Materials Science and Engineering C 45 (2014) 162–169

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

In vitro drug release and biological evaluation of biomimetic polymeric micelles self-assembled from amphiphilic deoxycholic acid–phosphorylcholine–chitosan conjugate

Minming Wu, Kai Guo, Hongwei Dong, Rong Zeng *, Mei Tu, Jianhao Zhao

Department of Materials Science and Engineering, College of Science and Engineering, Jinan University, Guangzhou 510632, PR China

A R T I C L E I N F O

Article history:
Received 9 June 2014
Received in revised form 7 August 2014
Accepted 10 September 2014
Available online 16 September 2014

Keywords:
Polymeric micelles
Biomimetic
Phosphorylcholine
Drug release
Biological evaluation

A B S T R A C T

Novel biomimetic amphiphilic chitosan derivative, deoxycholic acid–phosphorylcholine–chitosan conjugate (DCA–PCCs) was synthesized based on the combination of Atherton–Todd reaction for coupling phosphorylcholine (PC) and carbodiimide coupling reaction for linking deoxycholic acid (DCA) to chitosan. The chemical structure of DCA–PCCs was characterized by ^{1}H and ^{31}P nuclear magnetic resonance (NMR). The self-assembly of DCA–PCCs in water was analyzed by fluorescence measurements, dynamic laser light-scattering (DLS), zeta potential and transmission electron microscopy (TEM) technologies. The results confirmed that the amphiphilic DCA–PCCs can self-assemble to form nanosized spherical micelles with biomimetic PC shell. In vitro biological evaluation revealed that DCA–PCCs micelles had low toxicity against NIH/3T3 mouse embryonic fibroblasts as well as good hemocompatibility. Using quercetin as a hydrophobic model drug, drug loading and release study suggested that biomimetic DCA–PCCs micelles could be used as a promising nanocarrier avoiding unfavorable biological response for hydrophobic drug delivery applications.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Polymeric micelles have attracted considerable attention in the field of therapy and diagnosis for effectively delivering therapeutic and/or diagnostic agents such as low molecular weight drugs, DNA, RNA, peptides, or contrast/imaging agents. They have several favorable physicochemical and biological properties over other carriers including: nanoscale size suitable for drug delivery, hydrophobic core–hydrophilic shell structure allowed for loading water insoluble drugs or protecting sensitive cargo, as well as tailored architecture for sustained or controlled release, and targeting to specific sites in vivo [1–3]. In the past decades, by adjusting the chemical nature and length of polymer component, the hydrophobic/hydrophilic balance, and the conditions of self-assembly, polymeric micelles with various well-defined architectures have been achieved for improving therapeutic efficacy [4,5], including hydrophilic–hydrophobic diblock copolymer micelles (e.g. poly(ethylene glycol) (PEG)–poly(ε-caprolactone) (PCL) [6], PEG-*b*-poly(lactic acid) (PLA) [7]), double hydrophilic (nonionic–ionic) diblock copolymer micelles (e.g. PEG–poly(L-lysine) (PLL) [8], PEG–poly[N-(N-citraconyl-2-aminoethyl)aspartamide] (PAsp(EDA-Cit)) [9]), triblock (hydrophilic–hydrophobic–hydrophilic) copolymer micelles (e.g. PEG–poly(propylene oxide) (PPO)–PEG (Pluronic) [10]), and graft (hydrophilic-g-hydrophobic) copolymer micelles (e.g. hydrophobically modified polysaccharides [11]).

In order to avoid the undesired biological response to polymeric micelles, a biocompatible hydrophilic shell suppressing non-specific interactions with biological components is essential, which is commonly composed of PEG mostly due to its non-toxicity, low immunogenicity, and tightly bound water layer [6–10]. However, several unfavorable biological responses related to PEGylation have been reported recently. Koide et al. reported that PEG-containing polymeric micelles around 50 nm or more would induce an accelerated blood clearance (ABC) phenomenon like PEGylated liposomes [12], though the mechanism of the ABC phenomenon induced by PEG has not been completely elucidated yet [13]. Additionally, PEGylation may inhibit the binding and uptake of the nanocarriers into the target cells [14]. Considering the above problems of PEGylation, cell membrane mimicking phosphorylcholine (PC), due to its excellent hemocompatibility and protein-resistant property, has received great interest in recent years as an alternative approach to cover nanocarriers for avoiding the undesired biological response [15,16]. Salvage et al. reported that 2-methacryloyloxyethyl phosphorylcholine (MPC)–2-(diisopropylamino) ethyl methacrylate (DPA) diblock copolymers could form novel non-toxic biocompatible micelles of appropriate nanosize and good colloidal stability with pH-modulated drug uptake and release for drug delivery [17]. More interestingly, PC-containing nanostructures can enter living cells through fusogenic interaction with the plasma membrane [18], which was advantageous for intracellular trafficking of the nanocarriers. And Ji et al.

* Corresponding author.
E-mail address: tzengronga@jnu.edu.cn (R. Zeng).

http://dx.doi.org/10.1016/j.msec.2014.09.008
0928-4931/© 2014 Elsevier B.V. All rights reserved.

demonstrated that PC-modified nanoparticles were selectively uptaken by different types of cancer cells than normal cells due to the over-expressed choline receptors on the cancer cell membranes, compared to PEG [19,20]. However, most of PC-containing nanoparticles reported were non-biodegradable, which greatly limited their pharmaceutical and biomedical application in vivo.

Recently, we have successfully synthesized biodegradable biomimetic PC–chitosan conjugates with different degrees of substitution (DS) via a phosphoramide linkage between glucosamine and PC through Atherton–Todd reaction and subsequent hydrolysis under the mild conditions [21]. These PC–chitosan conjugates can form nanohydrogels suppressing the non-specific interactions with proteins by ionic crosslinking [22], or directly self-assemble to form PC-covered spherical micelles, but their stability was limited due to their relatively larger critical micelle concentration (CMC) values [23]. In order to improve the physicochemical and biological characteristics of PC–chitosan based micelles for drug delivery, in this work, hydrophobic modification was conducted by further introducing deoxycholic acid (DCA) to PC-chitosan conjugates to adjust their hydrophilicity/hydrophobicity balance [24]. The self-assembly behaviors of the obtained deoxycholic acid–phosphorylcholine–chitosan conjugate (DCA–PCCs) were investigated by fluorescence techniques, dynamic light scattering (DLS) and transmission electron microscopy (TEM). The cytotoxicity and hemocompatibility of DCA–PCCs micelles were evaluated in vitro. Using quercetin (QUE), a flavonoid widely found in plant sources with anti-inflammatory and antioxidant properties as a hydrophobic model drug [25], in vitro drug release behaviors of DCA–PCCs micelles were investigated.

2. Experimental

2.1. Materials

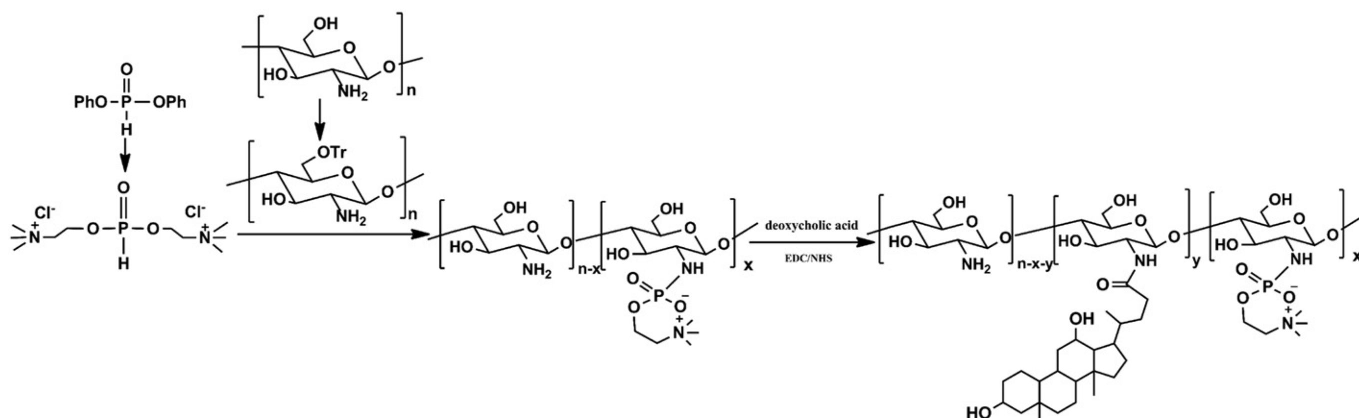
Chitosan (low molecular weight, Brookfield viscosity: 20 cps) was purchased from Sigma-Aldrich, then treated with 40% aqueous sodium hydroxide at 110 °C for 1.5 h three times to reach 100% deacetylation degree (confirmed by no signal associated with acetyl groups in ^{1}H NMR spectra). Diphenyl phosphate was also purchased from Sigma-Aldrich. Choline chloride was obtained from Acros. N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethyllaminopropyl) carbodiimide hydrochloride (EDC), deoxycholic acid (DCA) and quercetin were obtained from Aladdin Reagent Company (China) and used as received. All other reagents were commercially available reagents of analytical grade.

2.2. Synthesis and characterization of deoxycholic acid–phosphorylcholine–chitosan conjugate (DCA–PCCs)

Deoxycholic acid–phosphorylcholine–chitosan conjugate (DCA-PCCs) was synthesized according to the procedure based on the combination of Atherton–Todd reaction for coupling PC and carbodiimide coupling reaction for linking DCA to the amino groups of chitosan (Scheme 1), and all experiments involving water-sensitive compounds were conducted under dry conditions. Briefly, 732 mg (5.2 mmol) of choline chloride was added to 0.475 mL (2.5 mmol) of diphenyl phosphite in 10 mL of freshly distilled pyridine/DMSO (1:10) at room temperature; the solution was stirred for 2 h. The crude product of dicholinyl H-phosphonate dichloride was obtained after the solvent was evaporated, then dissolved in 10 mL of 2-propanol, and then added dropwise to 6-*O*-trityl chitosan obtained by Nishimura's method [26] (200 mg, 0.50 mmol of free NH_2) in a mixed solution of dimethylacetamide (DMA, 10 mL), triethylamine [1.05 mL (7.5 mmol)], and tetrachloromethane [0.475 mL (5.0 mmol)]. After stirring overnight, the resulting solution was evaporated to dryness, 10 mL of formic acid was added to the residue, and stirred for 1 h, then formic acid was removed by rotary evaporation. The residue was dissolved in 10 mL of 0.1 mol/L NaOH and stirred for 2 h, and then centrifuged. The supernatant was dialyzed with distilled water for 3 days and lyophilized to provide the PC–chitosan conjugate. PC–chitosan (100 mg) was dissolved in 1% w/v acetic acid solution (20 mL), then DCA was added at 2.0 mol/mol glucosamine residue of chitosan after activation by EDC and NHS (DCA:EDC:NHS = 1:1.5:1.2, mol/mol) in 50 mL methanol and stirred at room temperature. After 48 h, the reaction mixture was dialyzed against methanol for 1 day and then distilled water for 3 days, then lyophilized to obtain the DCA–PCCs product (95.3 mg).

^{1}H and ^{31}P NMR spectra were recorded in D_2O at 400 MHz using a Bruker UX-400 NMR spectrometer (Germany). The ^{1}H NMR spectra at 293 K (D_2O, δ/ppm) contained the following peaks: 0.68–1.12 (m, H of DCA), 2.98 (br, H2 of GlcN-PC and GlcN), 3.14 (s, $–N^+(CH_3)_3$), 3.50–3.71 (m, $N^+CH_2–$, H3, H4, H5, H6 of GlcN and GlcN-PC), 4.21 (br, $–N^+(CH_3)_3–CH_2–\underline{CH_2}–$), 4.67 (br, H1 of GlcN and GlcN-PC). The ^{31}P NMR spectra at 293 K (D_2O, δ/ppm) contained a single peak: 6.00 (–NH–P(O)O2–).

The degree of substitution (DS, defined as the number of PC per 100 sugar residues) of PC moiety was calculated by the amount ratio of H of $–N^+(CH_3)_3$ (from PC) to H1 (from glucosamine units of GlcN and GlcN-PC). The DS of DCA (defined as the number of DCA per 100 sugar residues) is determined spectrophotometrically according to the method described by Wang et al. [27]. Briefly, the DCA–PCCs sample (4–10 mg) was accurately weighed in an ampoule and dissolved in 0.5 mL DMSO, then 0.5 mL of 60% aqueous acetic acid solution and



Scheme 1. Synthetic route of DCA–PCCs conjugate.

Download English Version:

https://daneshyari.com/en/article/1428544

Download Persian Version:

https://daneshyari.com/article/1428544

Daneshyari.com