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Amphiphilic copolymers with pendent carboxyl groups for high-efficiency loading and controlled release of doxorubicin

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ABSTRACT

In this paper, biodegradable amphiphilic block copolymer based on methoxy poly(ethylene glycol)-*b*-poly(5-allyloxy-1,3-dioxan-2-one) (mPEG-*b*-PATMC) was successfully synthesized in bulk using immobilized *porcine pancreas* lipase (IPPL) as the catalyst. After thiol-ene "click" reactions occur between thiol group of thioglycolic acid and carbon–carbon double bonds of PATMC segments, the pendent carboxyl-modified copolymer mPEG-*b*-PATMC-*g*-SCH_2COOH was obtained for high-efficiency loading and controlled release of doxorubicin (DOX) to cancer cells. Both the carboxyl-modified and unmodified copolymers could self-assemble to form nano-sized micelles in aqueous solution, while transmission electron microscopy (TEM) observation showed that the micelles dispersed in spherical shape with nano-size before and after DOX loading. Compared with the unmodified copolymer, the pendent carboxyl-modified structure in mPEG-*b*-PATMC-g-SCH_2COOH could markedly enhance the drug-loading capacity and entrapment efficiency *via* the electrostatic interaction. The *in vitro* release studies showed more sustained drug release behavior of mPEG-*b*-PATMC-*g*-SCH_2COOH without an initial burst, which could be further adjusted by the conditions of ionic strength and pH. Confocal laser scanning microscopy (CLSM) indicated efficient cellular uptake of DOX delivered by mPEG-*b*-PATMC-*g*-SCH_2COOH, while MTT assays also demonstrated potent cytotoxic activity against HeLa cells.

1. Introduction

In recent decades, with the aim to improve the cancer therapy, tremendous effort has been directed to the development of polymer nanoparticles, for the anticancer drugs delivery and controlled release *in vivo*, such as doxorubicin (DOX), paclitaxel (PTX), and other insoluble anticancer drugs [1,2]. Due to its special structure with a hydrophobic core and hydrophilic shell, the biodegradable polymeric micelles as drug delivery carriers have received significant attentions [3,4], and a series of micellar formulations of anticancer drugs have already advanced into clinical trials [5]. Amphiphilic copolymers could self-assemble to form micelles in aqueous solution. The hydrophobic polymeric core could contain poorly water-soluble drugs *via* hydrophobic interactions [6], whereas the hydrophilic polymeric shell could protect the drug from the aqueous environment [7]. Polyethylene glycol (PEG) is one of the best choice as hydrophilic segment for its several interesting features, including prolonged circulation time, enhanced permeability and retention effect (EPR effect) improved drug tolerance and so on [8,9]. Biodegradable aliphatic polycarbonates have been used as the hydrophobic core of copolymer micelles due to their low toxicity, favorable mechanical properties and biodegradability [10], while the properties of aliphatic polycarbonates could be further controlled for different applications by introducing pendant functional groups [11]. 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 5-allyloxy-1,3-dioxan-2-one (ATMC), dimethyltrimethylene carbonate (DTC) and other kinds of cyclic monomers [12–14], while amphiphilic block copolymers based on aliphatic polycarbonates could also be prepared by enzymatic methods using PEG as a macroinitiator [15–17]. Immobilized enzymes (such as IPPL) would not only maintain enzyme properties of nontoxicity, high catalytic, and high selectivity under mild reaction conditions, but also present promising stability and recyclability, which will be good for its applications in the polymer synthesis systems [12,14].

On the other hand, achieving high drug-loading capacity and a controllable drug-release property are two main challenges for the design of efficient drug carriers [18,19]. It has been reported that the drug contents generally cannot exceed 10% in nanoparticles [20], leading to large amounts of carriers needed for administration of

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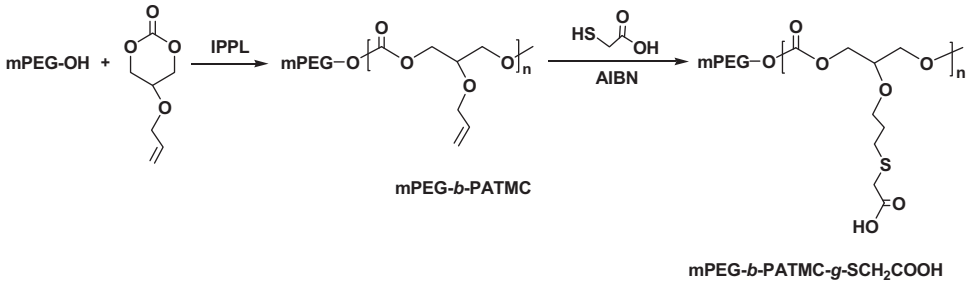

Scheme 1. Synthesis of mPEG-*b*-PATMC and mPEG-*b*-PATMC-*g*-SCH_2COOH.

high-dose of anticancer drugs, and thereby severe toxicity and a burden for the patients to absorb or excrete drug carrier materials [21]. Furthermore, in most cases, drugs such as DOX were entrapped into the hydrophobic core of micelles by hydrophobic interaction [6], while the initial burst release of drug from polymeric micelles was observed over a period of several hours and would limit potential clinical applications in drug delivery [19]. To overcome these problems, great efforts have been made to improve the drug loading capacity and the drug-release property. For example, drug molecules chemically conjugated to the carriers could enhance the drug loading capacity, but also possibly lead to lose their biological activity and affect the drug release into the tumor [22,23]. Another alternative strategy is the introduction of non-covalent interactions in drug delivery systems. The non-covalent interactions between drugs and carriers, such as hydrogen bond interaction, π–π interaction and electrostatic interaction could enhance the drug loading capacity [23], but also provide an effective approach for controlled drug-release due to their environmental sensitivity.

Doxorubicin (DOX), exhibits anticancer activity by intercalating DNA strands and subsequently inhibiting macromolecular biosynthesis, is one of the most common therapeutic drugs used in clinical studies because of it high antitumor efficacy [24]. In this paper, biodegradable amphiphilic block copolymer based on methoxy poly(ethylene glycol)-*b*-poly(5-allyloxy-1,3-dioxan-2-one) (mPEG-*b*-PATMC) was successfully synthesized in bulk using immobilized *porcine pancreas* lipase (IPPL) as the catalyst. The pendent carbon–carbon double bonds of PATMC segments facilitate further modification with thioglycolic acid by thiol-ene "click" reactions, which results in the pendent carboxyl-modified copolymer mPEG-*b*-PATMC-*g*-SCH_2COOH. As shown in Scheme 1, the hydrophilic long-circulating PEG block acted as the outer shell, while the biodegradable PATMC was employed as the hydrophobic core of the copolymer micelles. Based on the electrostatic interaction between exposed carboxyl groups of hydrophobic PATMC segments with amine groups of DOX molecules, mPEG-*b*-PATMC-*g*-SCH_2COOH were proposed as a novel powerful controlled drug delivery system for high-efficiency loading and controlled release of DOX to cancer cells.

2. Materials and methods

2.1. Materials

Methoxy polyethylene glycol (mPEG, M_n = 5000) was purchased from Acros. Doxorubicin hydrochloride (DOX·HCl) was obtained from Dalian Meilun Biology Technology Co., Ltd. Thioglycolic acid from Sinopharm Chemical Reagent Co., Ltd and 2,2′-azobis(isobutyronitrile) (AIBN) from Shanghai No.4 Reagent & H.V. Chemical Co., Ltd were used without further purification. IPPL was prepared according to He [12]. ATMC was synthesized by a procedure established in our laboratory [13]. 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 obtained 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 mPEG-b-PATMC-g-SCH_2COOH

2.2.1. Synthesis of mPEG-b-PATMC

mPEG-*b*-PATMC was synthesized in bulk by enzymatic ring-opening polymerization. The vessel containing mPEG, ATMC (EG:ATMC molar feed ratio of 6.0:1.0, 5.0:1.0, and 4.0:1.0, respectively) and IPPL (0.3 wt% of ATMC) 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 140 °C for 24 h. The reaction mixture was dissolved in CH_2Cl_2 and the insoluble IPPL was removed by filtration. The solvent was condensed and dissolved in THF, and then dialyzed in distilled water (MWCO 14,000) for 48 h at room temperature. The distilled water was refreshed every 4 h. The obtained solution was lyophilized to obtain mPEG-*b*-PATMC (Yield: 54%, 71% and 67%, respectively).

2.2.2. Synthesis of mPEG-b-PATMC-g-SCH_2COOH

mPEG-*b*-PATMC-*g*-SCH_2COOH was synthesized by the thiol-ene "click" reaction [25–27]. 0.28 g (0.801 mmol allyl groups) of mPEG-*b*-PATMC was dissolved in 3 ml of anhydrous tetrahydrofuran (THF) followed by adding 4.003 mmol (0.368 g) of thioglycolic acid and 4.003 mmol (0.657 g) of AIBN under a N_2 atmosphere. The mixture was stirred at 60 °C for 24 h. Then the solution was filtrated, condensed and precipitated into diethyl ether twice. The obtained copolymer mPEG-*b*-PATMC-*g*-SCH_2COOH was dried *in vacuo* at room temperature to constant weight (yield: 67%).

2.3. Measurement

^1H NMR spectra were performed on a Mercury VX-300 spectrometer using tetramethylsilane (TMS) as an internal reference and $CDCl_3$ as the solvent. The molecular weights and distributions were determined by a gel permeation chromatography (GPC) system equipped with a model 2690D separation module and a 2410 refractive index detector. THF was used as the eluent at a flow rate of 0.3 ml/min and 20 μl of 1.0% (w/v) sample solutions were injected for each analysis. Fluorescence spectra were recorded using a RF-5301 PC (Shimadzu) spectrofluorometer (slit widths: 5 nm). Transmission electron microscope (TEM) observations were conducted on a JEM-2100 (HR) electron microscope at an acceleration voltage of 200 kV. The samples were prepared by placing a drop of micellar solution (0.1 mg/ml) onto the copper grid with formvar film and dried at room temperature. Dynamic light scattering (DLS) (Nano-ZS 3600, Malvern Instruments, UK) was performed to measure size and zeta potential of copolymer micelles. The micelle