)	50
Dendritic cell	Metalorganic framework	T cell activation	Mouse breast cancer (4T1)	102

tumors and neutralize PD-ligand 1 expression on tumors and TGF-β1 in the tumor environment. In addition, T cell-derived membrane-coated nanoparticles enhanced dacarbazine delivery to tumors and induced tumor cell apoptosis.

Chimeric antigen receptor-engineered T cell (CAR-T cell) therapy has recently emerged as an innovative treatment for cancer. CAR-T cells are generated *ex vivo* by genetically modifying the TCR to recognize an antigen of interest without requiring antigen presentation. The resulting *ex vivo*-amplified CAR-T cells are reinfused into cancer patients, where they serve a tumor cell surveillance function. CAR-T cell targeting of the CD19 antigen has been approved by the FDA for treatment of acute lymphoblastic leukemia or relapsed/refractory diffuse large B-cell lymphoma. The potential of CAR-T cell therapy against cancer prompted a recent effort to use CAR-T cell membranes for drug delivery (Fig. 6B)[96]. In this study, CAR-T cells were engineered to express single-chain variable fragment, an antibody light chain specific for glypican-3 expressed in hepatocarcinomas. Mesoporous silica nanoparticles loaded with IR780, a photo-thermal agent, were encapsulated into vesicles derived from Glypican-3-loaded-CAR-T cell membrane, providing a tumor-targeting feature.

3.4.3.   Macrophage membrane-derived vesicles
Macrophages—major components of the innate immune system—are the main phagocytes that detect, engulf, and destroy pathogens[97]. In disease, macrophages also play important roles in regulating inflammation and disease progression. Macrophages express a wide array of cell-type-specific proteins that function in phagocytosis, pathogen recognition and tissue infiltration, as well as communication with other immune cells. These properties have motivated interest in macrophage membrane-derived vesicles for use in drug-delivery systems.

Macrophage membrane-coated gold nanoshells have been studied for tumor phototherapy (Fig. 6C)[98]. In this application, coating gold nanoshells with mouse RAW 264.7 macrophage membranes were shown to prolong the circulation time of the nanoparticles, such that 30% of the injected dosed of coated nanoshells remained in the circulation after 48 h; by contrast, naked gold nanoshells were completely eliminated within 24 h of dosing. Compared with RBC membrane-coated gold nanoshells, macrophage membrane-coated gold nanoshells also promoted greater tumor accumulation and photothermal therapeutic efficacy. Moreover, intravenous administration of macrophage membrane-

coated gold nanoshells followed by NIR irradiation-ablated tumors in 4T1 tumor-bearing mice.

Macrophage membranes have been further used to functionalize silicon nanoparticles for treatment of rheumatoid arthritis[99]. Macrophages are among the key immune cells that contribute to joint inflammation. Coating silicon nanomaterials with macrophage membrane provided biocompatibility. The resulting macrophage membrane-coated nanoparticle inhibited further activation of the immune system and reduced expression of maturation markers in antigen-presenting cells.

3.4.4.   DC membrane-derived vesicles
DCs are professional antigen-presenting cells that play a pivotal role in connecting innate and adaptive immune systems[100]. Resident DCs are found in organs and lymphoid tissues. They are also phagocytic cells that engulf pathogens or cell debris, and process and present antigens to T cells. Thus, DCs activate T cells *via* cytokine communication or cell−cell contact to generate a humoral or cellular immune response.

In practice, DC therapy still faces certain challenges, such as low efficacy owing to the limited migration of DCs to lymph nodes and poor cell survival post-injection. To overcome these challenges, researchers have extracted DC membranes for immunotherapy[43]. In this recent study, DCs were pulsed with tumor antigen and matured by treating with monophosphoryl lipid A. Membrane-derived vesicles obtained from mature DCs were able to prime T cells and promote the expansion of adoptively transferred T cells up to 4-fold. To the same end, DC membranes were fused with 4T1 breast cancer cell membranes, generating a hybrid vesicle that mimics the behaviors of both antigen-presenting cell and tumor cell. These hybrid cell membrane-derived vesicles were also able to activate T cells and induce immune responses through DC activation.

3.4.5.   NK cell membrane-derived vesicles
NK cell membrane-derived vesicles have been used for delivery of doxorubicin (Fig. 6D)[49]. These vesicles, hybrids of doxorubicin-loaded liposomes and NK-92 cell membranes fabricated by co-extrusion, retained the immunosurveillance properties of NK cells and specifically interacted with MCF7 breast cancer cells, but not with normal human osteoblast cells. After intravenous administration, doxorubicin-loaded hybrid membrane vesicles exhibited a higher tumor-inhibition rate (78.5%) compared with free drug (63.4%) in MCF7 tumor-bearing mice. Table 3 shows examples of immune cell membrane-derived vesicles.

Table 4 Cancer cell membrane-derived vesicles with core particles.

Core particle	Purpose	Disease	Ref.
Polyamidoamine dendrimer	Tumor-targeting	Human lung cancer (H1975)	111
Bovine serum albumin-drug nanocrystal	Tumor-targeting	Mouse breast cancer (4T1)	112
Copper sulfide	Tumor-targeting	Mouse melanoma (B16−F10)	113
Gelatin	Tumor-targeting	Patient-derived squamous carcinoma	108
Gold nanocage	Tumor-targeting	Mouse breast cancer (4T1)	114
Iron oxide	Tumor-targeting	Human squamous carcinoma (UM-SCC-7)	107
Lipoplex	Tumor-targeting	Breast cancer (4T1, MDA-MB-831)	106,110,115
PLGA	Tumor-targeting	Human breast and liver cancer (MDA-MB-231, HepG2)	66,116
Poly (epsilon-caprolactone)/pluronic F68 polymer	Tumor-targeting	Human glioblastoma (U87)	105
Polyethyleneimine-modified (2-hydroxypropyl)-γ-cyclodextrin	Tumor-targeting	Mouse melanoma (B16−F10)	117
Porphyrin-based metal organic framework	Tumor-targeting	Mouse breast cancer (4T1)	55
Rare-earth doped nanoparticles	Tumor-targeting	Human breast cancer (MDA-MB-231)	63
Silica	Photoacoustic imaging of tumor miRNA	Human breast cancer (MCF-7)	118
Thermally oxidized porous silicon @acetalated dextran	Immunostimulating	Human breast cancer (MDA-MB-231)	119

3.5. Cancer cell-derived vesicles

Cancer is characterized by the abnormal growth of cells with the potential to metastasize. These cells have some distinct properties—including the ability to escape the immune system[103] and homotypic cell adhesion, which is important for organizing metastatic lesions[104]—that collectively serve a self-protective purpose. Because of the unique characteristics of cancer cells, membranes derived from them have attracted attention as a coating material for anticancer nanoparticles. Coating nanoparticles with cancer cell membranes can confer the various attributes of cancer cells onto nanoparticles. Cancer cell membrane-bound tumor antigens have been delivered as part of a variety of nanoparticles, including PLGA nanoparticles, liposomes, gelatin particles, and titanium oxide particles.

Cancer cell membrane-coated nanoparticles have been studied for a variety of cancer therapy applications. Some of these studies have exploited the cancer cell membrane's ability to penetrate the blood−brain barrier. For example, Wang and colleagues[105] coated polycaprolactone/F68 nanoparticles with brain metastatic tumor cell membranes and loaded the resulting nanoparticles with indocyanine green, used as an imaging and photothermal agent. Intravenously injected nanoparticles were shown to distribute to the brain in U87MG-Luc glioma cell-bearing mice. In another study, MDA-MB-831 cancer cell membrane-coated PEG-PLGA nanoparticles were investigated as a possible theranostic agent against brain metastatic breast cancer[106]. These researchers reported that cancer cell membrane-coated nanoparticles showed greater accumulation in the brain compared with uncoated nanoparticles.

The homologous targeting feature of cancer cell membranes has also been studied[107]. In this application, iron oxide nanoparticles were coated with UM-SCC-7 and HeLa cell membranes and co-incubated with four cell lines: UM-SCC-7, HepG2, HeLa, and COS7 cells. The UM-SCC-7 and HeLa cell membrane-coated nanoparticles showed high affinity for the corresponding source cells. They also possessed the capacity for self-targeting to the homologous tumor, and efficiently suppressed tumor growth *in vivo*. Another study applied these ideas to personalized cancer therapy, engineering tumor cells from head and neck cancer patients to coat cisplatin-loaded gelatin nanoparticles[108]. The resulting patient-derived tumor cell membrane-coated nanoparticles showed patient-specific cancer targeting capability and anticancer efficacy in patient-derived xenograft models, with no notable toxicity.

Some researchers have taken advantage of this homologous targeting ability to support imaging technology. For example, MDA-MB-435 cancer cell membrane-coated upconversion nanoparticles were studied as nanoprobes for tumor imaging[109]. These nanoparticles emit green upconversion luminescence under 980 nm NIR laser excitation, making them ideal for use as probes. In a tumor xenograft mouse model, these cancer cell membrane-coated upconversion nanoparticles provided specific tumor imaging without notable *in vivo* toxicity. In a recent study, rare-earth-doped nanoparticles were coated with cancer cell membranes, creating an imaging nanoplatform for tumor surgery navigation[63]. These nanoparticles, which contain neodymium and yttrium, displayed an emission peak at 1060 nm corresponding to the NIR-II window under 808 nm laser excitation and enabled tumor tissue dissection by NIR-II imaging.

Cancer cell-derived vesicles have been studied for application to cancer immunotherapy. The presence of tumor-associated antigens on the surface of cancer cell membrane-coated nanoparticles can lead to presentation with MHC molecules and activation of antigen-specific T cells following uptake by antigen-presenting cells. Building on this idea, Kroll et al.[110] used B16F10 melanoma cell membranes to coat CpG-PLGA nanoparticles as an anticancer vaccine. Western blots showed the presence of the membrane-bound tumor antigens, MART1, TRP2 and GP100, on the particles. Subcutaneously injected melanoma cell membrane-coated particles effectively activated DCs, stimulating antigen-specific T cells. Both TRP2 and GP100 antigen-specific cytotoxic T cells proliferated extensively after treatment. Co-administration of these cancer cell membrane-coated nanoparticles with immune checkpoint inhibitors was shown to increase the anticancer therapeutic effect. Table 4 shows examples of cancer cell membrane-derived vesicles.

3.6. Other cell membrane-derived vesicles

Beyond the cell types mentioned above, some other cells have been studied as sources of cell membrane for delivery systems of therapeutics cargoes. Endothelial cells, gastric epithelial cells, fibroblasts, and bacteria have been used as sources of cell membrane for such work. For example, endothelial cells from the inner wall of blood vessels were studied as a source of membrane-derived vesicles[38]. The vesicles were generated via cytochalasin B treatment of human endothelial cells, and in some cases were modified with the aptamer, AS1411, to enable them to target tumor cells. The AS1411-modified endothelial cell membrane-derived vesicles showed greater distribution to tumor tissues compared to plain vesicles. Doxorubicin loaded to these vesicles inhibited tumor growth with lower toxicity than seen with free doxorubicin.

Cell membranes from genetically engineered TRAIL-expressing umbilical vein endothelial cells were studied as a potential means to target inflamed M1 macrophages in rheumatoid arthritis[33]. PLGA nanoparticles loaded with the antirheumatic drug, hydroxychloroquine, were coated with umbilical vein endothelial cell membrane. The endothelial cell membrane-coated PLGA nanoparticles were shown to bind to macrophages and trigger apoptosis via an interaction between TRAIL and death receptor-5. Vesicles harboring these core nanoparticles localized to inflamed paw sites and ameliorated the pathological state in a collagen-induced mouse model of arthritis.

Gastric epithelial cell membrane-coated PLGA nanoparticles were investigated for targeting to Helicobacter pylori[54]. The study was inspired by the natural interactions between pathogen and hosts, as gastric epithelial cell membranes were known to contain receptors that can be recognized by H. pylori. Oral administration of antibiotic clarithromycin-loaded PLGA nanoparticles with gastric epithelial cell membrane coating was found to provide greater bactericidal activity than free clarithromycin or membrane-uncoated nanoparticles.

Fibroblasts have also been studied as a source of cell membrane for vesicles[120]. Semiconducting polymeric nanoparticles were coated with activated fibroblast membranes, with the goal of targeting cancer-associated fibroblasts. The fibroblast membrane-coated polymeric nanoparticles exhibited greater tumor accumulation and antitumor effects compared with cancer cell membrane-coated nanoparticles. The fibroblast membrane-coated vesicles were speculated to exhibit higher penetration of tumor microenvironments. However, penetration through solid tumor tissues may remain a major challenge in this field.

In addition to mammalian cells, bacteria have been used as sources of cell membrane. For example, membranes derived from Escherichia coli were used to coat gold nanoparticles[121]. Unlike plain gold nanoparticles, bacterial membrane-coated gold nanoparticles retained their stability in physiological conditions. In another study, bacterial membrane coating was studied for its ability to enhance the adjuvant effect of CpG[122]. Indeed, bacterial membrane-coated CpG polyplexes were found to stimulate DC and increase antitumor T cell responses.

Bacterial membrane-derived vesicles may offer advantages in stimulating antigen-presenting immune cells because bacterial membranes are enriched with pathogen-associated molecular patterns, including TLR agonists. However, the presence of lipopolysaccharides in bacterial membranes may cause side effects, such as fever. The contents of lipopolysaccharides in bacterial membranes should therefore be assessed and controlled when researchers seek to use bacterial membrane-derived vesicles for in vivo applications.

4. Challenges and future directions

Cell membrane-based drug-delivery platforms are an emerging technology that takes advantage of a natural source: living cell membranes. One intriguing aspect of cell membrane-based delivery is its manipulation of complex natural cellular membranes as a source of multifunctional biomaterial. The complexity of cell membranes, with their inserted functional proteins, may not be possible to reproduce synthetically. Clinical realization of the benefits of membranes derived from live cells, however, faces several challenges (see Table 5).

Since cell membrane-derived vesicles represent an emerging field, information from clinical trials remains limited. A clinical study of drug-encapsulated tumor cell vesicles (NCT02657460, phase II) was conducted for treatment of malignant pleural effusion. Another trial (NCT01854866, phase II) is in progress for treating malignant pleural effusion and malignant ascites with tumor cell-derived microparticles containing methotrexate, hydroxycamptothecin, or cisplatin. These microparticles, which originated from apoptotic tumor cells, were found in preclinical trials to ablate tumor cells without the severe side effects of chemical anticancer drugs[122].

Choosing the proper cell source is important for safe and effective cell membrane-derived, nanoparticle-based therapy. In the case of an autologous source, cells isolated from the same patient are guaranteed to be ideal materials that avoid mismatched

Table 5 Challenges in the clinical translation of cell membrane-derived vesicles.

Challenge	Concern	Requirement
Cell source	Host immune responses	Autologous cell source
Manufacturing process	Cost-consuming and labor-intensive	Master and working cell banks
Quality control	Heterogeneity by the type of donor cells	Donor eligibility, screening, and monitoring protocols
Purification	Contamination with other organelles and nuclei	Standard operating procedures
Coating efficiency of core nanoparticles	Limited quality control	Quantification of the amounts of cell membranes on nanoparticles
Ratio of dual cell membranes	No rationale for the ratio	Exact compositions of hybrid cell membrane by efficacy
Multi-components of cell membranes	Limited quality control	Profiling total protein, lipid, and carbohydrate components of cell membranes
Stability	Lack of product for testing	Stability testing plan in appropriate time and condition

antigens and thus reduce the risk of host immune responses due to differences in MHC class types. However, the use of an autologous cell source may limit the timely availability of cells for preparation of membrane-derived particles. Because preparation of cell membrane-based drug products is a multi-step process that requires a certain amount of time and additional quality-control procedures, treatment plans may be delayed or interrupted if patients need to wait for cell isolation and product synthesis. In contrast, the use of allogenic (donor) cells may eliminate such delays, providing a readily available source of cell membranes for treatment when needed. However, similar to the case of organ transplantation, antigen matching needs to be performed to avoid host immune responses while maximizing the therapeutic effects of cell membrane-derived nanoparticles.

One possible direction for future research would be to build donor cell banks that can be easily accessed for the selection of suitable MHC type cell sources. In addition, the development of gene-editing techniques may enable deletion of specific genes encoding unwanted immunogenic proteins. For example, several studies have demonstrated the feasibility of using various gene-editing platforms, including zinc finger nuclease and CRISPR/Cas9, to delete HLA genes and generate more immune-compatible stem cells[123,124]. Cell membrane nanoparticles derived from these cells could overcome immune attack, making them suitable for long-term treatment.

The type of donor cell can also influence the homogeneity of the resulting membrane-derived vesicles. Various cell types, ranging from blood cells (RBCs, platelets) to diverse immune cells, stem cells and cancer cells, have been exploited to produce cell membrane-derived vesicles as delivery systems. RBCs and platelets are the most ubiquitous cell source for membrane isolation. Because these cells lack nuclei, they are not readily amenable to genetic engineering approaches for pre-modification of cell membranes. Although this obstacle can be circumvented in theory, as evidenced by a previous study demonstrating genetic modification of RBCs in pre-embryo stage animals, in practice, translating it for human use may be an insurmountable challenge[35]. Moreover, the different features of cell membranes in various growth phases and cell cycles may result in batch-to-batch variation that could affect therapeutic outcome. Therefore, parameters and protocols for quality control testing of cell sources (raw materials) and membrane-derived vesicles (final products) should be an emphasis of future investigations.

From a manufacturing standpoint, another challenge is the large-scale preparation of sufficient amounts of cell membrane-derived vesicles. To date, most investigational studies have used extrusion and sonication methods to produce cell membranes. The yields of pure cell membrane isolated, the coating efficiency of core nanoparticles, and the efficiency of hybridization with other cell membranes would be critically influenced by initial cell densities, cell membrane fluidity, and processing variables. In the case of preparations obtained from nucleated cells, membranes free from contaminating organelles and nuclei need to be isolated in pure form. Increasing the yield of high purity cell membranes necessary to generate high yields of homogeneous cell membrane-derived vesicles will require optimizing purification methods and processing variables for each cell source. Implementation of optimal workflow and suitable quality control assays in the near future should help overcome these challenges.

In current practice, cell membranes are coated onto the surfaces of core nanoparticles, chemically modified, or hybridized with other cell membranes. However, to date, the homogeneity of cell membrane-coated nanoparticles after preparation has rarely been tested and should be characterized in detail. Such information would be a crucial part of quality control tests of nanoparticles for translational studies. An additional parameter affecting the quality of the membrane coating that requires characterization is the amount of cell membranes on nanoparticles. In this context, the ratio of phosphate to polymeric nanoparticles would be one way to characterize vesicles, given that phospholipids are dominant features of cell membranes.

Another challenge is ensuring the specificity of conjugation during chemical conjugation of cell membrane-derived vesicles. Unlike synthetic nanoparticles, with their controllable composition of chemically reactive components, natural cell membranes are susceptible to nonspecific chemical modifications. Conjugation of chemicals onto membrane surfaces can alter the integrity of cell membrane components, reduce the original functionality, and alter biological behavior. One approach for overcoming nonspecific and uncontrollable chemical modifications on natural cell membrane is highlighted by a recent study in which cells were metabolically engineered to express functional groups on the parent cell surface, allowing specific interaction with modifying agents[34]. Additional approaches for controlling the specificity of ligand tagging need to be investigated.

In cell membrane hybridization studies, optimization of the ratio of dual cell membranes is necessary to maximize the performance of hybrid cell membrane-derived vesicles. In most recent studies, hybridization is qualitatively established based on enhanced interaction with target cells or improved pharmacological activity of entrapped therapeutic cargoes. To provide greater insight into the design of hybrid cell membrane-derived vesicles as delivery systems, researchers will need to quantitatively characterize the precise composition of hybrid cell membrane-derived vesicles.

Because the bioactivity of cell membrane-derived vesicles relies on multi-component membrane materials, effective quality control will require complete profiles of the major cell membrane components (total proteins, lipids and carbohydrates). The development of mass spectroscopy and advanced analysis techniques may allow high-throughput screening of cell membrane components based on proteomic, lipidomic or glycomic analyses[81,125,126]. In the case of cancer cell membrane-derived vesicles, the cell source is more homogeneous and easier to scale up. However, one of the main concerns associated with the use of cancer cell membranes is safety. Thus, membrane preparation and purification procedures must guarantee the complete removal of any components from cancer cells that could potentially promote cancer cell growth.

Long-term stability is an important factor in the potential of cell membrane-derived nanomaterials for clinical translation. To support predictions on the long-term stability and shelf-life of nanomaterials, it could be helpful to develop a database on the physicochemical stability profiles of cell membrane components. Knowledge regarding the stability features of cell membrane components under various conditions (e.g., temperature, oxygen, pH, and light) would be a foundation from which researchers could formulate final products. Given the high contents of lipids in cell membranes, it could be useful to use of excipients that can prevent oxidation of lipid components. For long-term stability, cell membrane-derived vesicles may need to be stored in the frozen state. However, given that low-temperature storage can damage the integrity of membranes, researchers should study relevant cryoprotectants with the goal of minimizing membrane damage during storage.

5. Conclusions

The development of cell membrane material-derived therapeutics is an emerging research field that is particularly attractive because it is an organic cellular networking system. However, exploiting the natural mechanisms of living matter—the greatest advantage of biomimetic technology—is a double-edged sword. One serious obstacle is how to identify which of the multiple components confers cell membrane functionality, and then adjust the ratio of each component as needed. In the case of pharmaceutical agents, even if there are ingredients that hinder safety and effectiveness, there are questions regarding how to manipulate and remove them. A similarly demanding production process will be required to develop drug-containing cell membrane components. Despite challenges of quality control, manufacturing, and processing variables that must be overcome, natural cell-derived vesicles have the unique feature of customizable bioactivity that reflects the properties of the parent cells. Given their unprecedented advantages, which cannot be matched using synthetic nanomaterials, natural cell membrane-derived vesicles will continue to evolve as a new delivery system modality.

Author contributions

Gayong Shim, Quoc-Viet Le, Jaiwoo Lee, Hobin Lee, and Yu-Kyoung Oh wrote the manuscript. Gayong Shim, Quoc-Viet Le, Jaiwoo Lee, and Hobin Lee prepared Tables and Figures. Gayong Shim, Quoc-Viet Le, and Yu-Kyoung Oh revised the manuscript. All of the authors have read and approved the final manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

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