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Cell Membrane-Camouflaged Nanoparticles for Drug Delivery

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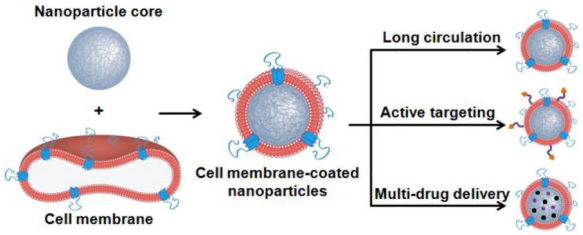
Abstract

Nanoparticles can preferentially accumulate at sites of action and hold great promise to improve the therapeutic index of many drugs. While conventional methods of nanocarrier-mediated drug delivery have focused on primarily synthetic approaches, engineering strategies that combine synthetic nanoparticles with natural biomaterials have recently gained much attention. In particular, cell membrane-camouflaged nanoparticles are a new class of biomimetic nanoparticles that combine the unique functionalities of cellular membranes and engineering versatility of synthetic nanomaterials for effective delivery of therapeutic agents. Herein, we report on the recent progress on cell membrane-coated nanoparticles for drug delivery. In particular, we highlight three areas: (i) prolonging systemic circulation via cell membrane coating, (ii) cell-specific targeting via cell membrane coating, and (iii) applications of cell membrane coating for drug delivery. The cell membrane-camouflaged nanoparticle platform has emerged as a novel delivery strategy with the potential to improve the therapeutic efficacy for the treatment of a variety of diseases.

Graphical Abstract



Cell membrane-coated nanoparticles present a novel emerging strategy for drug delivery. The cell membrane coating approach is multifaceted, offering long circulation times, targeting capabilities, and simultaneous multi-drug delivery.

Keywords

Nanomedicine; biomimetic nanoparticle; cell membrane; drug delivery; targeted delivery

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1. Introduction

Most drug molecules currently used in the clinic are non-targeted and tend to have poor bioavailability [1], resulting in quick excretion and non-specific toxicity along with other adverse side effects [2]. Free drugs tend to distribute evenly throughout the body following administration, thereby requiring large dosages to achieve sufficient concentration at desired sites of action. Due to these drawbacks of traditional therapeutic strategies, newer and more improved approaches are necessary to achieve improved therapeutic index of drug molecules.

Nanoparticle-based delivery systems offer several distinct advantages over free drug molecules [3–5]. Nanoparticles are able to preferentially accumulate at tumor sites by extravasation through the leaky vasculature of tumor sites via the well-known enhanced permeability and retention (EPR) effect [6–9]. Additionally, synthetic nanoparticles can be tailored to have desirable characteristics, such as prolonged circulation half-life, improved drug encapsulation, and sustained or triggered drug release [10, 11]. Nanoparticles can also be engineered to have specific physicochemical properties, including size, surface charge, hydrophobicity/hydrophilicity, and geometry, depending on their application [12–15]. These features allow for more effective delivery of therapeutic agents to desired sites of action. Preferential accumulation of nanoparticles at diseased sites can be further enhanced using active targeting strategies by incorporating targeting ligands, such as small molecules, peptides, antibodies, and aptamers, onto the nanoparticle surface [16–18]. In addition, many biocompatible and biodegradable materials, such as poly(D,L-lactic-*co*-glycolic acid) (PLGA), poly(lactic acid), (PLA), poly(glutamic acid) (PGA), poly(caprolactone) (PCL), N-(2-hydroxypropyl)-methacrylate (HPMA), and poly(amino acids), provide a safe and non-toxic means of delivery for *in vivo* administration [19, 20].

With strong efforts devoted to polymer synthesis and engineering methods, polymeric nanoparticles can now be manufactured reliably and fine-tuned for optimal properties at large scale [21–23]. Such improved manufacturing techniques aid in the bench-to-bedside translation of therapeutic nanocarriers, resulting in a growing number of nanoformulations in clinical trials. Among the most well-known nanotherapeutic candidates are CRLX101[24, 25], BIND-014 [22], CALAA-01 [26], and Genexol-PM [27, 28], all of which have demonstrated favorable pharmacological profiles as well as promising effects against a variety of cancers in clinical trials. Building on the success of such encouraging clinical results, researchers have continued to develop myriad new nanomaterials and nanostructures for drug delivery. In particular, new engineering strategies have emerged that combine synthetic nanoparticles with natural biomaterials to create nature-inspired biomimetic delivery systems [29–32]. These hybrid systems possess advantages from both fields—the tailorability and flexibility of synthetic materials, and the functionality and complexity of natural materials. Along these lines, the use of natural cellular membranes to coat synthetic polymeric nanoparticles for biofunctionalization has recently gained much interest [33–35]. Using this strategy, intact cellular membranes are collected in their entirety from natural cells and subsequently coated onto synthetic nanoparticle surfaces. The resulting cell membrane-coated nanoparticles possess the highly tunable physicochemical properties of

synthetic nanomaterials as well as the highly complex functions of the host cell membranes. In addition, the cellular membrane coating provides a bilayered medium ideal for transmembrane protein anchorage, allowing for the fabrication of highly functional biomimetic nanoparticles.

By exploiting the natural functionalities of source cell membranes, cell membrane-coated nanoparticles have huge potential in the delivery of therapeutic agents for a variety of diseases. Though a relatively recent development in the field of nanomedicine, these biomimetic nanoparticles have shown great promise in nanoparticle drug delivery [36–38]. In this article, we provide an overview of the recent advances of cell membrane-coated nanoparticles for drug delivery. We highlight three areas in particular: (i) cell membrane coating for the extension of systemic circulation, (ii) cell membrane coating for active targeted delivery, and (iii) applications of cell membrane coating for drug delivery. Taking advantage of the complex properties of natural cell membranes, membrane-camouflaged nanoparticles have emerged as a novel class of nanotherapeutics with the capability to improve drug delivery and treatment efficacy.

2. Extension of systemic circulation via cell membrane camouflage

One of the main goals of nanomedicine is to achieve long circulation of therapeutic nanocarriers. Long-circulating nanoparticles have significant clinical impact due to their potential for sustained systemic delivery and improved targeting via both passive and active mechanisms [4, 10]. The current gold standard to increase systemic circulation via “stealth” coating is to use polyethylene glycol (PEG). PEG improves circulation by stabilizing nanoparticles and protecting them from opsonization. PEG has been used with great success and has been incorporated into several clinical products [38]. However, recent observation of anti- PEG immunological responses has triggered researchers to explore other options to stealth coating [39]. Other synthetic zwitterionic materials, such as poly(carboxybetaine) and poly(sulfobetaine), have been proposed as alternatives to PEG due to their ability to form a hydration layer that prevents nonspecific protein adsorption [40, 41].

Recent advances in molecular and cellular biology have inspired scientists to move more towards using and mimicking natural materials. In particular, researchers have taken inspiration from red blood cells (RBCs), which are nature’s long-circulating delivery vehicles. Properties of RBCs such as their structure, surface proteins, and functionalities, have been taken as design cues to develop next-generation delivery platforms [42–45]. Though significant efforts have been made to bridge the gap between synthetic and natural biological materials, an RBC-mimicking delivery vehicle has remained elusive to biomedical researchers. The major challenge facing this goal is the difficulty in functionalizing nanoparticles with the complex surface chemistry inherent to a biological cell. Conventional chemical conjugation strategies would be impractical in achieving this goal due to the abundance, variety, and complexity of proteins associated with RBC membranes. These bottom-up approaches remain largely inadequate in duplicating the complex composition of natural cellular membranes on a nanoscale substrate.

The cell membrane coating technique provides a top-down method that addresses the above challenges by directly translocating RBC membrane in its entirety onto synthetic nanoparticles for long circulation. Preparation of these RBC membrane-camouflaged nanoparticles (RBC-NPs) is divided into two parts: membrane vesicle derivation from RBCs and vesicle-particle fusion (Fig. 1a). RBCs are isolated from whole blood and subjected to hypotonic treatment to remove their intracellular components. The emptied RBCs are then washed and extruded through porous membranes to created RBC membrane-derived vesicles. To synthesize RBC-NPs, the RBC vesicles are then fused with preformed poly(lactic-co-glycolic acid) (PLGA) nanoparticles through mechanical extrusion. The resulting RBC-NPs exhibit a core-shell structure, with the RBC membrane forming a single bilayer around the polymeric core (Fig. 1b).

From a nanoengineering perspective, this approach to stealth functionalization provides unprecedented control in enabling biomimetic functionalities on nanoscale substrates. By translocating cellular membranes in their entirety to nanoparticles, the complex biochemistry of cell surfaces can be faithfully translocated as well [33]. Careful study of the surface chemistry of RBC-NPs demonstrated that the nanoparticles possess the same density of CD47 as its RBC source [46]. More importantly, the proteins were shown to be oriented almost exclusively in the right-side-out fashion, with the extracellular portion displayed on the RBC-NP surface. This right-side-out orientation was attributed in part to the electrostatic repulsion between the negatively charged PLGA core and the negatively charged sialyl moieties on the extracellular side of the source RBC membranes [47]. The exoplasmic glycans present on the RBC membrane also serve to orient the membrane correctly on the RBC-NPs and provide a stabilizing effect; unlike bare PLGA nanoparticles, RBC-NPs remained stable in phosphate buffered solution and serum. In addition, the correctly oriented membrane coating was able to significantly impede macrophage uptake of the RBC-NPs *in vitro* [46].

Perhaps the most important property of RBC membrane coating is that the technique bestows impressive stealth properties onto nanoscale substrates. The RBC-NP possessed a significantly longer elimination half-life compared to an analogous PEGylated formulation, demonstrating superior suppression of *in vivo* clearance conferred by RBC membranes in mice (Fig. 1c) [33]. Based on a two-compartment model, the elimination half-life of RBC-NPs was calculated to be 39.6 h, compared with 15.8 h for PEG-coated nanoparticles. Overall, when coated onto nanoscale substrates, the RBC membrane coating confers immune-evasive properties, allowing for long circulation properties vital for drug delivery. The RBC membrane coating technique has been extended to materials beyond polymers as well, including gold [48, 49] and gelatin [50]. These findings highlight the strength of membrane-cloaked nanoparticles, whose self-camouflage presents a comprehensive evasion strategy against the multifaceted nature of immune clearance.

3. Cell-specific targeting via cell membrane coating

Cell-specific targeting is a desirable feature for nanocarriers in disease treatments, as it holds promise in reducing off-target side effects. A variety of chemical conjugation techniques have been employed to functionalize nanoparticles with targeting ligands that bind to

overexpressed antigens at diseased sites, including carboxyl-, amine-, and sulfhydryl-based chemistry [16, 51, 52]. Actively targeted nanoparticles have demonstrated preferential accumulation at specific disease sites and have shown encouraging results in clinical studies. In functionalizing membrane-coated nanoparticles, however, different functionalization strategies must be used in order to preserve the integrity of the carbohydrates and proteins located on the cell membranes, as the biomimetic capabilities of the membrane-cloaked nanoparticle depend on the presence of fully functional membrane moieties.

As a non-disruptive functionalization strategy to incorporate targeting ligands to membrane-coated nanoparticles, a lipid insertion approach was recently developed [53]. In this method, targeting moieties were first tethered to lipid molecules and then inserted into RBC membranes (Fig. 2a). The intrinsic fluidity and dynamic conformation of the membrane bilayers allow for the lipid tether to physically insert into the membrane coating on the nanoparticles. Furthermore, targeting ligands of different molecular weights can be functionalized onto membrane-coated nanoparticles. For example, small molecules such as folate (M_W = 441 Da) (Fig. 2b) and macromolecules such as the nucleolin-targeting aptamer AS1411 (M_W = 9000 Da) (Fig. 2c) were inserted into RBC membranes without damaging the existing RBC surface proteins. Via lipid insertion, targeting ligands can be integrated into the cell membrane-coated nanoparticle platform in a simple yet robust way. This strategy also allows for control over ligand density, offering a means to improve the applicability and effectiveness of developing biomimetic nanocarriers with complex surface chemistry.

In addition to the lipid insertion method to introduce targeting capabilities to cell membrane-coated nanoparticles, the cell membrane cloak itself can be utilized for targeting. By leveraging the natural homotypic or heterotypic adhesion properties of source cells, targeted membrane-cloaked nanoparticles can be fabricated without additional synthesis steps. Intrinsic cell adhesion properties play a significant role in biology, and, in particular, cancer. For example, many cancer cells exhibit homotypic targeting via surface antigens with homophilic adhesion domains such as carcinoembryonic antigen and galectin-3 [54]. Such adhesion properties are critical in the steps leading to metastasis and tumor formation [55].

To exploit the natural ability of cancer cells to interact with each other, cancer cell membrane was collected and then coated onto PLGA nanoparticles (Fig. 3a) [56]. Membrane was collected from MDA-MB-435 cells, a human estrogen-independent breast carcinoma model cell line, via a combination of hypotonic treatment, mechanical membrane disruption, and differential centrifugation. During the membrane purification process, nuclear components were removed, thereby alleviating concerns with the administration of genetic material. Cancer cell membrane-derived vesicles were formed from the collected membrane and fused onto PLGA cores to form cancer cell membrane-coated nanoparticles (CCNPs) using physical extrusion. Fluorescence imaging showed that CCNPs had significantly higher binding and uptake by live MDA-MB-435 cells compared with bare PLGA cores and RBC-NPs (Fig. 3b). Indeed, quantification using flow cytometry revealed that CCNPs had 40-fold and 20-fold increase in uptake compared with bare PLGA and RBC-NPs, respectively (Fig. 3c). This association was cell-specific, as the CCNPs did not

show increased binding to a heterotypic human foreskin fibroblast cell line compared with bare PLGA cores.

The cell membrane coating approach is a versatile one, in which a variety of targeting ligands and cell membranes can be utilized to achieve cell-specific binding and uptake. Targeting ligands with a lipid tether can be easily inserted into RBC membranes to provide a targeted long-circulating nanoparticle formulation. Alternatively, coating nanoparticles with cell membrane can take advantage of the natural homing ability that many cells possess as an intrinsic biological function. Regardless of the method used, cell membrane-camouflaged nanoparticles show great potential for targeted delivery of therapeutic agents to reduce adverse off-target effects.

4. Applications of cell membrane coating for drug delivery

In bridging synthetic and natural materials, scientists are able to take advantage of the best of both worlds. The natural cellular membrane component of membrane-cloaked nanoparticles provides functionalities that would otherwise be very difficult or even impossible to achieve via traditional chemical means, while the synthetic core can be finely tuned to have specific properties and to load certain therapeutic agents. Polymeric nanoparticle cores, for example, can be formulated by conjugating multiple functional units to soluble macromolecules or by co-polymer self-assembly. In a typical self-assembled formulation, the nanoparticle core can be loaded with a variety of therapeutic or imaging agents, where surface or bulk erosion, diffusion, swelling followed by diffusion, or local environmental stimulation, results in the sustained and controlled release of the encapsulated cargo [4]. In addition, direct conjugation of drug molecules to the polymer backbone allows for precise drug loading and added control over drug release kinetics [57]. To further tailor the membrane-coated nanoparticle platform to specific needs, researchers have developed a large arsenal of biocompatibility and biodegradable materials, each with their own strengths [58–61].

As part of continuing efforts to further develop the biomimetic cell membrane-cloaked nanoparticle platform for advanced drug delivery applications, small molecule chemotherapy drugs such as doxorubicin (DOX) have been loaded into the polymeric core of RBC-NPs [62]. Two strategies were utilized to load DOX into the core: physical encapsulation and chemical conjugation. Physical encapsulation of DOX led to lower drug loading yield but quicker drug release when compared to chemical conjugation of DOX to the polymer backbone. The more sustained release profile from the drug-polymer conjugated nanoparticle formulation suggests that chemical linkers responsive to environmental triggers can achieve better-controlled drug release when developing RBC-NPs for drug delivery [63, 64]. In both cases, the RBC membrane coating acted as a diffusional barrier, serving to slow the release of DOX from the polymeric core. By comparing DOX release from RBC-NPs and PEGylated NPs, it was found that the RBC-NPs loaded with DOX demonstrated more sustained release; approximately 20% of DOX was released within the first 72 h from RBC-NPs compared with 40% from PEGylated NPs, when DOX was covalently conjugated to the polymeric cores. When DOX was physically encapsulated into the particles, approximately 85% was released from RBC-NPs in 72 h

compared with 100% released from PEGylated NPs. This observation is in accordance with previous studies demonstrating that phospholipid coatings can act as barriers to drug diffusion [65]. The membrane coating reduced the drug diffusivity by 1.2 times based on calculations using a diffusion-dominant Higuchi model. This ability of the membrane cloak to slow drug release suggests that strategies aimed at engineering lipid membrane coatings may allow for responsive drug release from RBC-NPs under certain environmental cues. The drug-loaded RBC-NPs also demonstrated impressive therapeutic potential *in vitro*. Whether physical loading or chemical conjugation was used, the RBC-NPs loaded with DOX exhibited higher toxicity against Kasumi-1 acute myeloid leukemia cells in comparison to free DOX over a 72-h incubation period. The RBC membrane coating provides desirable characteristics for drug delivery applications.

The nanoparticle core can be tailored to encapsulate any number of drugs, and can even be used to encapsulate multiple drugs simultaneously for enhanced therapeutic efficacy. Combined therapy with two or more drugs provides a promising strategy to suppress cancer drug resistance, as different drugs may have effect on cells at varying stages of their growth cycles [66]. Different combinations of drugs can also function synergistically to prevent disease recurrence [67–69]. The major challenge to current combinatorial therapy is the issue of unifying the pharmacokinetics and cellular uptake of different drug molecules, which would allow for precise control of the dosage and scheduling of multiple drugs. The nanoparticle platform addresses this issue by co-delivering multiple drugs simultaneously. In this way, multiple drugs are localized to the same site of action to maximize their combinatorial effects.

Along these lines, paclitaxel (PTXL) and gemcitabine (GEM) were covalently conjugated through hydrolysable linkers and loaded into nanoparticles in a precisely controllable manner [70]. The synthesized PTXL-GEM drug conjugates were readily encapsulated into lipid-coated polymeric nanoparticles, and the resulting drug-loaded nanoparticles demonstrated improved cytotoxicity compared to the free drug conjugates. In a similar vein, camptothecin (CPT) and DOX were dually loaded into a single nanoparticle carrier in a precisely controllable manner (Fig. 4a) [57]. Each drug was conjugated directly to a poly-L-lactide backbone, and the conjugates were used to prepare nanoparticles at highly precise ratios with over 90% efficiency. The resulting nanoparticles were uniform in size, size distribution, and surface charge, and demonstrated superior cellular cytotoxicity against cancer cells compared with corresponding cocktail mixtures of single-drug loaded nanoparticles (Fig. 4b). This dual drug delivery approach offers a solution to the long-standing challenge in ratiometric control over multiple drugs loaded within the same drug delivery vehicle. Combined with the biofunctionality of a membrane coating, multiple drug-loaded nanoparticles present a formidable approach to disease treatment.

5. Conclusions and outlook

Using a novel top-down approach, natural cellular membranes can be faithfully translocated in their entirety, including both lipids and membrane-associated proteins and carbohydrates, onto nanoparticle substrates. Bringing together the benefits of both synthetic and natural platforms, this new class of biomimetic nanoparticles has significant therapeutic potential.

The membrane coating retains the original biological properties of the source cell membrane, serving to prolong systemic circulation. Long *in vivo* circulation is essential to drug delivery, as it provides nanocarriers with greater opportunity to accumulate at sites of action. Cell-specific targeting can be achieved via lipid insertion method, or by utilizing specific membrane derived from selected cells. In both cases, active targeting functionality is accomplished without the need for chemical reactions that may disrupt the protein makeup on cell membrane-coated nanoparticles. The synthetic aspect of the platform provides a biocompatible vessel in which one or more types of therapeutic agents can be concurrently carried. The prospect of combinatorial drug delivery using cell membrane-coated nanoparticles holds great promise for the treatment of many diseases.

Since its conception, the membrane coating technique has been rapidly applied to a variety of nanostructures including PLGA [34, 35, 45, 71, 72], gelatin [50], gold [48, 49, 73], and silica [74]. The application of the cell membrane coating method to a variety of materials demonstrates the engineering flexibility of the platform. Individual formulations can be custom made and tailored to address the particular needs for a certain treatment. The nanoparticle core can be modified to implement precise characteristics to aid in efficient and effective drug delivery. Further, the cell membrane coating can be selected for each specific treatment protocol. For example, RBC membrane can be used to extend *in vivo* circulation time or other cell membranes can be utilized as a targeting strategy for a variety of diseases, including solid tumor cancers (*e.g.* breast cancer, prostate cancer, colorectal cancer, lymphomas, etc.), circulating and metastatic cancers (*e.g.* leukemias), and potentially even systemic bacterial infections. In addition to drug delivery, cell membrane coating can be applied towards other areas as well, such as detoxification of pathogenic virulence factors [34] and anti-virulence vaccination [35] to treat deadly bacterial infections such as those by *Staphylococcus aureus*, *Streptococcus pyogenes*, Enterobacteriaceae, and others.

Cell membrane camouflaged nanoparticles have emerged as a novel and versatile strategy to integrate the advantages of both synthetic and biological systems. The resulting nanoparticles formed using the membrane coating technique possess the unique functionalities and complex surface chemistry of the original cell membranes, features which would otherwise be impossible to achieve via traditional chemical conjugation methods. With such desirable properties, cell membrane-coated nanoparticles hold great potential for effective disease intervention. Towards the future, the cell membrane-cloaked nanoparticle platform will continue to inspire the development of new nanotherapeutics that bridge the gap between manmade and natural biological materials.

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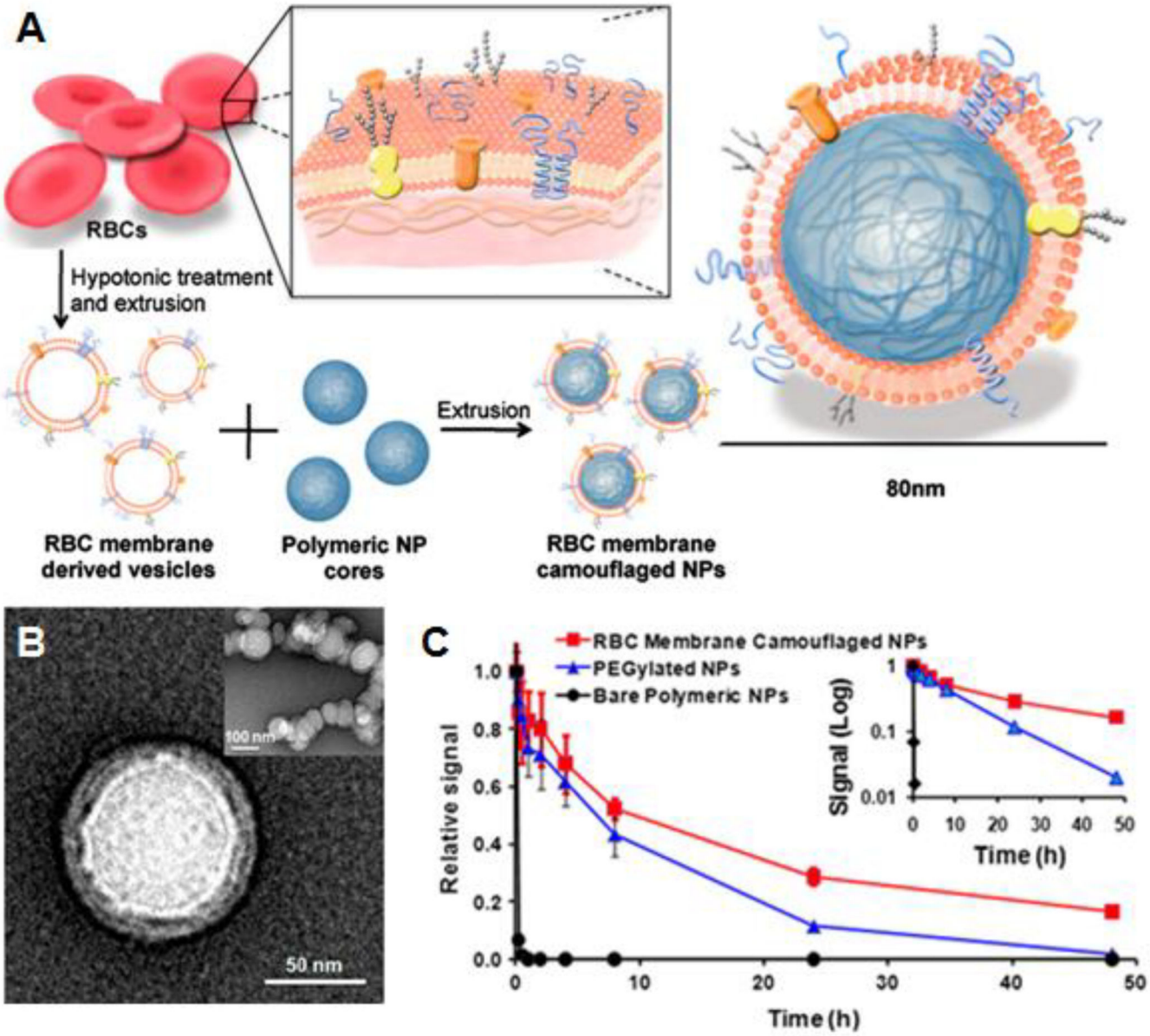

Fig. 1.

(A) Schematic of the preparation process of RBC membrane-camouflaged nanoparticles (RBC-NPs). RBC membrane-derived vesicles are extruded together with polymeric nanoparticle cores to form the final RBC-NPs. (B) TEM image of RBC-NPs that have been negatively stained with uranyl acetate. The clear core-shell structure of the RBC-NPs can be seen. (C) *In vivo* circulation time of RBC-NPs in mice. Fluorescently labeled RBC-NPs were injected intravenously through the tail vein of mice. At various time points blood was drawn intraorbitally and measured for fluorescence to evaluate the systemic circulation lifetime of the nanoparticles (n = 6 per group). Reproduced with permission from Ref. [33].

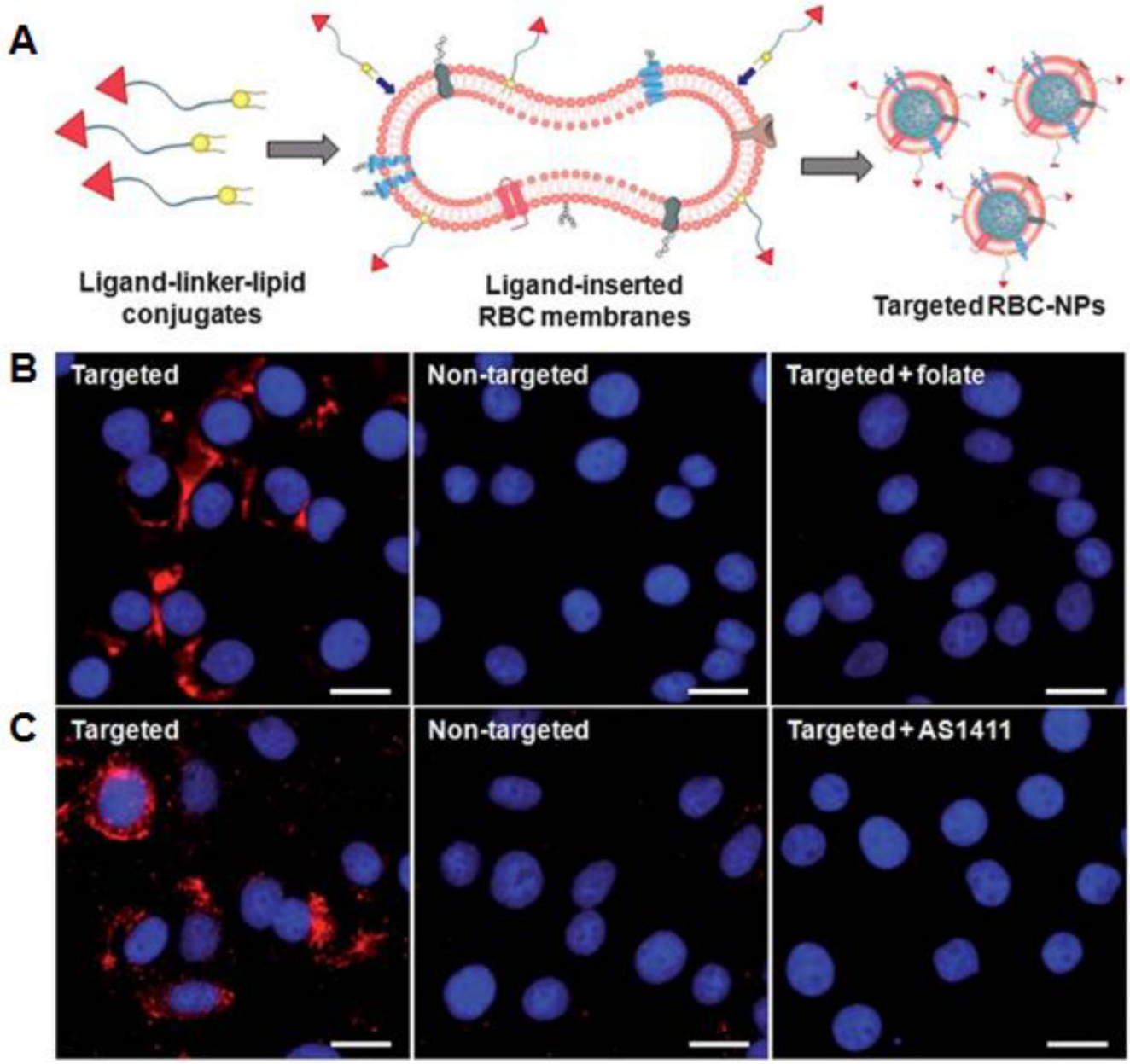

Fig. 2.

(A) Schematic of the preparation of RBC-NPs with targeting ability. Lipid-tethered ligands are synthesized and then inserted into RBC membrane ghosts, resulting in ligand-functionalized RBC membranes which are ultimately used to coat polymeric cores to form targeted RBC-NPs. (B) Fluorescence microscopy imaging of KB cells incubated with folate-functionalized RBC-NPs, non-targeted RBC-NPs, and folate-functionalized RBC-NPs together with free folate. The targeted RBC-NPs demonstrated significantly higher uptake by cells overexpressing the folate receptor. Scale bars = 25 μm. (C) Fluorescence microscopy imaging of MCF-7 cells incubated with AS1411-functionalized RBC-NPs, non-targeted RBC-NPs, and AS1411-functionalized RBC-NPs together with free AS1411 aptamer. The AS1411- functionalized RBC-NPs demonstrated significantly higher uptake in

cells overexpressing nucleolin. Scale bars = 25 μm. Reproduced with permission from Ref. [53].

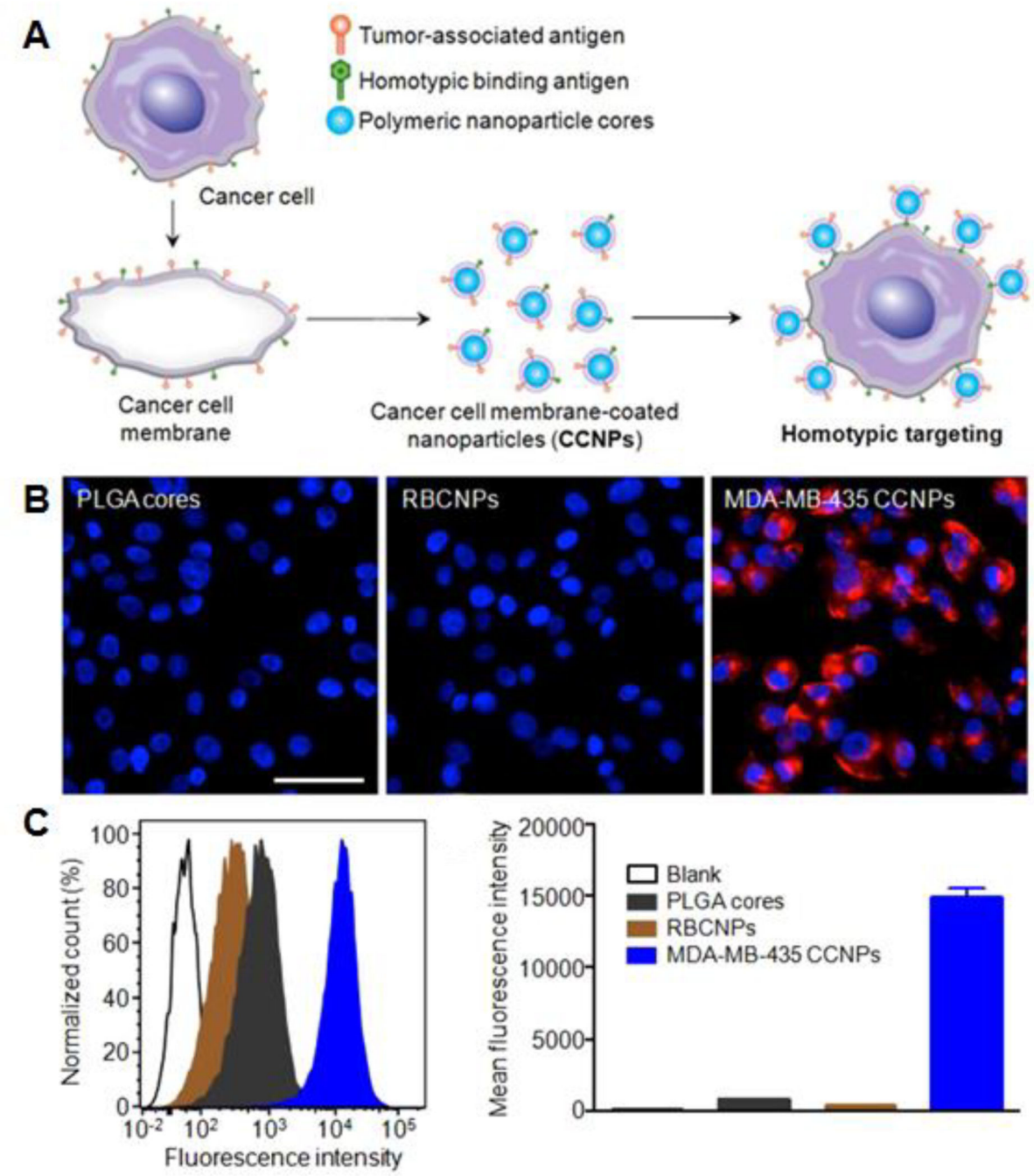

Fig. 3.

(A) Schematic of the preparation of cancer cell membrane-coated nanoparticles (CCNPs) for homotypic targeting. (B) Fluorescent imaging of MDA-MB-435 cells incubated with PLGA cores, RBC-NPs, or CCNPs coated with membrane derived from MDA-MB-435 cells. CCNPs demonstrated significant homotypic targeting ability. Scale bar = 50 μm. (C) Flow cytometric histogram (left) and quantification (right) of MDA-MB-435 cells incubated with blank solution, PLGA cores, RBC-NPs, or CCNPs. Reproduced with permission from Ref. [56].

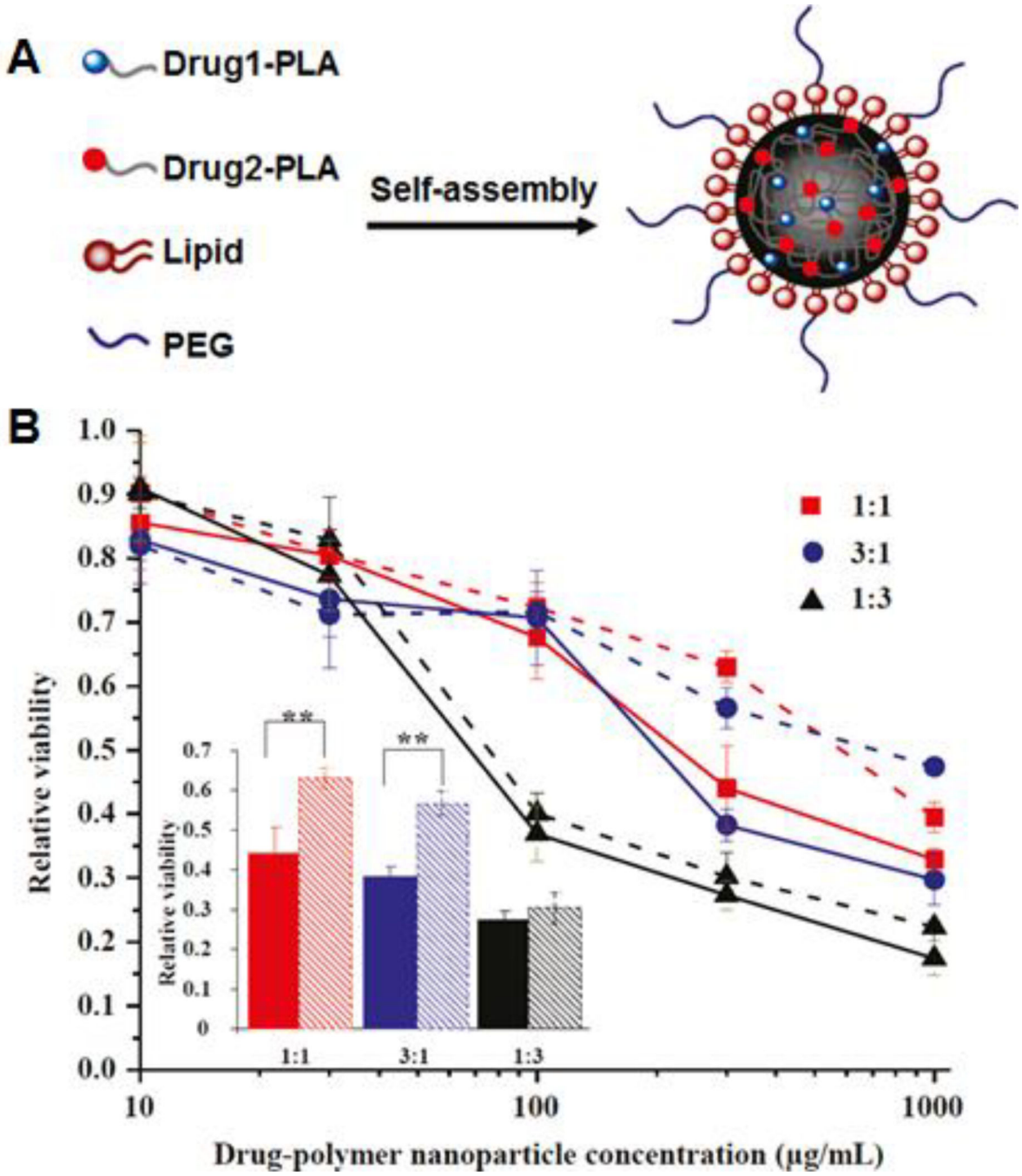

Fig. 4.

(A) Schematic of the preparation of dual-drug loaded lipid-polymer hybrid nanoparticles, of which the polymeric core consists of two distinct drug-polymer conjugates with ratiometric control over drug loading. (B) Comparative cytotoxicity study of DOX-PLA and CPT-PLA loaded dual-drug nanoparticles at different DOX-PLA:CPT-PLA ratios against MDA-MB-435 breast cancer cells. Solid lines represent the dually loaded nanoparticles, and dashed lines represent the cocktail mixture of DOX-PLA and CPT-PLA single drug-loaded nanoparticles. The inset highlights the cytotoxicity comparison of dual-drug loaded

nanoparticles and cocktail mixtures of single-drug loaded nanoparticles at 300 μg/mL of nanoparticle concentration. Reproduced with permission from Ref. [57].