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Self-assembly of pH-sensitive mixed micelles based on linear and star copolymers for drug delivery

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ABSTRACT

Comicellization of a star block copolymer poly(ε-caprolactone)-*block*-poly(diethylamino)ethyl methacrylate (S(PCL-*b*-PDEAEMA)) and a linear block copolymer methoxy poly(ethylene glycol)-*block*-poly(ε-caprolactone) (mPEG-*b*-PCL) was developed to enhance the stability and lower the cytotoxicity of the micelles. The two copolymers self-assembled into the mixed micelles with a common PCL core surrounded by a mixed PDEAEMA/mPEG shell in aqueous solution. This core–shell structure was transformed to the core–shell–corona structure at high pH due to the collapse of the PDEAEMA segment. The properties of the polymeric micelles were greatly dependent on the weight ratio of the two copolymers and the external pH. As increasing the mPEG-*b*-PCL content, the size and the zeta potential of the mixed micelles were lowered while the pH-dependent stability and the biocompatibility were improved. Moreover, an increase in pH accelerated the release of indomethacin (IND) from the mixed micelles *in vitro*. These results augured that the mixed micelles could be applied as a stable pH-sensitive release system.

1. Introduction

In the past decades, polymeric micelles have been extensively studied for their great potential applications in the biomedical field [1–6]. The prominent features of such micelles are the abilities to solubilize lipophilic drugs, avoid non-selective uptake by the reticuloendothelial system (RES), and utilize the enhanced permeability and retention effect (EPR effect) for passive targeting [1,2].

Up to now, a variety of amphiphilic polymers, including diblock or multiblock copolymers, graft copolymers, branched or hyperbranched copolymers and nonlinear copolymers, have been used to construct the ordered micelles from self-assembling polymers [7–13]. However, traditional polymeric micelles formed from a single polymer are often lack of multiple functionalities due to the limitation in the number of building blocks. In order to overcome this limitation, the polymeric micelles consisted of more than one amphiphilic polymer have aroused great interest recently. The combination of two or more species of block copolymers to produce the multifunctional mixed micelles is a straightforward and promising strategy. Firstly, the intentional block copolymers are much easier to be tailored by the conventional polymerization and the controlled radical polymerization techniques or can be obtained straightway from the industrial products compared with the multicomponent copolymers [14–16]. Secondly, the properties of the mixed micelles, such as stability, size, morphology, drug loading level and responsiveness, can be readily tuned by varying the relative content of the two block copolymers or altering another similar substitution [15,17–21]. Furthermore, many functional molecules, such as targeting ligand (e.g. galactosamine, folate), fluorescein isothiocyanate (FITC) and Cy5.5 dye, can be separately or simultaneously clung to the surface of micelles by a facile chemical method [22–24].

Based on these advantages, herein a large number of mixed micelles have been designed to be superior to their individual constituents. Current researchers have focused on the stimuli-responsive mixed micelles based on two linear copolymers [14,17,25]. However, little attention has been paid to the star copolymers, especially the ones with stimuli-responsive properties. It has been known that the star copolymers exhibit a smaller hydrodynamic radius, lower intrinsic viscosity and denser functional groups when compared with the linear copolymers of similar composition and molecular weight [26]. Therefore, star copolymers are expected to optimize the properties of the micelles to better meet the various requirements. Poly(2-(diethylamino)ethyl methacrylate) (PDEAEMA) is one kind of potential polycationic gene carriers [27]. The star-shaped PDEAEMA has higher charge density than the linear one, which is facilitated to bind and condense DNA [28]. However, this charge property can also evoke cytotoxicity and needs to be improved by the chemical modification. In this

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study, we proposed a new mixed micelle comprised of a star block copolymer poly(ε-caprolactone)-*block*-poly(2-(diethylamino)ethyl methacrylate) (S(PCL-*b*-PDEAEMA)) and a linear block copolymer methoxy poly(ethylene glycol)-*block*-poly(ε-caprolactone) (mPEG-*b*-PCL). Such a mixed micelle was expected to exhibit low cytotoxicity and good stability at the whole pH range and to realize the drug controlled release. Each component in the micelles displayed particular functional characteristics that rendered the chains to be integrated into the multifunctional mixed micelles. PDEAEMA is known to be a cationic polyelectrolyte which is sensitive to the external pH. mPEG is a common hydrophilic polymer which is extensively used in the biomedical field due to its excellent physicochemical and biological properties [29]. Poly(ε-caprolactone) (PCL), a biodegradable polymer, has attracted much attention due to its good biocompatibility and degradability. Therefore, the micelles containing these three components would be an efficient carrier for the drug delivery. Indomethacin (IND), a water-insoluble anti-inflammatory drug, was employed as a model drug in this study to evaluate the release property of the IND-loaded micelles.

2. Experimental

2.1. Materials

Indomethacin (IND) was supplied by Aladdin (Shanghai, China). The designed amphiphilic star-shaped block copolymer poly(ε-caprolactone)-*block*-poly(diethylamino)ethyl methacrylate (S(PCL-*b*-PDEAEMA)) was synthesized by ring-opening polymerization (ROP) and atom transfer radical polymerization (ATRP) as described in an earlier publication [30]. The linear block copolymer methoxy poly(ethylene glycol)-*block*-poly(ε-caprolactone) (mPEG-*b*-PCL) was synthesized by ROP using mPEG as a macroinitiator, according to our previous report [31]. Other reagents were purchased from Shanghai Chemical Reagents Co. (Shanghai, China) and used as received unless otherwise mentioned. Data of molecular weight and molecular weight distribution of each sample are summarized in Table 1.

2.2. Preparation of micelles

The mixed micelles were prepared by the solvent-removal procedure. 20 mg of mPEG-*b*-PCL (L) and S(PCL-*b*-PDEAEMA) (S) with different L/S weight ratios was dissolved in 4 mL of tetrahydrofuran (THF). Subsequently, the copolymer solution was added dropwise into 10 mL of water with stirring. After stirring for 2 h, the solution was placed into the dialysis membrane (MWCO: 3.5 kDa) and dialyzed against ultrapure water for 24 h at room temperature to remove organic solvent. The concentration of the mixed micelle solution was adjusted to 1 mg/mL. The pure mPEG-*b*-PCL or S(PCL-*b*-PDEAEMA) micelles were prepared by the same method.

Table 1
Composition and characterization of each copolymer.

Code	Structure	M_n of hydrophilic segment[a] (g/mol)	M_n of hydrophobic segment[a] (g/mol)	M_w/M_n [b]
L	mPEG-*b*-PCL	5000	4900	1.15
S	S(PCL-*b*-PDEAEMA)	38,600	12,400	1.38

[a] M_n of hydrophilic segment and M_n of hydrophobic segment were calculated from ^{1}H NMR determined.
[b] Polydispersity indexes were determined from SEC.

2.3. Drug loading and release in vitro

Drug-loaded mixed micelle formulations were also prepared in the similar way using indomethacin (IND) as a model drug. 10 mg of IND and 20 mg of the two copolymers were dissolved in 4 mL of THF, and then added dropwise into 20 mL of water. After stirring for 2 h, the solution was placed into the dialysis membrane (MWCO: 3.5 kDa) and dialyzed against ultrapure water for 24 h at room temperature to remove organic solvent and unloaded IND dissolved in aqueous solution. After filtering through a 0.45-μm microporous membrane to remove the aggregated particles, a part of the solution was frozen and lyophilized to calculate the concentration and yield of solution prepared in this process. The remaining part of the solution was stored before use.

The release behavior of the mixed micelles loaded with IND was conducted in pH 5.0 and pH 7.4 buffer solutions at 37 °C, respectively. At predetermined intervals, 5 mL aliquot of the test solution was withdrawn periodically and replaced with an equal volume of the fresh release medium. The drug concentration in the removed solution was detected by measuring the absorbance at 320 nm in a UV–vis spectrometer (725, Shanghai). All the drug release tests were performed in triplicate.

2.4. In vitro cytotoxicity assay

The *in vitro* cytotoxicity of the polymeric micelles was evaluated by the standard MTT assay against mesenchymal stem cells (MSCs). The cells were seeded in 96-well plates at the density of 5×10^3 cells per well and incubated at 37 °C with 5% CO_2 for 24 h to permit cells attachment. After removing the culture medium, the micelle solutions were added to the cells with a final concentration of 0.1 mg/mL. Control wells were treated with an equivalent volume of the culture medium without polymeric micelles. The cells were then incubated for 24 h and 48 h before adding MTT (20 μL, 5 mg/mL in sterile-filtered PBS) into the medium of each well. After incubation for another 4 h, the medium was aspirated and the precipitated formazan was extracted with 200 μL of DMSO. The optical density (OD) value of the solution was measured using microplate reader (Bio-Rad 550) at 570 nm. The relative cell viability compared to the control cell culture was calculated as: Cell viability (%) = $(OD_{test}/OD_{control}) \times 100\%$, where $OD_{control}$ and OD_{test} were obtained in the absence and in the presence of polymeric micelles, respectively. All the testing was performed in triplicate.

2.5. Characterization

^{1}H NMR spectra were measured on a Bruker AM 400 (400 MHz) spectrometer using $CDCl_3$ or D_2O as the solvent. The size exclusion chromatography (SEC) measurements were carried out on a Waters 1515 system with a series of PS gel columns and refractive index detector. Tetrahydrofuran (THF) was used as the eluent at a flow rate of 1.0 mL/min. Monodispersed polystyrene standards were used to generate the calibration curve.

Size and size distribution were determined by dynamic laser light scattering (DLS) with a PSS Nicomp submicron particle analyzer (NICOMP 380 ZLS, Santa Barbara, California, USA). The mean diameter of the mixed micelles was obtained from the intensity distribution curves produced by the particle analyzer. The micelle solutions were filtered through a 0.45-μm cellulose membrane before analysis. The concentration of each sample was 1 mg/mL. Light transmittance was fixed at 633 nm with the scattering angle of 90°. The pH of the solution was adjusted by HCl or NaOH solution (1 M) and detected by a pH meter (640 pH, China).

The zeta potential of the mixed micelle solution was determined at 25 °C with a Malvern Instruments Nano-ZS Nanosizer.