Acta Pharmaceutica Sinica B 2019;9(4):675–689

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

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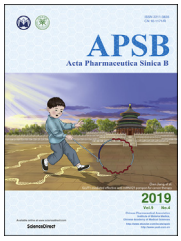

REVIEW

Red blood cell membrane-camouflaged nanoparticles: a novel drug delivery system for antitumor application

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Received 15 September 2018; received in revised form 30 October 2018; accepted 28 November 2018

KEY WORDS

Red blood cells;
Membrane;
Nanoparticles;
Drug delivery;
Antitumor;
Biomimetic nanoparticles

Abstract Erythrocytes (red blood cells, RBCs) are the most abundant circulating cells in the blood and have been widely used in drug delivery systems (DDS) because of their features of biocompatibility, biodegradability, and long circulating half-life. Accordingly, a "camouflage" comprised of erythrocyte membranes renders nanoparticles as a platform that combines the advantages of native erythrocyte membranes with those of nanomaterials. Following injection into the blood of animal models, the coated nanoparticles imitate RBCs and interact with the surroundings to achieve long-term circulation. In this review, the biomimetic platform of erythrocyte membrane-coated nano-cores is described with regard to various aspects, with particular focus placed on the coating mechanism, preparation methods, verification methods, and the latest anti-tumor applications. Finally, further functional modifications of the erythrocyte membranes and attempts to fuse the surface properties of multiple cell membranes are discussed, providing a foundation to stimulate extensive research into multifunctional nano-biomimetic systems.

Abbreviations: ABC, accelerated blood clearance; APCs, antigen presenting cells; AuNCs, gold nanocages; AuNPs, gold nanoparticles; C8bp, C8 binding protein; CR1, complement receptor 1; DAF, decay accelerating factor; DDS, drug delivery systems; DLS, dynamic light scattering; Dox, doxorubicin; ECM, extracellular matrix; EpCam, epithelial cell adhesion molecule; EPR, enhanced permeability and retention; ETA, endothelin A; FA, folic acid; GA, gambogic acid; H&E, hematoxylin and eosin; HRP, homologous restriction protein; MCP, membrane cofactor protein; MNCs, magnetic nanoclusters; MNs, magnetic nanoparticles; MPS, mononuclear phagocyte system; MRI, magnetic resonance imaging; MSNs, mesoporous silica nanoparticles; NIR, near-infrared radiation; PAI, photoacoustic imaging; PBS, phosphate buffered saline; PCL, poly(caprolactone); PDT, photodynamic therapy; PEG, polyethylene glycol; PFCs, perfluorocarbons; PLA, poly(lactide acid); PLGA, poly(D,L-lactide-*co*-glycolide); PPy, polypyrrole; PS, photosensitizers; PTT, photothermal therapy; PTX, paclitaxel; RBCM-NPs, RBCM-coated nanoparticles; RBCMs, RBC membranes; RBCs, red blood cells; RES, reticuloendothelial system; rHuPH20, recombinant hyaluronidase, PH20; ROS, reactive oxygen species; RVs, RBCM-derived vesicles; SEM, scanning electron microscopy; SIRPα, signal-regulatory protein alpha; TEM, transmission electron microscopy; TEMPO, 2,2,6,6-tetramethylpiperidin-1-yl oxyl; TPP, triphenylphosphonium; UCNPs, upconversion nanoparticles; UV, ultraviolet

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Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

https://doi.org/10.1016/j.apsb.2019.01.011

1. Introduction

Over previous decades, nanoparticle-based drug delivery systems (DDS) have been successfully applied experimentally and clinically to improve the efficacy of many drugs and therapeutic molecules[1,2]. Such DDSs can achieve passive tumor targeting owing to the enhanced permeability and retention (EPR) effect[3,4]. Compared to the free-drug, nanoparticle-based drugs are characterized by low toxicity, good stability, outstanding biocompatibility, higher drug release efficiency, higher blood retention time, and long shelf-life for biomedical applications[5]. However, especially for cancer treatments, ideal nanoparticles should also possess features of extended blood circulation, tumor tissue targeting, and cancer cell binding.

Therapeutic molecules are often released early or captured by the immune system *in vivo*; however, compensation by increasing drug dosage may not be a viable option as overdosage often results in unexpected side effects in normal tissues. Therefore, polyethylene glycol (PEG) functionalization has been widely used in various nanocarriers, as this can effectively avoid phagocytosis through the mononuclear phagocyte system (MPS) to achieve long-term circulation in the body. The principle is that these hydrophilic polymers hide the nanoparticles behind a hydration layer to reduce their immune clearance[6], resulting in a modified nanoparticle half-life of several tens of hours whereas that of nonPEGylated nanoparticles is only a few minutes[7]. However, the appearance of anti-PEG immune responses in some patients has shifted research focus to the new field of bio-inspired nanocarriers. These novel biomimetic strategies commonly utilize features that imitate the body's internal components, and mimicking many natural mechanisms to achieve targeted effects[8–12].

Various kinds of cell membranes have been inspired from nature to realize long-term circulation or tumor targeting including membranes from cancer cells, bacteria, lymphocytes, platelets, leukocytes, and erythrocytes (red blood cells, RBCs). Affording blood circulation up to 120 days, RBCs were studied initially and have been used as an ideal carrier to deliver various bioactive compounds, such as enzymes, drugs, proteins, and macromolecules[13]. Moreover, mature RBCs lack a nucleus and various organelles, which is very favorable for their extraction and purification[14]. As the most abundant cells in the human body, human blood contains 5 billion RBCs per milliliter on average, providing rich coating materials for drug carrier functionalization. RBCs constitute biconcave discs averaging 7.8-μm diameter, 2.5-μm edge and 1-μm center thickness, and 85–91 fl (μm^3) volume[15]. Upon decreased osmotic pressure of the surrounding medium, RBCs become cupular and eventually spherical[15]. This swelling feature is a key to the feasibility of loading RBCs with drugs or other chemicals. In summary, the advantages of RBCs as carriers to load nanoparticles include[15–17]: a) escaping the immune system and achieving long-term circulation; b) marked inherent biocompatibility and biodegradability; c) avoiding some intrinsic nano-preparation toxicities; d) lifespan up to 120 days; e) readily achieving high load capacity owing to the large quantities of cell membranes; and f) improving nanoparticle stability, enhancing *in vitro* storage time, and discouraging aggregation.

Notably, erythrocyte membrane-coated nano-formulations have been widely adopted in antitumor research to considerable achievement[18–20]. Some properties of RBCs, such as structure and surface proteins, have also been utilized as design clues to devise the next generation of drug delivery platforms[21–23]. In this review, we narrow our focus to various aspects of the field of erythrocyte membrane-coated nano-cores, particularly emphasizing coating mechanisms, preparation methods, verification methods, and the latest anti-tumor applications. Current advances provide confidence toward their clinical application in the near future. However, because this platform is composed of biological materials, strict disinfection and rigorous blood group matching are required to maximize compatibility and avoid the risk of immunogenicity.

2. History of erythrocytes as drug carriers

RBCs were first described in human blood samples in the 17th century by Dutch scientist Lee Van Hock, with their flat disc rather than spherical shape identified after another century by Howson. In 1953, Gardos attempted to load the "erythrocyte ghosts" with ATP, with this attempt laying the foundation for subsequent coating of the erythrocyte membrane with various active ingredients, opening up a whole new area of drug delivery strategies. In 1959, Marsden and Ostling[24] reported the entrapment of dextrans in erythrocytes, followed by the use of RBC loading with therapeutic agents for delivery purposes by Ihler et al.[13]. Subsequently, the term "carrier red blood cells" was introduced in 1979[25].

Following a groundbreaking study of the treatment of Gaucher's disease with β-glucosidase and β-galactosidase using carrier erythrocytes in the 1970s[13], many enzyme replacement therapeutic methodologies have been developed, such as gentamicin, daunorubicin, and L-asparaginase loading into erythrocytes for respectively treating bacterial infections, leukemia, and asparagine-dependent leukemia[26]. In 1994, Lejeune et al.[27] reported the preparation of RBC membrane-derived liposomes, also termed "nanoerythrosomes", which was obtained by physically squeezing RBC ghosts through membranes with defined pore sizes. However, simply conjugating active molecules onto the surface of erythrocytes has been found to trigger a rapid clearance from the bloodstream, in addition to a tendency to cause vesicle aggregation, indicating that these erythrocyte ghosts lacking structural integrity are highly unstable [28]. In 2013, the Zhang's group[29] proposed the definition of nanosponges, providing a biomimetic toxin nanosponge that functioned as a toxin decoy *in vivo*. Composed of polymer nanoparticle cores and surrounding erythrocyte membranes, the nanosponges could absorb membrane-damaging toxins, then transfer them away from the cell targets. Considered as one of the most exciting breakthroughs in this field, this work instigated a boom of bionic nano-medicine research (Fig. 1).

3. Immune escape mechanism of RBC membranes (RBCMs)

The long-term circulatory effect of erythrocytes is mediated by a series of membrane proteins on the membranes surface. Among these, CD47 constitutes an integral membrane protein with five

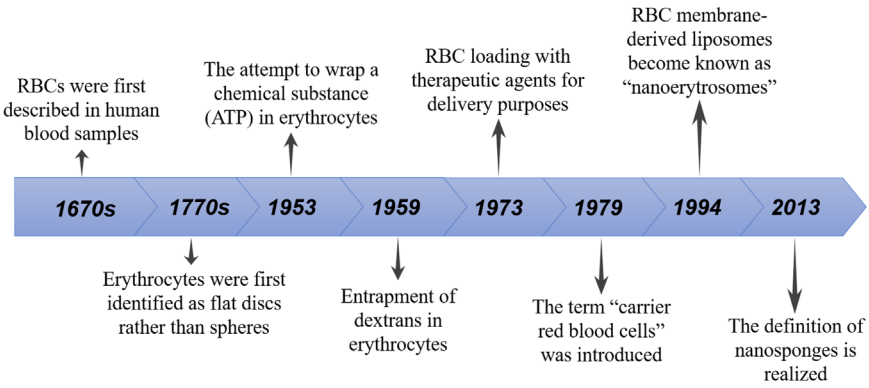

Figure 1 The historical process of RBCs as drug carriers.

membrane-spanning regions, firmly embedding it in RBCMs, along with an IgV-like extracellular domain that contributes to RBCM survival in circulation[30]. CD47 also serves as a self-marker that can actively signal macrophages to prevent erythrocyte uptake[31]. Specifically, the signal-regulatory protein alpha (SIRPα) glycoprotein, which is expressed by phagocytic cells, interacts with and recognizes CD47 as a "do not eat me" signal, thereby inhibiting immune cell engulfment of RBCs[32,33].

Other membrane proteins such as C8 binding protein (C8bp), homologous restriction protein (HRP), decay accelerating factor (DAF), membrane cofactor protein (MCP), and complement receptor 1 (CR1) and CD59 on the surface of RBCMs also play a role in the defense of complement system attacks[26,34–37]. Thus, these RBCM surface proteins can reduce the immune response by presenting drug nanocarriers as "self" to achieve long-term systemic circulation. Compared to PEGylated nanoparticles with half-lives of 15.8 h, RBCM-coated nanoparticles (RBCM-NPs) exhibit a half elimination of 39.6 h, an over two-fold improvement[38]. These data demonstrated that the RBCM coating improves the circulation time and reduces the uptake of RBCM-NPs by the reticuloendothelial system (RES), thereby increasing the chances of nanoparticles entering the tumor through the EPR effect.

4. Preparation methods of RBCM-NPs

Various methods including physical and chemical property-based techniques have been reported for encapsulating drugs or other bioactive agents in erythrocytes, such as hypotonic hemolysis[39,40], hypotonic dilution[41,42], hypotonic dialysis[43], hypotonic preswelling[44,45], and osmotic pulse[46] or chemical perturbation of the membrane[47,48] along with electrical breakdown[49]. In addition, endocytosis, lipid fusion, and intrinsic uptake of substances by erythrocytes are used to encapsulate different compounds[15]. To achieve successful covering, the encapsulated compounds may require a considerable degree of water solubility as well as erythrocyte inactivity; *i.e.*, lack of physical and chemical interactions with erythrocyte membranes to avoid leakage from the loaded RBCs, which could result in toxicological problems. Therefore, drugs are commonly prepared into a nano-preparation with less toxicity and higher stability as the core, followed by the use of RBCMs to disguise the nano-preparations to avoid identification by immune systems.

4.1. *Preparation of RBCM-derived vesicles (RVs)*

In general, the optimized and common preparation of RBCM-NPs can be divided into two parts: membrane-derived vesicles from RBCs and vesicle-particle fusion[44,48,50]. RVs are obtained by combining two steps, hypotonic treatment and sequential extrusion. Fresh whole blood obtained from the organism (*e.g.*, mouse) is centrifuged at 4 °C to maintain protein activity, and then the serum and buffy coat are removed to collect erythrocytes. The resulting RBCs are repeatedly washed with phosphate buffered saline (PBS) and re-collected by centrifugation to remove residual plasma and other unwanted cells. RBC ghosts are then acquired by hypotonic treatment, wherein the washed RBCs are gently mixed with an excess of 0.25 × PBS[50] and held to release the intracellular RBC components. Following high-speed centrifugation to remove hemoglobin, the RBC ghosts comprising the resulting pink precipitate are sonicated in a bath sonicator, and then passed through different pore size polycarbonate porous membranes using an Avanti mini-extruder to obtain the target size of RVs (Fig. 2). To keep the membrane bio-active, protease inhibitors are usually added to the samples and the samples are stored at 4 °C[51–53].

4.2. *Coating mechanism of RBCM-NPs*

The highly flexible RBC structure depends on the cell membrane viscoelasticity, cell content viscosity, and cell surface to volume ratio, which allow RBCs to pass through fairly narrow capillary networks and "sieving organs" such as the spleen and the liver[37]. The dense polysaccharide coating on the RBC surface, termed glycocalyx, is important to cell stability and immune escape characteristics[54,55]. These complex polysaccharides on the cell surface are equivalent to a hydrophilic coating for achieving spatial stability[56,57]. Polymeric nanoparticles with higher surface energies are more likely to interact with the stabilized membranes of the polysaccharide to minimize total energy, whereas the stabilized RBCM-NP surface can effectively exclude further membrane interactions[58]. This stabilization mechanism ensures the occurrence of monolayer film coating, even in the presence of excess RBCMs.

Additionally, the negatively charged sialyl residues in the polysaccharide terminal confer a charged asymmetry on the cell membranes that plays a key role in the interfacial interactions between RBCMs and nanoparticles. Analysis of positively and negatively charged nanoparticles coated with RBCMs by Luk et al.[58] demonstrated that negatively charged nanoparticles could form nuclei-shells with distinct particles, whereas positively charged nanoparticles only formed polydisperse aggregates (Fig. 3). These results could be attributed to the dense negatively charged sialic acid moiety of the outer membrane side, with the strong affinity from positively charged nanoparticles likely collapsing the lipid bilayer and hindering the local arrangement necessary for lipid coverage. In contrast, the negatively charged

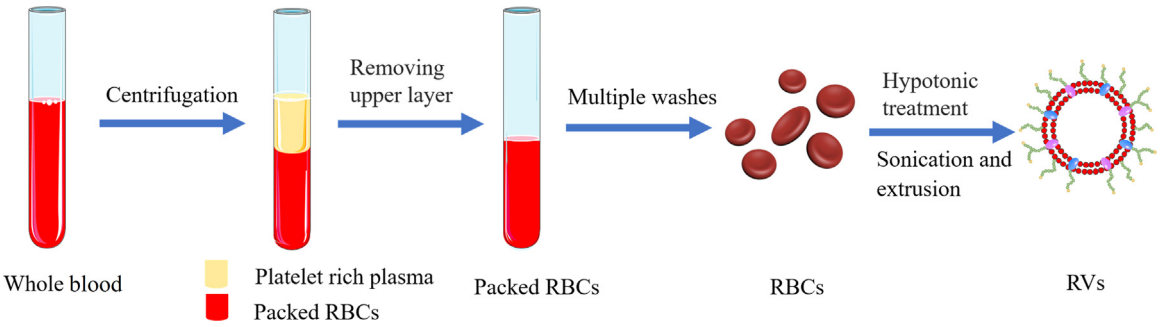

Figure 2 Schematic preparation of red blood cell membrane-derived vesicles (RVs). Fresh whole blood was centrifuged and repeatedly washed to obtain clean RBCs, and then RVs were obtained through further hypotonic and extrusion treatment.

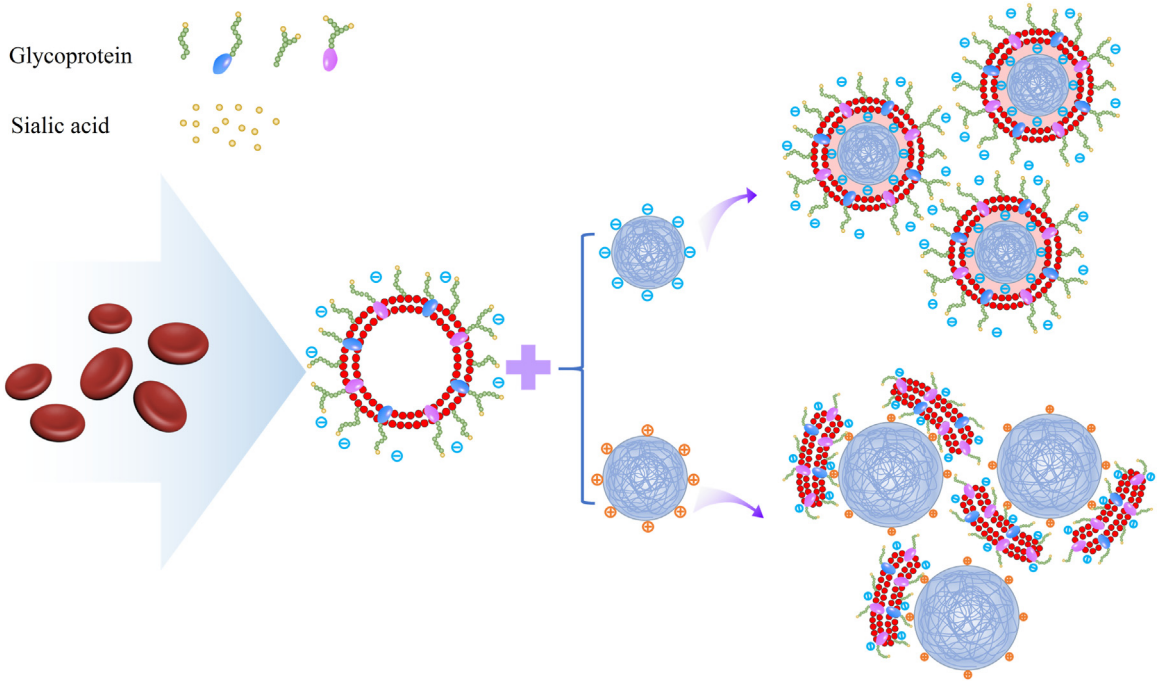

Figure 3 Schematic diagram of electrostatic interactions between negatively and asymmetrically charged RVs with negatively and positively charged polymeric cores, respectively. The negatively charged nanoparticles and the negatively charged RVs produce strong electrostatic repulsion, resulting in the fusion of the nanoparticles with the intracellular membrane side, while the positively charged nanoparticles and the negatively charged RVs produce a strong affinity to collapse the lipid bilayer. Adapted with permission from Ref. 58. Copyright © 2014 Royal Society of Chemistry.

nanoparticles and sialic acid moiety produced a strong electrostatic repulsion that caused the nanoparticles to fuse with the intracellular membrane side, forming a right-side-out-membrane orientation structure[59] to retain cell surface glycocalyx.

4.3. *Methods of vesicle-particle fusion*

Early efforts devoted to bridging nanoparticles and RBCMs involved the use of "bottom-up" approaches, *i.e.* functionalizing nanoparticles with the surface chemistry of RBCs. However, the RBC-mimicking delivery vehicles formed by chemistry-based bioconjugation techniques often result in protein denaturation. Besides, the nanoparticles could hardly be adequately duplicated into a complex protein to anchor RBCMs. In 2011, Zhang et al.[38] reported a "top-down" method for producing RBCM-camouflaged nanoparticles. By extruding nanoparticles with nanoscale RVs prepared in advance, they successfully coated the sub-100-nm PLGA nanoparticles with the RBCMs. This "top-down" method is promising for a large-scale production of RBCM-NPs. Herein, several main methods for RV-nanoparticle fusion are summarized.

4.3.1. *Co-extrusion method*

Prepared nanoparticles are usually fused with obtained RVs through mechanical extrusion. The principle for the coating process is the interfacial interactions mentioned above. Depending on the prepared nanoparticle size, the mixture is repeatedly extruded through different-sized porous membranes before bath sonication for several minutes[58] (Fig. 4A). The mechanical force promotes the nanoparticles to pass through the lipid bilayer, generating the vesicle-particle fusion. In particular, the RBCM phospholipid bilayer structure should be as complete as possible throughout the preparation process to minimize membrane protein

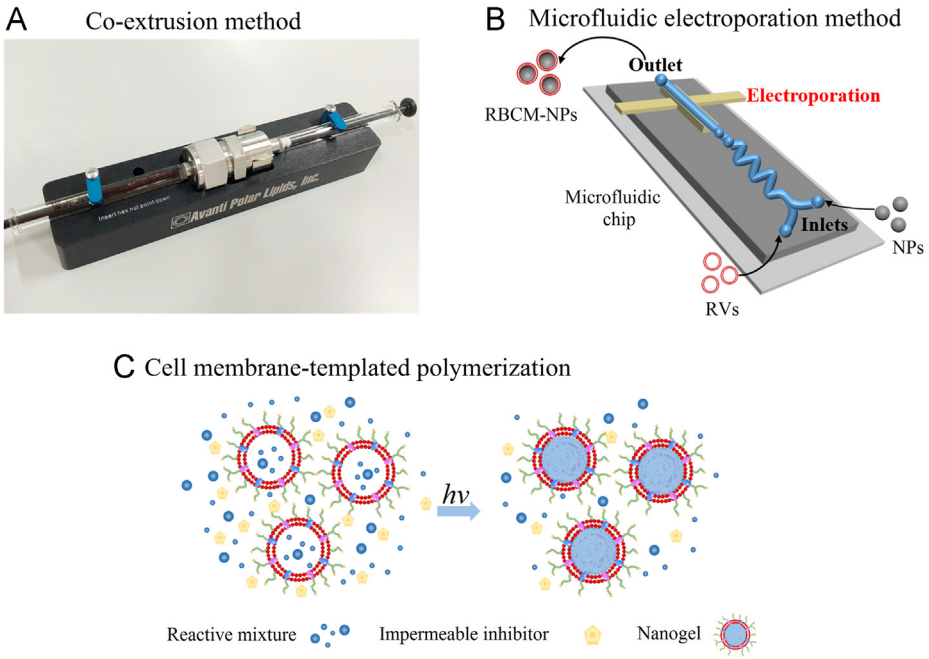

Figure 4 Schematic of RBCM-NP preparation by three different methods. (A) Co-extrusion method; (B) microfluidic electroporation method; Adapted with permission from Ref. 60. Copyright © 2017 American Chemical Society. (C) cell membrane-templated polymerization. Adapted with permission from Ref. 62. Copyright © 2015 Wiley Online Library.

loss and damage[38]. After repeated extrusions, the excess vesicles are removed by centrifugation, with the collected precipitate representing the final product and redispersed for future use[50].

The ratio of the amount of RBCMs to nanoparticles is based on RBC membrane volume and the total membrane volume required to completely coat 1 mg of nanoparticles[38,58]. Brian et al.[58] used different sizes of PLGA nanoparticles with diameters between 65 and 340 nm and coated by erythrocyte membranes, which resulted in different amounts of erythrocyte membranes as required. For the same weight of nanoparticles, the smaller the particle size, the fewer the red blood cell membranes needed. To compensate for cell membrane loss during preparation and the fusion process with nanoparticles, excessive amounts of blood are usually used to ensure that all nanoparticles are coated with RBCMs.

4.3.2. *Microfluidic electroporation method*

With the widespread use of biomimetic membrane-coated nanoparticles in the biomedical field, microfluidic electroporation has also been demonstrated to effectively promote RBCM-NP synthesis. Rao et al.[60] mixed Fe_3O_4 magnetic nanoparticles (MNs) and RVs in a microfluidic device. The microfluidic chip for electroporation consists of five sections: two inlets for the RVs and nanoparticles, respectively, a Y-shaped merging channel, an S-shaped mixing channel, an electroporation zone, and an outlet. When the mixture of MNs and RVs flows through the electroporation zone, the electrical pulses can break down the dielectric layer on the cell membranes and create multiple transient pores[61], providing a pathway for the entry of Fe_3O_4 MNs. During this process, pulse voltage, duration, and flow rate should be optimized. After integration, the RBCM-MN are collected from the chips and injected into experimental animals for a series of *in vivo* performance tests (Fig. 4B).

The microfluidic electroporation strategy perfectly combines biology with physics, excluding the requirement of a very large force to repeatedly squeeze the nanoparticles through porous membranes compared with the co-extrusion method, maintaining the membrane integrity to some extent and reducing cell surface protein loss, to achieve a better therapeutic effect. In addition, RBCM-MNs prepared by microfluidic electroporation exhibit better colloidal stability and magnetic resonance imaging (MRI) and photothermal therapy (PTT) performance *in vivo* than those prepared by conventional extrusion methods. Thus, the utilization of the microfluidic electroporation method in bio-inspired cell membrane coating of nanoparticles appears to have bright prospects.

4.3.3. *Cell membrane-templated polymerization*

The majority of existing cell membranes covering nanoparticles are prepared *via* a nanoparticle-templated coating routes, such as the co-extrusion method and microfluidic electroporation method, wherein the nanoparticle core is pre-synthesized then the outer layer coated with cell membranes. In this process, the interfacial interactions[58] between the membranes and the cores may hinder the application of some non-compliant nanomaterials. This led us to ponder the possibility of nanoparticle cores being grown *in situ* in cell-derived vesicles. Zhang et al.[62] successfully implemented the first example of using a cell membrane-template polymerization method to synthesize polymer cores by *in situ* polymerization to form cell membrane-coated nanogels. They used acrylate polymerization as a model system, with the key to the study being the addition of a membrane-impermeable macromolecular inhibitor during membrane-templated formation, which was formed by the combination of a popular membrane-permeable free radical scavenger, 2,2,6,6-tetramethylpiperidin-1-yl oxyl (TEMPO), and PEG. The macromolecular inhibitor could effectively inhibit extracellular aggregation while maintaining the internal response of the vesicles, and reduce the risk of cell membrane protein denaturation and content leakage[62]. After the addition of the

macromolecular inhibitor, ultraviolet (UV) irradiation induced gelation, resulting in the formation of cell membrane-coated hydrogels, termed nanogels (Fig. 4C).

This method offers many advantages over the nanoparticle-templated coating route, including complete coating of the nanocores and easy control of the final biomimetic nanoparticle size and stiffness. Moreover, it overcomes the limitation requiring nanoparticles to be coatable, and provides a unique platform for a wide range of biomedical applications. Thus, the cell membrane-template method will likely be applicable to coating various nanostructures aside from nanogels.

5. *In vitro* verification of RBCM-NPs

In vitro evaluation of these cellular vectors is necessary as the chemical structures and surface proteins of the RBCMs play a vital role in their immune escape and circulation. The main characterization parameters are as follows.

5.1. Surface morphology

Nanoparticle size and potential values before and after RV encapsulation were examined using dynamic light scattering (DLS) measurement. The diameter of the coated nanoparticles is usually increased within the range of $10-20$ nm, as the cell membrane lipid bilayer thickness is about 8 nm. The surface charge is close to the RVs after surface covering[50,63,64]. In addition, a stabilizing effect on the RBCM-NPs in comparison with the naked nanoparticles has been demonstrated[37]. Transmission (TEM) and scanning (SEM) electron microscopy are usually used to observe RV, nanoparticle, and RBCM-NP shape and morphology. Upon repeated extrusion, the original irregular cell membrane fragments are extruded into 100–200 nm hollow spherical vesicles, which differ completely from untreated RBC morphology, comprising a micron-level concave disc under electron microscopy. The hollow spherical vesicles also provide sufficient space for further nanoparticle encapsulation. Successful nanoparticle coating after negative staining can clearly be observed as a layer of membrane coated on the nanoparticle surface (*i.e*, the core-shell structure) upon TEM visualization[38,53,58,59].

5.2. Verifications of surface proteins

Another proof of successful coating is the determination of whether the encapsulated nanoparticles carry the specific RBCM proteins, which determine whether the coated nanoparticles will have immune escape and long-term circulatory effects. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by Coomassie staining is used to visualize the RBC, RV, and RBCM-NP proteins, wherein all protein bands should show a similar profile to that of the RBCs[53,59]. To further analyze specific protein markers, western blotting analysis is performed. CD235a, also known as glycoprotein A[65], the main RBC sialic acid glycoprotein and the blood group A antigen, was shown to be present in RVs and RBCM-NPs, with CD47 also found to an almost equal degree on RBCs, RVs, and RBCM-NPs[66]. The biological activity of membrane proteins expressed on the RBCM-NP surface can also be examined at the cellular level, especially the ability of CD47 to evade macrophage phagocytosis. Specifically, RBCM-NP uptake by mouse macrophage RAW264.7 cells was found to be 59.0% lower than that of naked nanoparticles[53]. In addition, a saturation in CD47 functionalization by the RBCM coating has been identified, below which approximately 92% of the input membrane proteins are used for particle functionalization, suggesting that the entire coating process is both reasonable and feasible[59]. Determining the position and orientation of RBCM functional proteins after being coated on the nanoparticles is highly desirable. Membrane protein interactions with lipids were successfully investigated using cell blebs as an intermediate and determined using single molecule tracking and moment scaling spectrum analysis[67]. Moreover, a geometry-based approach was developed by automatically inserting a membrane protein with a known 3D structure into membranes[68]. These approaches may be consulted in revealing RBCM protein orientation.

5.3. Fluorescence colocalization

To further verify core-shell particle structure integrity, Hu et al.[38] designed a method wherein hydrophobic red DiD dyes and lipophilic green rhodamine-DMPE dyes were respectively loaded into the polymeric cores and RVs prior to their fusion. The resulting dual-fluorophore-labeled nanoparticles were incubated with HeLa cells for 6 h, after which DiD and rhodamine DMPE were observed to overlap at the same position using a fluorescence microscope. Fluorescence colocalization showed that the nanoparticles had a complete core-shell structure after being internalized by the cells, demonstrating the success of RBCM coating.

5.4. UV–vis absorption spectra

Encapsulation conditions can be also characterized by UV–vis absorption spectroscopy. Compared with the original nanoparticle absorption pattern, the encapsulated nanostructures retain the original absorption peak and obtain an extra absorption peak equivalent to the characteristic RV absorption peak[50,69]. This result indicates a successful shift of the RVs on the nanoparticle surface without affecting the properties of the original nanoparticles.

Together, these *in vitro* characterizations verify the ideal state of RBC separation into vesicles and integration with nanoparticles.

6. RBCMs coated with different core nanoparticles for anti-tumor application

Subsequent research has allowed development of the coated core nanoparticles from the initial polymer nanoparticles to MNs, mesoporous silica nanoparticles (MSNs), upconversion nanoparticles (UCNPs), and gold nanoparticles (AuNPs) (Fig. 5).

6.1. Polymeric nanoparticles

6.1.1. Poly(caprolactone) (PCL) nanoparticles

PCL, with properties of biodegradability, biocompatibility, and low glass transition temperature, is used as a kind of polyester to construct core nanoparticles[70]. Paclitaxel (PTX), a broad-spectrum antineoplastic agent, constitutes a commonly used model drug for treating various cancers including breast, ovarian, and lung cancers. Recently, RBCM-coated poly(caprolactone) nanoparticles loaded with PTX co-administered with a tumor penetrating peptide iRGD have been utilized for metastatic breast cancer treatment[53]. The modified NPs showed significantly prolonged blood

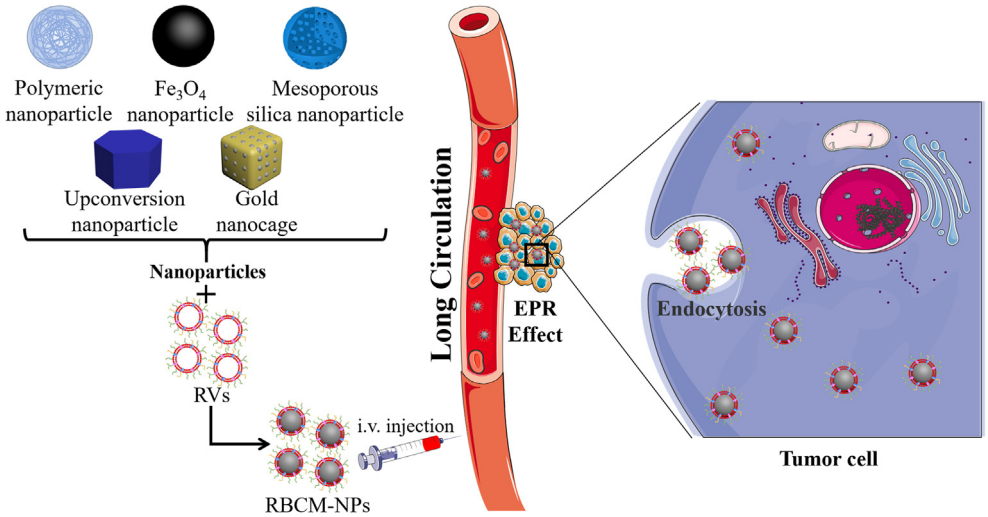

Figure 5 Red blood cell membrane-camouflaged nanoparticle achievement of antitumor effect. Different core nanoparticles are coated with RVs and then enter into the blood by intravenous (i.v.) injection, evading the immune system to realize long-term circulation, penetrate into the tumor tissues owing to the EPR effect, and finally enter into the tumor cells *via* endocytosis to achieve diagnosis and treatment of cancers.

circulation with a half elimination time 5.8-times that of the original polymer NPs. In addition, RBCM-mimetic nanoparticle co-administration with iRGD inhibited over 90% of tumor growth and suppressed 95% of lung metastases. The experimental data reveals the tremendous importance of long-term circulation and tumor penetration in the treatment of cancers, offering a new paradigm for nanocarrier design for delivering drugs to lung metastases-derived tumors.

However, lack of RBCM targeting ability for solid tumors is an obstacle to tumor cell internalization. Moreover, the diffusion barrier caused by complete RBCMs on the NP surface limits the effective release of loaded drugs[71–73]. Therefore, some attempts have been implemented to solve these shortcomings, such as membrane surface modification to enhance its targeting and the combination of chemotherapy and phototherapy to promote the ablation of erythrocyte membranes, thereby promoting drug release. Su et al.[74] further designed novel near-infrared radiation (NIR) laser-responsive RBCM-NPs, in which the thermosensitive lipid DPPC is used in composite polymer cores. Under NIR laser stimulation, powerful thermal effects trigger DPPC phase change, destroying core structure and releasing PTX, which increased tumor cell uptake by 2.1-fold. The resulting antitumor effect representing marked optimization of previous RBCM-NPs and tumor penetration chemotherapy, suggesting that synergistic chemotherapy has advantages over single chemotherapy and may become an effective option for clinical treatment of metastatic breast cancer.

6.1.2. *Polypyrrole (PPy) nanoparticles*

PPy nanoparticles are considered as highly promising materials owing to their excellent photothermal and photoacoustic effects and are often used in PTT for tumors and as photoacoustic imaging (PAI) contrast agents for cancer diagnosis[75–77]. Wang et al.[69] masked a layer of RBCMs on photothermal PPy nanoparticle surfaces; to further improve the photothermal effect, they used the endothelin A (ETA) receptor antagonist BQ123 to regulate the tumor microenvironment. BQ123 blocks ETA receptors, induces tumor vasodilatation, and increases blood perfusion by modulating the ET-1/ETA transduction pathway[78,79], which causes rapid of tumor vessel diameter expansion by 20%–30%, thereby improving biomimetic PPy NP-directed targeted tumor delivery. The average tumor weight on day 13 was only 0.136 g, showing a better antitumor effect compared to other groups. The authors successfully achieved PAI and PTT under the dual action of RBCM-PPy NPs and ETA receptor antagonists, significantly increasing local PPy NP concentration in the tumor site.

6.1.3. *Poly(lactide acid) (PLA) nanoparticles*

PLA constitutes a promising material owing to its excellent biocompatibility, variable biodegradability, and commercial availability[80–82]. Aryal et al.[74] used PLA nanoparticles as the core to compare the strategies of physically encapsulating and chemically conjugating doxorubicin (Dox) into RBCM-NPs as assessed *in vitro* using the myeloid leukemia cell line Kasumi-1 acute. When the Dox molecules were physically encapsulated in the polymer, 20% were released within the first 2 h, whereas only 5% were released following chemical conjugation. This suggests that chemical linkers responsive to the environment can achieve better controlled drug release[83,84]. Moreover RBCM-NP release rate over about 72 h was about 20% whereas PEGylated NPs released 40% of Dox, demonstrating that RBCM cloaking provides a barrier that slows the outward diffusion of encapsulated drug molecules. These results indicate that nanoparticles covered by RBCMs represent a potential drug delivery platform for sustained release.

6.1.4. *Poly(D,L-lactide-co-glycolide) (PLGA) nanoparticles*

PLGA constitutes an FDA-approved polyester with adjustable biodegradability and good biocompatibility[85], which can be used to prepare nanoparticles by methods such as nanoprecipitation and emulsification[72,86–88]. Hypoxia is a common pathological feature of most solid tumors[89]. In cancer radiation therapy, oxygen can react with DNA and prevent DNA repair by tumor cells, thereby enhancing the killing effect[90]. Perfluorocarbons (PFCs) are inert chemicals with extremely high oxygen solubility and can be used as an alternative to artificial blood[91–93]. Gao et al.[94] obtained RBCM-PFC@PLGA-NPs by encapsulating PFCs in biocompatible PLGA and then coating with RBCMs. With efficient oxygen loading and drastically longer blood circulation times, RBCM-

PFC@PLGA-NPs could effectively delivery oxygen to the tumor after intravenous injection, thereby greatly relieving the tumor hypoxia and enhancing the therapeutic effect during radiotherapy. Zhang et al.[95] combined gambogic acid (GA)-loaded PLGA nanoparticles and RBCMs to assess the ability to preserve and enhance colorectal cancer treatment effects. RBCM-GA@PLGA-NPs were absorbed by gastrointestinal tract cells and inhibited SW480 cell proliferation *in vitro via* effects on apoptosis and cell cycle. *In vivo*, treated of mice with free GA prolonged survival for 4 days compared to that of untreated mice, whereas treatment with RBCM-GA@PLGA-NPs extended survival for at least 30 days. Moreover, Luk et al.[8] designed Dox-loaded PLGA nanoparticles coated with RBCMs for treating lymphomas and conducted a series of safety evaluations. This platform exhibited many necessary features for clinical DDS such as safety and biocompatibility, providing support for the continued development and clinical application of the RBCM-NP platform.

PH-sensitive polymeric nanoparticles (PGSC-PTX NPs) encapsulated with RBCMs have also been used in non-small cell lung cancer treatment strategies[96]. Furthermore, the study of encapsulating polymer nanoparticles as nano-vaccines for the induction of antitumor immunity against melanoma[97] has also achieved remarkable results.

6.2. *Fe_3O_4 nanoparticles*

Fe_3O_4 core nanoparticles have proven to be useful for MRI, drug delivery, hyperthermia, and tissue repair owing to their unique magnetism, low toxicity, controlled size, good biodegradability, and biocompatibility, and have been intensively studied for diagnostic and therapeutic applications[98–100]. To evade immune clearance and improve their systemic circulation time, Rao et al.[101] designed an experimental program using RBCM-coated Fe_3O_4 NPs (RBCM-Fe_3O_4-NPs). Compared with Fe_3O_4 and PEG-Fe_3O_4-NPs, RBCM-Fe_3O_4-NPs effectively reduced nanoparticle uptake by RAW 264.7 cells. In addition, RBCM-Fe_3O_4-NPs and PEG-Fe_3O_4-NPs respectively showed blood retentions of 14.2% and $<6\%$ ID/g after 24 h, further confirming that RBCM-NPs exhibit better blood retention than biopolymer-coated nanoparticles. With regard to biodistribution, RBCM-Fe_3O_4-NP accumulation in the two major organs of the RES/MPS, the liver and spleen[102], was 49.2 ± 7.1 and 100.1 ± 16.3 μg/g respectively, whereas PEG-Fe_3O_4-NP accumulation was 117.4 ± 30.3 and 174.5 ± 43.7 μg/g, demonstrating that RBCM coating can reduce RES/MPS absorption more effectively than PEGylation. Toward accelerating blood clearance, the second RBCM-Fe_3O_4-NP injection had similar pharmacokinetic trends as the first; however, the second PEG-Fe_3O_4-NP dose showed a dramatic decrease in the Fe content, suggesting a serious accelerated blood clearance (ABC) phenomenon in Fe_3O_4@PEG nanoparticles, with anti-PEG antibody production likely responsible for the ABC phenomenon. Together, these data indicate that the RBCM coating can provide nanoparticles with a longer circulation time without causing ABC or inducing immune responses at the cellular (myeloid-derived suppressor cells) or humoral (IgM and IgG) level, thereby representing promising new technologies with tremendous clinical potential.

6.3. *Magnetic nanoclusters (MNCs)*

Highly crystallized iron oxide nanoparticles generated by pyrolysis have an exquisite polymer coating that shows a photothermal effect at higher concentrations[103,104]. Ren et al.[105] in a comparative study on individual Fe_3O_4 nanocrystals and Fe_3O_4 MNCs, found that MNCs have a better PTT effect than individual Fe_3O_4 nanoparticles. On this basis, they coated MNCs with RBCMs followed by intravenous injection in a xenograft mouse model of breast cancer assisted with PTT[50]. In the cell uptake assay, 10^6 RAW264.7 cells absorbed 10.309 mg Fe in the MNC group, whereas only 4.417 mg Fe were taken up in the RBCM-MNC group, with 2.34-fold difference in cellular extrinsic Fe level. Owing to the diversity of membrane components, RBCM coatings can significantly reduce cell-level uptake of nonspecific nanoparticles. In the pharmacokinetic study, the MNCs were rapidly cleared from the bloodstream and could not be detected after 8 h, whereas the RBCM-MNCs exhibited a blood retention rate of approximately 10% ID/g 24 h after injection. In biodistribution, RBCM-MNC tumor uptake was $4.937\pm1.473\%$ and $2.630\pm0.602\%$ ID/g after 1 and 48 h following nanoparticle injection, whereas the MNC group showed only 1.282 ± 1.036 and $0.580\pm0.073\%$ ID/g. In addition, RBCM-MNC liver uptake at 1 and 48 h after injection was $17.743\pm5.547\%$ and $9.157\pm3.271\%$ ID/g, whereas that of MNC reached $32.110\pm5.812\%$ and $27.909\pm4.618\%$ ID/g, respectively. These data indicate that the RBCM coating strategy could significantly enhance tumor accumulation, reduce liver uptake, and achieve better biodistribution characteristics.

In the *in vivo* PTT antitumor effect study of RBCM-MNCs, the authors set up saline, MNC, RBCM-MNC, and RBCM-MNC with the magnetic field groups, and were illuminated with the same laser setting. Thermal images of mice from the different treatment groups showed that the temperature of both RBCM-MNC groups rose rapidly above 50 °C compared to that of the other two groups, which indicated that RBCM-MNCs could efficiently convert light into heat, and showed a higher photothermal effect than the original MNCs, demonstrating that the camouflage coating allowed for increased tumor accumulation and preferred biodistribution. Finally, the MNC, RBCM-MNC, and RBCM-MNC with magnetic field group tumor inhibition rates calculated by tumor weight were 21.43%, 93.81%, and 94.24%, respectively. RBC-derived membranes afford significantly increased MNC blood retention and invisibility, providing RBCM-MNCs with strong passive EPR effects even in magnetic-field free condition. These suggest the potential of iron-based nanomaterials for imaging and therapeutic applications and provide guidelines for combining nano-bionics with other physical techniques such as PTT and photodynamic therapy (PDT)[106] to treat cancer.

6.4. *Mesoporous silica nanoparticles (MSNs)*

MSNs have been widely used for anticancer drug delivery[107] because of their large surface area and adjustable pore size. However, MSNs are prone to aggregation, leakage, and limited circulation time in the blood, which limit their anti-cancer efficacy. Su et al.[108] achieved high loading capacity by masking MSNs with RBCMs to load the anticancer drug Dox and the near-infrared photosensitizer chlorin e6 (Ce6). Lipid-coated MSN (L-MSNs) were also used. Lipid coating did not reduce MSN cellular uptake, confirming the superiority of RBCM protein in reducing macrophage uptake. *In vitro* laser-induced RBCM-MSN-Dox/Ce6 respectively induced 1.9-, 2.0-, and 2.0-fold Dox accumulation after 1, 2, and 4 h of incubation compared to that without laser. This was suggested to arise from reactive oxygen species (ROS) produced by Ce6 laser stimulation, which oxidize and form defective phospholipid molecules in RBCMs and cancer cell

membranes, affecting cell membrane permeabilization, resulting in increased cellular uptake of released Dox[109]. In terms of cytotoxicity, RBCM-MSN-Dox and RBCM-MSN-Dox/Ce6+Laser IC_{50} values on 4T1 cells were 2.6 and 0.9 μg/mL, which was 1.4- and 1.5-fold lower than that of MSN-Dox (3.7 μg/mL) and MSN-Dox/Ce6+Laser (1.6 μg/mL), potentially because RBCM coating can prevent MSN drug leakage and premature release. In addition, the RBCM-MSN-Dox/Ce6 tumor inhibition rate was 91.4%, significantly higher than that of RBCM-Ce6+Laser (68.9%) and RBCM-Dox (73.7%). These results indicate that RBCM-MSN-Dox/Ce6, when combined with laser light stimulation, can combine the synergistic effects of chemotherapy and PDT to achieve the most efficient cytotoxicity. Moreover, as demonstrated by hematoxylin and eosin (H&E) staining, laser-induced RBCM-MSN-Dox/Ce6 obviously enhanced anti-metastatic function, with no metastases being found in the lungs.

6.5. *Upconversion nanoparticles (UCNPs)*

UCNPs have excellent chemical and optical properties, low toxicity, and good light stability compared to traditional down-conversion fluorescent nano-probes[110–116]. Ding et al.[106] have developed a new type of bionic PDT nanocarrier (F/P-RM-Us/PS) using UCNPs with incorporated photosensitizers (PS) as the cores, coated by the RBCMs modified with folic acid (FA) and triphenylphosphonium (TPP) to achieve dual targeting of cancer cells and mitochondria. Compared with other conventional coatings, RBCM coating significantly promoted ground state molecular oxygen ($^3O_2$) entry into the agent core for $^1O_2$ production. Specifically, the F/P-RM-Us/PS group exhibited over 4-fold higher intracellular $^1O_2$ concentration than the Us/PS group. At 20 days after PDT treatment using F/P-RM-Us/PS, tumor volume was only one tenth that using Us/PS. The design of PDT agents yielded a programmed boost in blood circulation, tumor accumulation, cellular uptake, mitochondria guidance, and $^1O_2$ transportation, providing the first example of RBCM-masked PDT nanocarriers with programmed delivery.

6.6. *Gold nanoparticles (AuNPs)*

Owing to their biocompatibility, optical properties, and easily modifiable surfaces, AuNPs are often used as imaging agents and drug carriers[117,118]. Gold nanocages (AuNCs) and gold nanoshells have been studied because hollow nanoparticles are more efficient at converting light to heat[71,119–121]. In studies by Piao et al.[71], RBCM coatings significantly prolonged AuNC half-life in the circulation, leading to two-fold in-tumor deposition at 24 and 48 h after injection compared with PVP-modified AuNCs. Moreover, tumor-bearing mice treated with RBCM-AuNCs achieved 100% survival over 45 days.

6.7. *Gelatin nanoparticles*

It is well-known that broad-spectrum bacteria secrete gelatinases, which can effectively hydrolyze gelatin nanoparticles to small biomolecules[122]. Therefore, Li et al.[123] proposed an antibiotic delivery system based on supramolecular gelatin nanoparticles (SGNPs) that activates its release mechanisms in the presence of gelatinase. The SGNPs were first decorated with vancomycin (Van), a model antibiotic, and then coated with RBCMs to obtain the novative and biomimetic antibiotic delivery system (Van ⊂ SGNPs@ RBC). Studies have shown that this delivery system effectively and specifically released antibiotics at the site of infection. By coating the RBCMs, Van ⊂ SGNPs@ RBC showed excellent immune evasion and alleviated symptoms caused by bacterial infection. It is believed that with the use of different antibiotics and bioresponsive materials, the system can achieve efficient and specific killing of various bacteria.

Notably, each type of core nanoparticles has its own advantages (Table 1). The choice of nanoparticle species plays an important role in the size and shape of the formulation as well as subsequent drug release. Different designs should therefore be developed according to the specific situation.

7. Surface modification of erythrocyte membranes

In the application of RBCM-NPs for the treatment of diseases, especially for cancers, it is desirable to overcome the obstacles in the process of tumor cell internalization. This is typically achieved by surface modification to increase the ability of the liquid to penetrate the tissue or by carrier functionalization with a ligand that targets the overexpressed tumor antigen to enable cancer targeting and minimize side effects[124]. Zhou et al.[64] stably anchored recombinant hyaluronidase, PH20 (rHuPH20) molecules on the RBCM outer domain by using a cell-impermeable linker, NHS-PEG-maleimide, while retaining their enzymatic activity. The functionalized RBCMs were then used to coat nanoparticles. The RBCM-NPs alone diffused little into extracellular matrix (ECM)-mimicking gels whereas rHuPH20-conjugated RBCM-NPs in the gel doubled the free rHuPH20 diffusion efficiency, with the same enzymatic activity. In PC3 cells, the conjugated rHuPH20 increased the amount internalized or bound to PC3 cells three-fold compared to that of RBC-NP with 10 U free rHuPH20. These results suggested that the conjugated rHuPH20 could more effectively assist NP diffusion in ECM-mimicking gels as well as in the cytoplasmic HA matrix of PC3 cells. Moreover, rHuPH20 modification did not reduce the RBCM-NP blood circulation time. This surface modification method thus preserves the inherent native cell membrane characteristics while adding other required functions.

Targeting selection is a necessary tool in the use of RBCM-NPs for the treatment of cancers, which can effectively avoid side effects of drugs on normal cells and tissues[125–128]. At present, there are many ongoing attempts to improve its targeting efficacy, which include chemical conjugation of carboxyl-, amine-, or sulfhydryl- groups on the surface of cell membranes[129–135]. However, these methods will lead to chemical reactions and inactivation of membrane proteins on the surface of RBCM membranes, consequently destroying its original immune escape function. Thus, Fang et al.[136] proposed a lipid insertion method for functionalizing RBCMs membranes, using ligand-linker-lipid conjugates as targeting ligands. In this study, folate with small molecular and nucleolin-targeting aptamer AS1411 with larger molecules was used as ligands to form targeting ligands. By flow cytometric and fluorescence imaging analysis, it was found that the model cancer cells' uptake of modified erythrocyte membrane-coated nanoparticles were respectively 8- and 2-fold as compared to unmodified cells, showing a pronounced targeting effect[136]. Through the aid of lipid chains and the dynamic membrane bilayer structure, the targeting ligand can spontaneously enter the surface of the cell membrane, effectively avoiding the exposure of the membrane proteins to chemical reactions. The complete release of the drug becomes a critical issue after the increasing the uptake of

Table 1 Studies on different inner cores of RBCM-NPs for cancer therapy.

Inner core		Advantage	Application	Ref.
Polymeric nanoparticles	PCL nanoparticles	Biodegradability; Biocompatibility; Low glass transition temperature	Loaded with PTX co-administered with iRGD/combined with PTT for the treatment of metastatic breast cancer	53,70,74
	PPy nanoparticles	Biocompatibility; Low cytotoxicity; Excellent photothermal and photoacoustic effects	Under dual action of biomimetic and ETA receptor antagonists to achieve PAI and PTT	69
	PLA nanoparticles	Biocompatibility; Biodegradability	Loaded with Dox for studies of controlled drug loading and release	73
	PLGA nanoparticles	Biocompatibility; Biodegradability; Sustained drug release	Loaded with PFC to relieve tumor hypoxia; Loaded with GA for the treatment of colorectal cancer	8,94,95
Fe_3O_4 nanoparticles (Fe_3O_4 NPs)		Unique magnetism; Low toxicity; Controlled size	For evading immune clearance and improving circulation time	101
Magnetic nanoparticles (MNs)		Better PTT effect	Curing breast cancer xenograft mice in conjunction with PTT	50
Mesoporous silica nanoparticles (MSNs)		Large surface area; Adjustable pore size	Loaded with Dox and Ce6 combined with *in vitro* laser triggering to treat metastatic breast cancer	108
Upconversion nanoparticles (UCNPs)		Low toxicity; Good photo-stability; Superior chemical and optical properties	Incorporated UCNPs with PS, and RBCMs modified with DSPE-PEG2000-FA and DSPE-PEG2000-TPP to develop a new type of bionic PDT nanocarrier	106
Gold nanoparticles (AuNPs)		Biocompatibility; Good optical properties; Easily modifiable surface	AuNCs coated with RBCMs to prolong half-life	71
Gelatin nanoparticles		Can be hydrolyzed into small biomolecules by gelatinases secreted by bacteria	For reducing clearance by the immune system and absorbing the bacterial exotoxin to relieve bacterial infection	123

the carrier by the tumor cells through targeting modification. It can be used in the form of phototherapy combined with chemotherapy, using light-sensitive nanocarriers[60,69,71,105] or co-loaded chemotherapeutic agents and PS to increase drug release, achieving excellent anti-tumor effects (Fig. 6).

In general, UCNPs are ligand-modified for targeting of cancers; however, nanoparticles in biological fluids would form "protein corona" that cover the ligands on the surface of the nanoparticles and reduce their targeting features. Recently, Rao et al.[137] reported that modification of FA with UCNPs coated with erythrocyte membranes effectively prevented protein adsorption, thereby enhancing targeting efficiency and *in vivo* tumor imaging. Besides, a brain-targeted delivery system that combines RBCM-NPs with unique neurotoxin-derived targeting moieties, "CDX peptides" has been shown to have potent brain targeting abilities, and to significantly reduce drug toxicity[138]. The highlight of this study is its effective combination of natural immune escape and cancer-targeted therapy, that are both pivotal in the treatment of cancers, and the next step may be to study the effect of targeting ligands on RBCM-NPs *in vivo* and to further demonstrate the promising cancer treatment prospects of this technique.

Apart from the membrane surface modifications (Table 2), the concept of membranes fusion to produce new bio-coatings by fusing two different sources of cell membranes has been proposed[17]. They coated the PLGA cores with the hybrid membrane fused by erythrocyte membrane and the platelet membrane that has been extensively used for nano-coating to enhance targeting in recent years. After a series of *in vitro* and *in vivo* characterizations, the authors confirmed the successful transfer of the protein markers on the membrane surface and further demonstrated that RBC-platelet hybridization membrane allows the PLGA cores to function simultaneously with immune escape and targeting[17]. This provides a strong guide to retain the natures of different cells by the fusion of other specific functional membranes, thereby overcoming the limitations of the current multi-functional modifications of nanoparticles. In addition, the technology can improve the applicability of emerging nano-carriers with complex surface chemistry, and opens up a new field for the further developments of biomimetic nanoparticles.

8. Conclusions

It is a daunting task to artificially replicate the biological interactions that occurs in the body. Researchers are more inclined to seek inspiration from nature instead of creating new solutions from scratch. Using erythrocyte membranes as drug carriers for novel DDS has become an interesting strategy for the functionalization of nanomaterials and will remain an active area in future researches. We can draw blood from the patient, coat the various nanoparticle cores with derivatized RBC vesicles and then inject them back into the same patient for treatment or diagnosis. Erythrocyte membrane-coated nanoparticles have achieved a transition from the "stealth" of original prevalent PEGylation to the "disguise" of erythrocyte membrane-coating, achieving the same surface conditions with the RBCs in the blood, thus escaping phagocytosis by the immune system. Coating of nanostructures with vesicles derived from native cell membranes imparts the intrinsic functions and characteristics of the source cells to the nanostructures due to the transport of many membrane proteins, glycans, and lipids to the surface of the original nanoparticles. These cell-membrane-coated nanoparticles can retain their cell-surface coating properties in interaction with other cells and pathogenic agents and

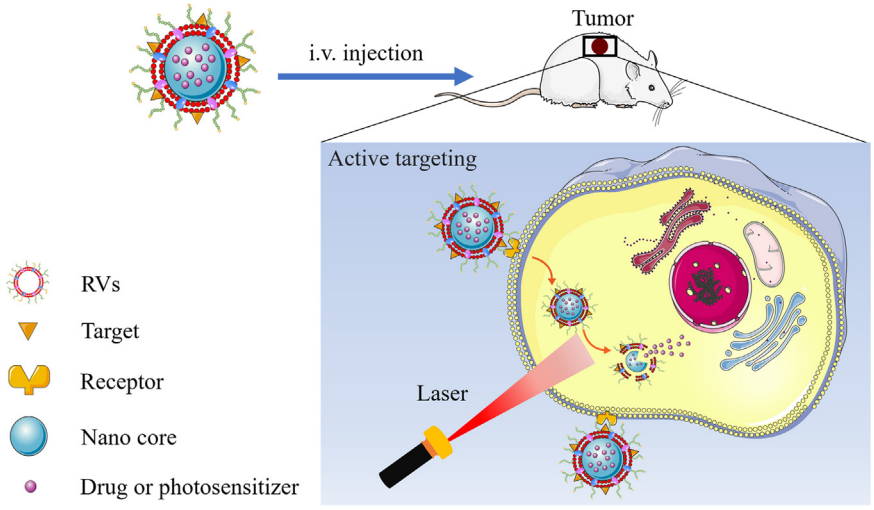

Figure 6 Schematic design of ligand-modified drug or photosensitizer-loaded RBCM-NPs combined with phototherapy for targeting and sequential drug delivery. Ligand-modified RBCM-NPs are injected into to mice to achieve active targeting into tumor cells. Under laser irradiation, light-sensitive nanocarriers or PS can provide strong thermal energy, triggering the destruction of cores and resulting in the release of PTX.

Table 2 Currently surface modifications of RBCMs for antitumor application.

Modified ligand	Function	Ref.
Folic acid (FA)	Selective recognition of cancer cells; effectively preventing "protein adsorption" on the surface of nanoparticles	106,136,137
Triphenylphosphonium (TPP)	Targeting to mitochondria and enhancing the yield of $^1O_2$	106
rHuPH20	Assisting nanoparticle diffusion in the cytoplasmic HA matrix of PC3 cells	64
Aptamer AS1411	Showing targeting capability against several cancer cell types	136
CDx peptides	Ability to traverse the blood–brain barrier and achieve marked therapeutic efficacy in glioma treatment	138
Mannose	Actively targeting APCs (antigen presenting cells) in lymphatic organs	97
Anti-EpCam (epithelial cell adhesion molecule) antibodies	Exhibiting better targeting capacity of 4T1 cells compared to unmodified nanoparticles	139
RGD (Arg–Gly–Asp)	Realizing stronger tumor growth inhibition effects than typical tumor-targeting peptides	140

achieve a wide variety of therapeutic purposes. In addition to the direct anti-tumor effect of the coated nano-preparation, there are already cases in which the polymeric nanoparticles encapsulated by the erythrocyte membranes as a nano vaccine to achieve anti-tumor immunity. At the same time, as it comes from homologous erythrocytes, it can bypass the complex processes of protein identification, purification, and conjugation. Prior studies focused on the development and applications of RBCMs coated with spherical metal oxide particles or PLGA nanoparticles. In future, we may be able to overcome this limitation and extend this coating technology to other types and shapes of nanostructures. At the same time, various external stimuli such as near infrared-mediated photothermal effects, ultrasound[141] and magnetocaloric therapies have now been tried to enable the drug to be released on demand, which will be a new and broader area to study.

The effective transportation and long-circulating half-life of erythrocytes demonstrate their unique advantages in anti-tumor drug delivery and have also stimulated scientists to develop and utilize other source cell membranes for nano-coating, based on the characteristics of the corresponding diseases. Various membranes including platelet membranes, neutrophil membranes, tumor cell membranes, and bacterial membranes have been used in many fields, such as anti-tumor, detoxification, and immunomodulation, and have achieved considerably good experimental results. In the successful case of the fusion of erythrocyte membrane and platelet membrane coated nanoparticles, we can also select two or more cell membranes with the corresponding functionalities for fusion experiments according to the needs of the treatment of the diseases, but their characterizations and verifications can be relatively complicated and require precise and elegant designs. With the assistance of nanotechnology and molecular biology, it is believed that researchers can design better synthetic delivery vehicles for more efficient loading.

At present, the technology for preparing nanoparticles and erythrocyte membrane-derived vesicles is relatively mature, and they can be produced independently without interfering with each other and have relatively high flexibility. Therefore, it is completely possible to realize large-scale production. However, the biggest challenge is getting acceptable batch-to-batch variation, and the fusion process must be scalable and optimized to maximize efficiency. Quality control of RBCM-NPs is another major challenge that must be addressed. It must be ensured that erythrocyte membranes are not contaminated by pyrogens and viruses, and that RBCM-NPs with denatured proteins should be eliminated to prevent potential immune responses to endogenous

antigens[51,142]. Meanwhile, it should be emphasized that since the systems are composed of biological materials, there are complex regulatory issues while using them for clinical applications. Despite various challenges that may come up in the future, this biomimetic nanosystem that combines natural and synthetic biomaterials provides a unique and exciting strategy for targeting tumor, and it is also a new paradigm of thinking.

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