Colloids and Surfaces B: Biointerfaces 115 (2014) 253–259

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Folate-conjugated amphiphilic block copolymers for targeted and efficient delivery of doxorubicin

Yin Lv, Bin Yang, Tao Jiang, You-Mei Li, Feng He*, Ren-Xi Zhuo

Key Laboratory of Biomedical Polymers of Ministry of Education, Department of Chemistry, Wuhan University, Wuhan 430072, China

ARTICLE INFO

Article history:
Received 25 September 2013
Received in revised form
22 November 2013
Accepted 29 November 2013
Available online 6 December 2013

Keywords:
Folate-conjugated
Polycarbonates
Micelle
Targeted delivery
Enzymatic polymerization

ABSTRACT

In this paper, novel biodegradable amphiphilic block copolymers based on folate-conjugated poly(ethylene glycol)-*b*-copolycarbonates (FA-PEG-*b*-P(MAC-*co*-DTC)) and methoxy poly(ethylene glycol)-*b*-copolycarbonates (mPEG-*b*-P(MAC-*co*-DTC)) were successfully synthesized for targeted and efficient delivery of doxorubicin (DOX) to cancer cells. Immobilized *porcine pancreas* lipase (IPPL) was employed as the catalyst to perform the ring-opening copolymerization in bulk, while the folate-conjugated poly(ethylene glycol) (FA-PEG) or methoxy poly(ethylene glycol) (mPEG) was used as the initiator. The resulting copolymers, characterized by ^{1}H NMR and GPC, could self-assemble to form nano-sized micelles in aqueous solution by dialysis method. P(MAC-*co*-DTC) acted as the hydrophobic core, thereby aggregating hydrophilic PEG chains as the outer shell with FA as targeting ligand located at the surface of the polymeric micelles. Transmission electron microscopy (TEM) observation showed that the micelles dispersed in spherical shape with nano-size before and after DOX loading. Both the FA-conjugated and non-conjugated block copolymers showed low cellular cytotoxicity. Furthermore, as compared to the non-conjugated copolymers, much more efficient cellular uptake of the FA-conjugated copolymers *via* FA-receptor-mediated endocytosis could be observed by confocal laser scanning microscopy (CLSM), while MTT assays also demonstrated highly potent cytotoxic activity against HeLa cells.

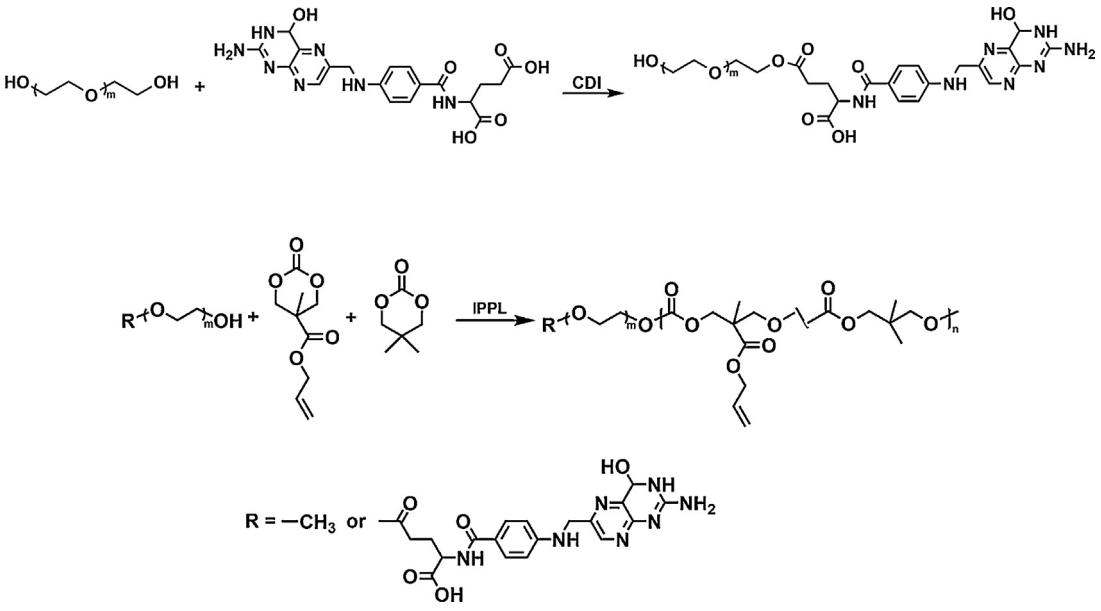
1. Introduction

Drug delivery system (DDS), which is driven by the need to maximize therapeutic activity while minimizing negative side effects, is the key to the clinical success of many potent drugs [1,2]. As one of the most important kind of DDS, polymeric micelles have received significant attention over the past decades, which are formed by self-assembling of amphiphilic block copolymers in aqueous solution with a core and shell structure [3,4]. The hydrophobic inner core could encapsulate the poorly water-soluble drug, whereas the hydrophilic outer shell or corona of amphiphilic block copolymers could protect the drug from the aqueous environment and stabilize the polymeric micelles against recognition *in vivo* by the reticuloendothelial system (RES) [5]. PEG is the most popular choice of hydrophilic segment [6], while biodegradable aliphatic polycarbonates have been used as the hydrophobic core of polymeric micelles due to their low toxicity, favorable mechanical properties and biodegradability [7–9]. Aliphatic polycarbonates are generally synthesized by ring-opening polymerization (ROP) of cyclic carbonates [8]. In our previous studies, immobilized *porcine pancreas* lipase on silica particles (IPPL) has proven to be a powerful catalyst for the ROP of cyclic carbonates such as trimethylene carbonate (TMC), dimethyltrimethylene carbonate (DTC), 5-methyl-5-allyloxycarbonyl-1,3-dioxan-2-one (MAC) and also their copolymers with other kinds of cyclic monomers [10–13]. Enzymes have remarkable properties such as natural kinds of protein without toxicity, high catalytic and high selectivity under mild reaction conditions, while immobilized enzyme (such as IPPL) present promising stability and recyclability which will be good for its applications in the polymer synthesis systems [11–13]. Furthermore, it is very interesting that the control synthesis of aliphatic polycarbonates with functional pendent groups by enzymatic methods would offer a wide range of opportunities for further modification and functionalization.

On the other hand, the drug in the blood circulation usual leads to drug degradation and loss upon administration, and subsequently decreased drug bioavailability and lower drug accumulation in the pathological zone [14]. To address this challenge, various drug targeting systems have been developed in the past decades. One of the well-known receptor mediated targeting moieties is folic acid (FA), which has been widely exploited for the tumor-targeting DDS because FA-receptors are frequently overexpressed on the surface of human cancer cells [15,16]. Polymers

* Corresponding author at: Wuhan University, Luo-jia-shan, Wuchang, Wuhan, China. Tel.: +86 27 6875 4061; fax: +86 27 6875 4509.
E-mail address: fenghe2002@hotmail.com (F. He).

0927-7765/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.colsurfb.2013.11.049



Scheme 1. Synthesis of folate-conjugated poly(ethylene glycol) (FA-PEG), folate-conjugated poly(ethylene glycol)-*b*-copolycarbonates (FA-PEG-*b*-P(MAC-*co*-DTC)) and methoxy poly(ethylene glycol)-*b*-copolycarbonates (mPEG-*b*-P(MAC-*co*-DTC)).

conjugated with FA could be efficiently taken up by malignant cells *via* FA receptor-mediated endocytosis. Many reports have focused on the FA-conjugated drug carriers based on polymeric micelles and nanoparticles [17,18].

In this paper, novel biodegradable amphiphilic block copolymers were successfully synthesized using IPPL as the catalyst. As shown in Scheme 1, the biodegradable copolymers of MAC with DTC acted as the hydrophobic core, while the hydrophilic long-circulating PEG block was employed as the outer shell with FA as targeting ligand located at the surface of the polymeric micelles. Considering the tumor-targeting properties of FA, anticancer drug of doxorubicin (DOX) was used as the model drug. The FA-conjugated amphiphilic block copolymers were proposed as a novel targeting DDS for efficient delivery of DOX to cancer cells.

2. Materials and methods

2.1. Materials

Methoxy poly(ethylene glycol) (mPEG, M_n = 2000 and 5000) were purchased from Acros and poly(ethylene glycol) (PEG, M_n = 2000 and 4000) were purchased from Shanghai Chemical Co., China. DOX hydrochloride (DOX·HCl) was obtained from Dalian Meilun Biology Technology Co. Ltd. MAC and DTC were synthesized according to the literature [19,20]. IPPL was prepared according to He et al. [21]. Folic acid (FA), 1,1-carbonyldiimidazole (CDI) were obtained from J&K Chemical Ltd. 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), Dulbecco's phosphate buffered saline (PBS), 3-Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were purchased from Invitrogen Corp. HeLa cells were incubated in DMEM containing 10% FBS and 1% antibiotics (penicillin–streptomycin, 10,000 U/mL) at 37 °C and a humidified atmosphere containing 5% CO_2. Other reagents were of analytical grade and purified by general methods.

2.2. Synthesis of FA-PEG-b-P(MAC-co-DTC) and mPEG-b-P(MAC-co-DTC)

2.2.1. Synthesis of folate-conjugated polyethylene glycol (FA-PEG)

FA-PEG was synthesized *via* CDI mediated ester formation [22]. At first, 2 mmol (0.883 g) of FA was dissolved in 40 ml of dried dimethylsulfoxide (DMSO) followed by adding 2.2 mmol (0.358 g) of CDI and reacted for 4 h at room temperature in the dark. Then, 0.5 mmol of PEG (2000 and 4000) was added into the above solution. The reaction was further carried out in a dark condition for 24 h at room temperature. The reaction mixture was transferred into a dialysis tube (MWCO: 3500 or 14,000 Da) and dialyzed for 48 h against distilled water, which was changed every 4 h. The resulting solution was filtered and then lyophilized to obtain FA-PEG, which was kept in a dry box for future use.

2.2.2. Synthesis of FA-PEG-b-P(MAC-co-DTC)

FA-PEG-*b*-P(MAC-*co*-DTC) was synthesized in bulk by IPPL-catalyzed ring-opening copolymerization. The vessel containing FA-PEG, MAC, DTC and IPPL (3 wt‰ of MAC and DTC) with a magnetic stirring bar was dried *in vacuo* with anhydrous phosphorus pentoxide at room temperature for 24 h. Then the vessel was sealed *in vacuo* and immersed into an oil bath at 100 °C for 48 h. The reaction mixture was dissolved in DMSO and the insoluble IPPL was removed by filtration. Then the solvent was dialyzed in distilled water (MWCO: 3500 or 14,000 Da) for 48 h at room temperature. The distilled water was refreshed every 4 h. The resulting solution was lyophilized to obtain FA-PEG-*b*-P(MAC-*co*-DTC).

2.2.3. Synthesis of mPEG-b-P(MAC-co-DTC)

In comparison to FA-PEG-*b*-P(MAC-*co*-DTC), mPEG-*b*-P(MAC-*co*-DTC) was also synthesized by enzymatic methods simultaneously. Typically, mPEG (M_n = 2000 and 5000), MAC, DTC and IPPL (3 wt‰ of MAC and DTC) were copolymerized *in vacuo* at 90 °C for 8 h. The other procedures correspond to those described in the synthesis of FA-PEG-*b*-P(MAC-*co*-DTC).

2.3. Measurement

^{1}H NMR spectra were performed on a Mercury VX-300 spectrometer using tetramethylsilane (TMS) as an internal reference and $CDCl_3$ or DMSO-d_6 as the solvent. GPC analysis was performed on a Waters HPLC system equipped with a model 2690D separation module and a 2410 refractive index detector. DMF was used as the eluent at a flow rate of 0.3 ml/min. 20 μl of 1.0% (w/v) sample solutions was injected for each analysis. Waters Millennium module software was used to calculate molecular