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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.

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 a 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

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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]. A 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,

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