Acta Biomaterialia 16 (2015) 94–102

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Long circulating micelles of an amphiphilic random copolymer bearing cell outer membrane phosphorylcholine zwitterions

Jing Zhao [a,b,1], Yu-Dong Chai [a,1], Jing Zhang [a,1], Peng-Fei Huang [a,1], Kenichi Nakashima [c,2], Yong-Kuan Gong [a,*]

[a] Key Laboratory of Synthetic and Natural Functional Molecule Chemistry of Ministry of Education, College of Chemistry and Materials Science, Northwest University, Xi'an 710127, Shaanxi, PR China
[b] School of Environmental and Chemical Engineering, Xi'an Polytechnic University, Xi'an 710048, Shaanxi, PR China
[c] Department of Chemistry, Faculty of Science and Engineering, Saga University, 1 Honjo-machi, Saga 840-8502, Japan

ARTICLE INFO

Article history:
Received 16 August 2014
Received in revised form 22 December 2014
Accepted 16 January 2015
Available online 27 January 2015

Keywords:
Polymeric micelles
Cell membrane mimetic
Phosphorylcholine
Long-circulation
Biodistribution

ABSTRACT

Polymeric micelles with cell outer membrane mimetic structure were prepared in water from amphiphilic random copolymers bearing both the hydrophilic phosphorylcholine zwitterions and hydrophobic octadecyl side chains of cell outer membrane. The polymeric micelles showed sizes ranging from 80 nm to 120 nm in hydrodynamic diameter and zeta-potentials from −6.4 mV to −2.4 mV by dynamic light scattering measurements. The micelles loaded with 6-coumarin as a fluorescence probe were stable to investigate their blood circulation and biodistribution. The *in vitro* phagocytosis results using murine peritoneal macrophages showed 10-fold reduction compared with a reference micelle. The *in vivo* blood circulation half-life of the polymeric micelles following intravenous administration in New Zealand Rabbits was increased from 0.55 h to 90.5 h. More interestingly, tissue distribution results showed that the concentration of the micelles in the kidney is 4-fold higher than that in the liver and other organs 48 h after administration. The results of this work show great promise for designing more effective stealth drug carriers that can minimize reticuloendothelial system clearance and circulate for long time to reach target by using simple cell membrane mimetic random copolymer micelles.

1. Introduction

Nanoparticles (NPs), including liposomes, polymeric micelles, polymer-drug conjugates, dendrimers and polymeric nanoparticles, have long been sought as promising drug delivery system for most cancer therapy. However, conventional drug-loaded nanoparticles can be recognized and entrapped by the monocyte phagocytic system (MPS), and then quickly eliminated following intravenous administration, resulting in short circulation lifetime and intolerable toxicity [1,2]. To increase circulation time and opportunity of nanoparticles reaching the target site in the body, the nanoparticle surface is commonly modified by polyethylene glycol (PEG) and other hydrophilic polymers to reduce plasma opsonization and MPS uptake [3–5]. PEGylation can be conducted by grafting, entrapping and adsorbing PEG chains onto the surface of a nanoparticle [5–8]. By providing a hydration layer and steric barrier surrounding the polymeric core, PEG coating can reduce non-specific binding of serum proteins to the particles, thereby reducing their clearance by MPS cells [9]. The stealth properties can be achieved and optimized by grafting a high density of PEG with MW ranging from 2k to 10k [3,10–13]. Although PEGylation has been extensively studied on a variety of nanoparticle systems to increase surface hydrophilicity and improve circulation half-life by decreasing interactions with blood proteins and MPS cells [14–17], detailed investigations also recognize its disadvantages [18–22]. The PEG coating not only interferes with nanoparticle–cell interactions and the endosomal escape of NPs after extravasation [23,26], but also induces rapid clearance of the PEGylated NPs from blood upon repeated injections [24–28]. This immune response to PEGylated NPs can be a significant issue for nano-medicines, adversely affecting their pharmacokinetics and biodistribution profiles.

Recently, red blood cell membrane-coated nanoparticles (RBC-NPs) were developed by coating poly (lactic-co-glycolic acid) (PLGA) nanoparticles (PLGANPs) with natural membranes derived from RBCs [28]. The RBC-NPs showed a superior long circulation time (~10 h) in mice compared to PLGA-PEG nanoparticles.

* Corresponding author. Tel.: +86 29 81535032; fax: +86 29 81535026.
E-mail addresses: nakashik@cc.saga-u.ac.jp (K. Nakashima), gongyk@nwu.edu.cn (Y.-K. Gong).
[1] Tel.: +86 29 81535032; fax: +86 29 81535026.
[2] Tel.: +81 952 28 8850.

http://dx.doi.org/10.1016/j.actbio.2015.01.019
1742-7061/

Anti-phagocytic properties demonstrated with RBC membrane-coated gold nanoparticles and white blood cell membrane-coated silica particles [29,30], suggested the broad applicability of the strategy. The cell membrane coating technique adopts the same immune-evasive strategy adopted by many viruses in nature, presents a unique biomimetic approach in nanoparticle stealth functionalization [31,32].

On the other hand, the cell membrane is a complicated biological system consisting of a phospholipid bilayer, different kinds of proteins and glycans. Different components of the cell membrane may contribute differently to the anti-phagocytic properties and long circulation time in the bloodstream. Inspired by the ideal biocompatibility of different cells in the body, the basic structure of phospholipid bilayer in a cell membrane should be greatly important for evading immune clearance. Although most liposomes of phospholipid are instable and have short lifetime *in vivo*, their survival time in body can be significantly prolonged to several hours by incorporating the components of red blood cell membranes such as cholesterol, glycolipids and glycoproteins [33–37]. Unfortunately, the *in vivo* circulation time of either the modified liposomes or the cellular membranes in their entirety coated nanoparticles is still far from that of red blood cells. In order to understand well and manipulate efficiently biomimetic surface functionalization on nanoparticles, the effects of cell membrane bilayer structure on evading immune clearance need to be investigated intensively.

Cell membrane mimetic polymers containing both the hydrophobic alkyl chains and hydrophilic phosphorylcholine (PC) zwitterions can form coating on a substrate. The hydrophilic PC groups of the polymer can reorient to the interface in aqueous environment forming a cell outer membrane mimetic structure [38–40]. The closely packed zwitterionic PC groups bind hydration water strongly through electrostatic interactions [41] and suppress non-specific protein adsorption and foreign body reactions [42–45]. This kind of amphiphilic cell membrane mimetic polymers has the ability to form micelles or aggregates bearing cell outer membrane mimetic structure in aqueous solutions [40].

We have synthesized a series of amphiphilic random-copolymers comprised of 2-methacryloyloxyethyl phosphorylcholine (MPC), stearyl methacrylate (SMA) and trimethoxysilylpropyl methacrylate (TSMA), and prepared stable micelles with cell membrane mimetic random-copolymers [46]. Here we report for the first time the long circulation lifetime and biodistribution results of the random-copolymer micelles bearing cell membrane mimetic phosphorylcholine interface. The results suggest that the cell membrane mimetic random copolymer micelles loaded with 6-coumarin may serve as a model nanoparticle to design and optimize for long-circulating and effective targeting drug nanocarriers.

2. Materials and methods

2.1. Materials and reagents

2-Methacryloyloxyethyl phosphorylcholine (MPC) was synthesized according to the method reported by Ishihara et al. [47]. Stearyl methacrylate (SMA), methacryloxyethyltrimethyl ammonium chloride (DMC), 3-(trimethoxysilyl)propyl methacrylate (TSMA) were purchased from Sigma–Aldrich Co. 2,2′-Azoisobutyronitrile (AIBN) was recrystallized from methanol and dried in vacuum. 6-coumarin of laser grade was purchased from J & K Reagent Company. L929 cells were obtained from the fourth military medical university. Bal b/c mice of 6–8 week-old, and New Zealand Rabbit of 2–2.5 kg body weight were obtained from Animal Center of Xi'an Jiaotong University. Fetal bovine serum (FBS) was purchased from Beijing Thermo Fisher Scientific Company. Deionized water was purified by a Millipore system (Direct-Q3UV, France).

Cell membrane mimetic random copolymers PMS and crosslinkable terpolymers PMST were synthesized according to previous report [46]. Briefly, MPC as the hydrophilic monomer, SMA as the hydrophobic monomer, and TSMA as the crosslinkable monomer were copolymerized by free radical polymerization through "monomer-starved" approach [39,48]. DMC and SMA were copolymerized to prepare cationic amphiphilic copolymer (PDS) as reference sample. The molar ratio of monomer units in the copolymers PMS, PDS and PMST was determined by ^{1}H NMR spectroscopy with an Inova 400 Hz NMR spectrometer (Varian, America). The polymer structural formulas are shown in Fig. 1. According to the ratio of monomer units in the copolymers, the polymers were denoted as PDS55, PMS37, PMS55, PMS73 and PMST553, respectively.

2.2. Preparation of 6-coumarin loaded polymeric micelles

Polymeric micelles with cell membrane mimetic structure loaded with 6-coumarin as fluorescent probe were prepared by the solvent evaporation method. A schematic diagram of the amphiphilic polymer micelle formation by self-assembly is shown in Fig. 2. The preparation procedure of 6-coumarin-loaded micelles was as follows. 45 mg of the amphiphilic copolymer was dissolved in 3 mL chloroform. Then, 100 μL 6-coumarin solution (0.45 mg/mL in chloroform) was added to the above polymer solution and the mass ratio of 6-coumarin to the polymer was 1:1000. The mixed solution was dropped into 45 mL pure water in a 100 mL flask under vigorous stirring and continued the agitation for 24 h in the dark. After filtration with a 0.45 μm filter, the micelle solution was centrifuged at 4000 rpm for 30 min using an ultrafiltration centrifuge tube with a molecular weight cutoff of 3000 Da. The ultrafiltration process was repeated until the fluorescent intensity of the filtrate reduced to 0.3% of the micelle solution.

2.3. Characterization of 6-coumarin loaded polymeric micelles

Particle size and polydispersity index of the polymeric micelle solutions were determined at 25 °C under an angle of 173° by Photon Correlation Spectroscopy (PCS) using a Malvern Zetasizer Nano ZS instrument. Morphology of polymeric micelles was observed with a scanning electron microscope (Quanta 200, Dutch philips-FEI Company). The SEM sample was prepared by coating 0.1 mg/mL micelle solution on a glass substrate and freeze dried at −20 °C. Before measurement, the sample was sprayed with a thin layer of gold by sputter coating.

The stability tests of the micelle suspensions in physiological medium were performed by PCS and fluorescence spectrophotometry (HITACHI F-4500, Japan). Polymeric micelle suspensions were placed for 12 h, 24 h, 48 h and 72 h at room temperature, and then the change of particle size and fluorescence intensity were measured.

The release of the loaded 6-coumarin probe from micelles was examined by ultrafiltration through a membrane with a molecular weight cutoff of 3000 Da. The equilibrated concentration of 6-coumarin in the filtrate was determined by fluorescence spectrophotometry.

Cytotoxicity of the polymeric micelles was determined by MTT assay to investigate biocompatibility using mouse connective tissue fibroblast cells (L929). L929 was seeded in 96-well culture plates at 1.0×10^4 cells per milliliter in DMEM medium containing 10% FBS followed by incubation in 5% CO_2 incubator at 37 °C for 24 h to allow for cell adherence. Culture medium was replaced with 100 μL of micelles/culture medium suspensions with different concentrations of 0.05, 0.2 and 0.8 mg/mL and incubated at 37 °C. The L929 cells without the micelles were used as a control for comparison. After 24 h incubation, 20 μL of MTT solution