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A smart micellar system with an amine-containing polycarbonate shell

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ABSTRACT

The present paper reports the design and preparation of an amphiphilic triblock co-polymer poly(ε-caprolactone) (PCL)–poly(6,14-dimethyl-1,3,9,11-tetraoxa-6,14-diaza-cyclohexadecane-2,10-dione) (PADMC)–PCL and the use of micelles composed of them as carriers for pH-sensitive drug release. The triblock co-polymers were synthesized via two-step ring-opening polymerization with catalysis by Novozym-435 lipase. By adjusting the feed ratio, three co-polymers with different PCL lengths and the same PADMC length were produced. The block structure of the co-polymers obtained was confirmed by comparative studies on PCL–PADMC–PCLs and the corresponding random poly(ε-caprolactone-random-6,14-dimethyl-1,3,9,11-tetraoxa-6,14-diaza-cyclohexadecane-2,10-dione) (poly(CL-*r*-ADMC)) by means of nuclear magnetic resonance and differential scanning calorimetry. Cell cytotoxicity tests showed that the co-polymer displayed no apparent cytotoxicity to 293T and HeLa cells. Transmissions electron microscopy indicates that the self-assembled micelles exhibited a well-defined spherical shape with a diameter between ~30 and 50 nm. The critical aggregation concentration was dependent on the block composition. Due to the presence of ionizable tertiary amine groups in the PADMC block, acid-induced variation in the micellar morphology was evident with respect to micelle size and size distribution. The size–pH curve was characterized by a smooth sigmoid form, and had a dramatic upward shift with decreasing pH from 6.5 to 4.5, which correlated well with the buffer range of hydrophilic PADMC. As a demonstration of the potential of PCL–PADMC–PCL micelles to control drug delivery, acid induced drug release for prednisone acetate-loaded micelles was explored. PCL–PADMC–PCL micelles show good promise as smart drug carriers, sensing the local specific pH decrease around lesion sites.

1. Introduction

Well-defined amphiphilic block co-polymers represent a major advance in the development of soft materials [1,2]. Those co-polymers consisting of hydrophilic and hydrophobic building blocks readily undergo microphase separation in aqueous media and self-assemble into versatile structures. As one typical example, nano-sized micelles have attracted significant attention for the formulation of continuous delivery systems for poorly water soluble drugs as well as therapeutic genes and proteins, leading to substantially improved drug bioavailability and stability [3–5]. The nanoscale dimension of the micelles is well documented to contribute to passive tumor targeting, enhanced efficiency in crossing biological barriers, and a prolonged lifetime in the blood circulation [6–9]. The gradual understanding of the virtues of micelle-based delivery systems has further stimulated the development of so-called "smart" micelles for stimulus-responsive drug release, in order to reduce drug-associated toxicity and promote therapeutic efficacy [10–13].

To date most amphiphilic block co-polymers have been based on the hydrophilic poly(ethylene glycol) (PEG), which is thermosensitive and has "stealth" properties, and structural optimization has mainly been directed towards the hydrophobic blocks [14–16]. Several other hydrophilic polymers, such as poly(N-isopropylacrylamide) (PNIPAAm), poly(acrylic acid) (PAA), poly(N,N-dimethylaminoethyl acrylate) (PDMAEA), poly(2-N-(morpholino)ethyl methacrylate) (PMEMA) have also been extensively explored and utilized for the construction of pH- or temperature-responsive micelles [10–13,17]. Although advantages and encouraging results have been shown when using those micelles for various drug delivery applications in vitro and in vivo, safety concerns remain regarding their non-degradable nature, latent immunogenicity and antigenicity, significant toxicity of residual monomers, and/or difficulties with renal excretion from the body [18–21]. On the other hand, several biodegradable, hydrophilic polymers have been utilized to develop amphiphilic block co-polymers, however, most of them lack stimulus-responsive function [22–25]. In this regard, the search for biodegradable and biocompatible polymers to act as the hydrophilic building block of "smart" micelles is appealing but still challenging at present.

Given the electrolyte enriched environment in the human body, specifically designed nanostructures surrounded by a polycationic

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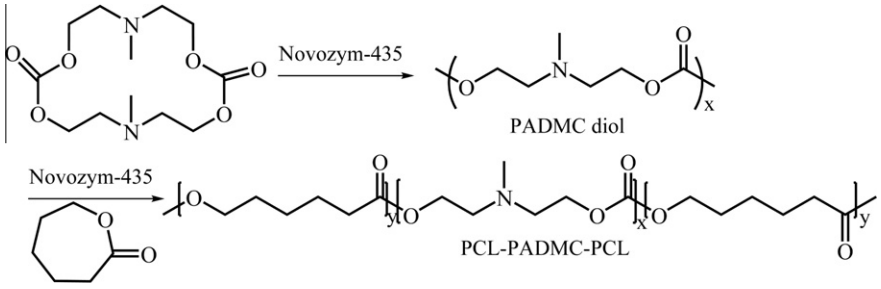

Scheme 1. Illustration of PCL–PADMC–PCL preparation.

shell have attracted great interest in the development of micelle-based delivery systems. The positively charged surface of drug-loaded micelles may increase cellular uptake through non-receptor-mediated endocytosis by increasing either the uptake rate or amount endocytosed, thus leading to improved therapeutic performance. Also, the changes in surface charge, water solubility, chain conformation, and aggregated state of polycations in response to local environmental stimuli (such as varied pH and ionic strength) provide opportunities to establish smart drug delivery systems [26,27]. Polycations investigated include branched and linear polyethylenimine (PEI), poly(L-lysine) (PLL), poly(L-histidine) (PLH), polyamidoamine (PAMAM) and poly((2-dimethylamino)ethyl methacrylate) [26–33]. Nevertheless, some issues, including non-degradability and undesirable acute or delayed toxicity hinder their further application in vivo to some extent [34,35].

Biodegradable aliphatic polycarbonates have found numerous applications in the biomedical field [36–39], and some of them have been utilized clinically [40]. A recent work reported a cationic polycarbonate poly(6,14-dimethyl-1,3,9,11-tetraoxa-6,14-diaza-cyclohexadecane-2,10-dione) functionalized with tertiary amine groups along the backbone, termed PADMC. This polymer demonstrated excellent properties in terms of water solubility, degradability and low cytotoxicity [41]. The aim of the present study was to develop a "smart" micellar drug delivery system based on thoroughly biodegradable, amphiphilic PADMC-block-polyester co-polymers (Scheme 1). Specifically, poly(ε-caprolactone) (PCL), a representative aliphatic polyester, was chosen as the hydrophobic block due to its leading status as a synthetic degradable polymer frequently used for drug delivery applications. The strong buffer capability of tertiary amine groups in PADMC may somewhat counterbalance the well-known side-effects of polyester degradation associated with the local acidic microenvironment, including the deactivation/denaturation of embedded biomolecules and aseptic inflammation [42–44].

Biosynthetic pathways such as metal-free enzymatic polymerization have recently attracted a great deal of attention as a new approach for biomaterial synthesis [45–48]. This straightforward strategy was utilized for the synthesis of PCL–PADMC–PCL triblock co-polymers via a two step ring opening polymerization (Scheme 1). The self-assembly behavior of PCL–PADMC–PCL in aqueous medium was systematically investigated. Furthermore, prednisone acetate was employed as a model drug to explore the potential of PCL–PADMC–PCL micelles as pH-sensitive vehicles for the controlled release of hydrophobic drugs.

2. Materials and methods

2.1. Materials

6,14-Dimethyl-1,3,9,11-tetraoxa-6,14-diaza-cyclohexadecane-2,10-dione $(ADMC)_2$ was prepared according to our previous work [41]. ε-Caprolactone (ε-CL) was purchased from Sigma, and distilled under CaH_2 before use. Lipase acrylic resin from *Candida antarctica* (Novozym-435) was used as received from Sigma and was vacuum dried over P_2O_5 (40 Pa, 25 °C, 4 h) in a dried pistol before use. 4 Å molecular sieves were dehydrated at 400 °C for 8 h before use. Toluene was received from Shanghai Chemical Reagent Co. and distilled under Na/K alloys before use. All other solvents and reagents were used as received without further treatment.

2.2. Preparation of PCL–PADMC–PCL triblock co-polymers

PADMC (M_n = 6000 g mol^{-1}) was prepared by Novozym-435 catalyzed ring opening polymerization of $(ADMC)_2$ in toluene [41]. A typical preparation of PCL–PADMC–PCL was as follows. 0.1 g of PADMC was placed in a tube with a magnetic stirring bar. Then ε-CL, dried toluene and molecular sieves were introduced into the tube. After stirring for 2 h at room temperature Novozym-435 was added. The tube was sealed and immersed in an oil bath thermostated at 60 °C. The toluene solution was diluted with 3 ml of dichloromethane following a 14 h reaction. After removing insoluble Novozym-435 by filtration the concentrated solution was dropped into a large amount of ether used as a poor solvent. This precipitation procedure was repeated for several times to isolate the polymer product.

PCL–PADMC–PCL block co-polymers with different chemical compositions were prepared by changing the molar ratio of ε-CL monomer to PADMC initiator. The unit ratios of CL versus ADMC, and the number average molecular weight (M_n) of those co-polymers were determined by comparing the peak intensities of the methylene proton in ADMC unit at δ 4.2 ppm (–OCH_2CH$_2$N(CH$_3$)CH$_2$-CH_2OCO–) (A_{ADMC}) with that of CL unit at δ 3.96 ppm (–OCH_2CH$_2$CH$_2$CH$_2$CH$_2$CO) (A_{CL}) in ^{1}H NMR spectra.

$$\text{unit ratio} = 2A_{CL}/A_{ADMC}$$

$$M_{n(\text{co-polymer})} = M_{n(PCL)} + M_{n(PADMC)} = 6000 + Fw_{(CL)} \times 41 \times \text{unit ratio}$$

where $M_{n(PADMC)}$ and $M_{n(PCL)}$ correspond to the M_n of the PADMC and PCL blocks, respectively, $Fw_{(CL)}$ is the formula weight of the CL unit and 41 is the number of repeat units of PADMC used (M_n = 6000 g mol^{-1}).

2.3. ^{1}H NMR characterization

^{1}H NMR spectra were recorded in a Mercury VX-300 spectrometer at 300 Hz using DMSO-d_6 or D_2O as the solvent at a concentration of ~4% (w/v).

2.4. GPC measurement

Gel permeation chromatography (GPC) was carried out in a Waters HPLC system equipped with a model 2690D separation

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