Materials Science & Engineering C 89 (2018) 401–412

Contents lists available at ScienceDirect

Materials Science & Engineering C

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

Bioinspired mimics: Self-assembly of redox-activated phosphorylcholine–based biodegradable copolymers for enhancing antitumor efficiency

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ARTICLE INFO

Keywords:
Biomimicking
Phosphorylcholine
Reduction-activated
Anticancer drug delivery

ABSTRACT

With the purpose of reducing side effects in anticancer therapy, the micelles of a novel reduction-activated copolymer with biomimicking phosphorylcholine, poly(ε-caprolactone)-ss-b-poly(2-methacryloyloxyethyl phosphorylcholine) (PCL-ss-PMPC) are developed. The well-suitable nanosize of micelles with good physiological stability (approximately 50 nm, 2.5 μg/mL) can be quickly internalized into cells due to bioinspired phosphorylcholine property and mainly located in endo/lysosomes. The reduction response of micelles is confirmed by size change and accelerated drug release under reducing environment, proved as better anticancer efficacy in comparison to insensitive micelles. Pharmacokinetics and in vivo studies demonstrate that redox-activated polymeric micelles can prolong blood transportation, facilitate passive target and accumulate in tumor site, and prompt drug release in cytoplasmic redox environment, behaving as much better antitumor efficiency than control and positive DOX·HCl groups. More importantly, the DOX-loaded micelles considerably reduce side effects and systematic toxicity. Therefore, this work fabricated an innovative bioinspired nanosystem via a facile strategy to achieve effective anticancer therapy.

1. Introduction

The polymeric micelles as drug delivery system have a lot of advantages, such as prolonged circulation time, better pharmacological profiles, enhanced drug accumulation in the tumor site via enhanced permeability and retention (EPR) effect, and reduced adverse effects and so on [1–6]. These micelles usually use PEG as the hydrophilic segment to form the shell, because a hydrophilic PEG layer can diminish RES clearance and thus prolong blood circulation [7,8]. However, the stealth behavior of PEG may reduce the cellular uptake of the micelles by tumor cells, resulting in decreased antitumor efficiency [7,9,10]. Moreover, because of the ether structure, PEG may suffer from oxidation damage in the presence of oxygen and transition metal ions which often exist in most biochemically relevant solutions [11]. More seriously, immune responses to PEG have been found in some cases [12,13].

In the past two decades, the polymers containing zwitterionic groups have received growing attentions in drug delivery systems, because these polymers usually show better anti-protein adsorption and non-bioadhesive properties than PEG [14–18]. Among these zwitterionic groups, phosphatidylcholine (PC) seems more attractive on account of its biomimic structure [19], which may be used to replace of other active target molecules [20,21]. Thanks to the design of a methacrylate monomer bearing PC, MPC, the poly (2-methacryloyloxyethyl phosphorylcholine) (PMPC) containing PC with a wide variety of molecular architecture was developed and used in various areas [11,22]. Recently, amphiphilic PMPC copolymers were designed for the delivery of hydrophobic drugs. Ishihara et al. used amphiphilic random copolymer poly(2-methacryloyloxyethyl phosphorylcholine-co-n-butylmethacrylate) (PMPC-PMB) as the carriers for PTX [23,24]. Armes et al. reported a series of pH responsive drug carriers based on amphiphilic PMPC block polymers [25,26]. Furthermore, biodegradable nanocarriers with PMPC have also been designed [27,28]. These researches showed that PMPC was capable for the hydrophilic block of drug carriers. Moreover, PMPC-based drug carriers showed higher cellular internalization efficiency and better inhibitory on tumor cells than PEG-based micelles. More importantly, some in vivo investigations revealed the excellent in vivo performance of the micelles with PC

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https://doi.org/10.1016/j.msec.2018.04.003
Received 14 August 2017; Received in revised form 21 February 2018; Accepted 4 April 2018
Available online 13 April 2018
0928-4931/

shells [29,30]. Zhao el al. has verified that the polymeric micelles with PMPC structures could be circulated for a long time in blood [30]. Therefore, the micelle with PMPC as the shell was a promising alternative to the micelles with PEG as the shell. Nevertheless, in comparison with PEG based carriers, investigations on the micelles with PC shell as drug carries are far from enough. Especially, there is less reports about PMPC carriers designed based on the microenvironment of tumor tissues and tumor cells.

As we know, various stimuli-responsive drug delivery systems with desired release have attracted more and more attentions [31–34]. Among them, polymer micelles with redox sensitiveness are promising for intracellular drug delivery since the redox process is widely present in physiological environments [35]. Specifically, there is a significant redox gradient for tumors between the intracellular components and the extracellular environments. The concentration of glutathione (GSH) is approximately 2–10 mM in intracellular space of tumor cells while it is only approximately 2–20 μM outside of the cells [36]. Making use of the difference, redox-responsive micelles are designed to achieve fast intracellular drug release. In these redox-responsive micelles, there is usually disulfide (ss) or diselenide structure, which is sensitive to reduction circumstance [37]. Many studies have reported that the disulfide structure was usually used as the sensitive linkage [38,39]. In most reduction-sensitive polymeric drug carriers, PEG serves as the hydrophilic component. Zwitterionic PMPC, as a promising alternative to PEG, may be a better choice as the hydrophilic shell of these reduction-sensitive drug carriers. Moreover, although in vitro investigations have been reported for most reduction-sensitive polymeric nanocarrier, only a few comprehensive in vivo experiments have been reported [40]. Therefore, to further demonstrate the in vivo performance of reduction-responsive drug carriers, more systemic in vivo studies should be deep investigated.

Guided by those thoughts above, we developed a new reduction responsive amphiphilic block polymer with zwitterionic PMPC and the disulfide bonds as the reduction sensitive linkage. The micelles from the polymer were prepared and their reduction-triggered properties were confirmed. In vitro drug release and the intracellular drug release of DOX-loaded micelles was explored. In vitro cell viability and intracellular location of those micelles were evaluated. More importantly, the pharmacokinetics, in vivo imaging and antitumor efficiency of DOX-loaded micelles in vivo were also thoroughly investigated.

2. Materials and methods

2.1. Materials

ε-caprolactone (CL) (New Jersey, USA) was dried by calcium hydride (CaH_2) (Chengdu Kelong Chemicals Ltd., China) and distilled in reduced pressure. Toluene and tetrahydrofuran (THF) (Chengdu Kelong Chemicals Ltd., China) were dried by refluxing over sodium and distilled before used. Triethylamine (TEA) (Chengdu Kelong Chemicals Ltd., China) was dried over CaH_2 and distilled. Stannous octanoate ($Sn(Oct)_2$, 95%), 2-bromoisobutyryl bromide (98%), copper (I) bromide (CuBr, 99%), 2,2′-bipyredine (bpy), pentaerythritol, 2,2′-disulfanediyldiethanol, 3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) were purchased from sigma (USA) and used as received. 2-methacryloyloxyethyl phosphorylcholine (MPC) was supplied by Nanjing Joy-Nature Ltd. (China) and used as received. Water-soluble DOX·HCl was neutralized by triethylamine to remove the hydrochlorideto make it become hydrophobic DOX [17]. All the other reagents and solvents were analytical grade and used without further purification.

2.2. Synthesis of HO-ss-iBuBr

2-bromo-2-methylpropanoyl bromide (1.49 g, 6.48 mmol) dissolved in dichloromethane (DCM) (10 mL) was added dropwise to a solution of excess 2, 2′-disulfanediyldiethanol (1.50 g, 9.72 mmol) and triethylamine (TEA) (1.95 g, 19.4 mmol) in DCM (20 mL) at −20 °C. The resulting solution was stirred overnight at room temperature. Subsequently, the solvent was removed by rotary evaporation, and the product was purified by silica column chromatograph with mixtures of petroleum ether/ethyl acetate (1/1, v/v). The product was isolated by evaporation of the solvents and further dried in a vacuum oven at 50 °C overnight to form oily residue. (δ 3.91, $HO\underline{CH_2}CH_2S$-; δ 2.90, $HOCH_2\underline{CH_2}S$-; δ 1.95, $(\underline{CH_3})_2CBrCOOCH_2$-; δ 4.46 $(CH_3)_2CBrCOO\underline{CH_2}$-; δ 2.99, $-COOCH_2\underline{CH_2}S$-).

2.3. Synthesis of PCL-ss-iBuBr

The initiator, HO-ss-iBuBr (0.440 g, 1.46 mmol), ε-caprolactone (4.28 g, 37.54 mmol) and $Sn(Otc)_2$ (0.004 g) were added into the reactor. Oxygen and residual water in the reactor were removed by three degassing in freeze-pump-thaw for 1.5 h. The reaction was carried out at 120 °C under vacuum for 24 h. The crude product was dissolved in dichloromethane and purified by precipitation in cold methanol. White powder was obtained. (δ 3.65, $HOCH_2$-; δ 4.06; $-COO\underline{CH_2}CH_2CH_2CH_2CH_2CO$-; δ 1.62 $-OCH_2\underline{CH_2}CH_2\underline{CH_2}CH_2CO$-; δ 1.40, $-OCH_2CH_2\underline{CH_2}CH_2CH_2CO$-; δ 2.33, $-OCH_2CH_2CH_2CH_2\underline{CH_2}CO$-; δ 4.34, $-CH_2COOCH_2CH_2S$-; δ 2.96, $-CH_2COOCH_2\underline{CH_2}S\text{-}S\underline{CH_2}$-; δ 4.44, $(CH_3)_2CBrCOO\underline{CH_2}$-; δ 1.94, $(\underline{CH_3})_2CBrCOOCH_2$-).

2.4. Synthesis of PCL-ss-PMPC

PCL-ss-iBuBr (1.00 g, 0.320 mmol), MPC (1.42 g, 4.80 mmol) and bpy (0.150 g, 0.960 mmol) were dissolved in mixed solvent of tetrahydrofuran (THF) and methanol (v/v: 2/1). After purging the solution with nitrogen for 30 min, CuBr (0.0689 g, 0.480 mmol) was added in the atmosphere of N_2. The reaction was performed for 72 h with the protection of N_2. The reaction mixture was exposed into atmosphere to stop the reaction. And then the reaction mixture was dialyzed against ultra-purified water. The product was obtained by freeze-drying method. (δ 2.92 $-OCH_2\underline{CH_2}SS\underline{CH_2}CH_2O$-; δ 1.83, $-\underline{CH_2}C(CH_3)$-; δ 0.89, $-CH_2C(\underline{CH_3})$-; δ 4.32, $-COO\underline{CH_2}CH_2O$-; δ 4.21 $-COOCH_2\underline{CH_2}O$-; δ 3.74, $-O\underline{CH_2}CH_2N^+(CH_3)_3$; δ 3.29, $-OCH_2CH_2N^+(\underline{CH_3})_3$).

2.5. Synthesis of PCL-PMPC

The control sample was prepared according to our previous study [28]. Using laurinol as the initiator, PCL was obtained by ring-opening polymerization and then 2-bromo-2-methylpropanoyl bromide was used to react with PCL to generate the maroinitiator, which would initiate the polymerization of MPC through ATRP to obtain PCL-PMPC. (δ 1.82, $-\underline{CH_2}C(CH_3)$-; δ 0.88, $-CH_2C(\underline{CH_3})$-; δ 4.32, $-COOCH_2CH_2O$-; δ 3.72, $-O\underline{CH_2}CH_2N^+(CH_3)_3$; δ 3.28, $-OCH_2CH_2N^+(\underline{CH_3})_3$).

2.6. Characterization of polymers

^{1}H NMR spectra recorded on a Unity Inova 400 spectrometer operating at 400 MHz and attenuated total reflection Fourier transform infrared (ATR-FTIR) spectra measured on a Nicolet Magna 560 FTIR spectrometer (Thermofisher, Massachusetts, USA) were chosen to characterize the structure of polymers. The molecular weight and polydispersity of the polymers, PCL and PCL-ss-iBuBr were determined by a Waters 1515 gel permeation chromatograph instrument system. The measurement was performed using THF as the eluent at a flow rate of 1.0 mL/min at 40 °C.

2.7. Critical micellar concentration

The critical micelle concentration (CMC) of copolymers in water was estimated by using pyrene as a hydrophobic fluorescence probe. A series of micellar solutions with different concentrations was prepared