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Dual-sensitive and folate-conjugated mixed polymeric micelles for controlled and targeted drug delivery

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ABSTRACT

For this study, we prepared a new type of drug carrier with the characteristics of stimuli-responsive transition and tumor-specific recognition through the co-assembly of two series of amphiphilic block copolymers, poly(ε-caprolactone)-*b*-poly[triethylene glycol methacrylate-*co*-*N*-methacryloyl caproic acid] and poly(ε-caprolactone)-*b*-poly[triethylene glycol methacrylate-*co*-*N*-(2-(methacrylamido)ethyl) folatic amide]. The pH-dependent thermal transition and the content of the targeting ligands of the mixed polymeric micelles are well correlated with the chemical structures and compositions of these two copolymers. Doxorubicin-loaded mixed polymeric micelles are stable at body temperature in the neutral condition for prolonged circulation in blood vessels, and demonstrated rapid drug release at acidic pH levels. The cumulative drug release profiles showed a relatively slow release at pH 7.4, and a quick release of 85% in 3 h at pH 5.3. The cytotoxicity tests against FA-positive (HeLa) and FA-negative (HT-29) tumor cell lines suggest that this mixed polymeric micelle system has potential merits as a controlled and targeted drug delivery system.

1. Introduction

Polymeric micelles have garnered considerable interest in the field of targeted and controlled drug delivery because of their promising potential as nanocarriers for both hydrophobic drugs and water-insoluble imaging probes [1–4]. This micelle-based nanocarrier system has a hydrophilic surface composed of biocompatible polymers and nanoscopic sizes of 10–100 nm, which allow them to avoid rapid renal filtration and undesired uptake by the reticuloendothelial system (RES) [5]. These features enable the drug-loaded polymeric micelles with prolonged circulation time in blood vessels as well as a passive target delivery through the enhanced permeability and retention (EPR) effect [6]. However, traditional polymeric micelles typically suffer from an inadequate drug release profile, in which the premature release of a drug from unstable micelles would cause a rapid drug clearance or undesired delivery to normal cells, whereas a slow drug release from highly stable micelles may result in reduced efficacy because of the low drug concentration at the pathological site.

Smart materials that display a significant and reversible property transition in response to specific stimuli have recently been employed to build stimuli-responsive polymeric nanocarriers capable of controlled drug release [7–9]. The spatiotemporal control of drug release from these nanocarriers could be triggered either by an environmental parameter (e.g., temperature, pH, or redox potential) [10–12] or by an external stimulus (e.g., light, a magnetic field, or ultrasound) [13,14]. Among the various types of stimuli, temperature is a critical parameter, and has been widely used to achieve controlled and site-specific drug release. Thermo-responsive nanocarriers have been designed based on temperature-sensitive polymers such as poly(N-isopropylacrylamide) (PNIPAAm) and poly(oligoethylene glycol acrylate) (POEGA) [15,16]. These polymers undergo a hydrophilic-hydrophobic transition at certain critical temperatures (lower or upper critical solution temperature, LCST or UCST), and this transition results in a disintegration or collapse of the thermo-responsive nanocarriers. Thus, the drug release from thermo-responsive nanoparticles can either be triggered by an abnormally high temperature (approximately 40 °C) at tumor tissues because of their proliferation [17,18] or be achieved in conjugation with hyperthermia treatment [19,20]. To obtain sophisticated polymeric nanocarriers for a more precise drug release and greater therapeutic efficacy, dual- and multi-stimuli responsive polymeric nanoparticles have been radically developed [21–23]. In our previous work, pH- and temperature-sensitive micelles that display a pH-dependent transition temperature have been employed to realize selective drug release in response to mildly acidic microenvironments at tumor sites [24].

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Polymeric micelles decorated with various targeting ligands have also been extensively explored to facilitate the selective internalization of carriers by a specific type of tumor cells through the receptor-mediated endocytosis [25,26]. Among various strategies using different combinations of targeting ligands and its corresponding receptors, folic acid (FA) is a well-known targeting ligand that can be easily recognized and internalized by numerous types of carcinoma cells overexpressed with the folate receptor (FR) [27–29]. The introduction of this targeting ligand has been routinely achieved by conjugating FA at the chain end of polymers, thereby constraining the facile manipulation of FA composition through the delicate design of drug carriers.

In this study, we report the development of a new mixed polymeric micelle system with the promising functionalities of dual sensitivity to temperature and the pH value as well as tumor-targeting ligands. These mixed polymeric micelles were co-assembled from two series of amphiphilic block copolymers, poly(ε-caprolactone)-*b*-poly[triethylene glycol methacrylate-*co*-N-methacryloyl caproic acid] [PCL-*b*-P(TEGMA-*co*-NMCA)] and poly(ε-caprolactone)-*b*-poly[triethylene glycol methacrylate-*co*-*N*-(2-(methacrylamido)ethyl) folatic amide] [PCL-*b*-P(TEGMA-*co*-NMFA)]. These two series of copolymers were prepared through post-functionalization from the same parent amphiphilic block copolymer, poly(ε-caprolactone)-*b*-poly[triethylene glycol methacrylate-*co*-N-hydroxysuccinimide methacrylate] [PCL-*b*-P(TEGMA-*co*-NHSMA)] (Scheme 1). The influences of the chemical structures of these copolymers and the composition of mixed polymeric micelles on their corresponding stimuli-responsive properties and nanostructures were investigated in this study. We loaded the mixed polymeric micelles with promising transition properties and different FA contents with an anticancer drug, doxorubicin (DOX), and the release profiles of the loaded drug were correlated with the stimuli-responsive characteristics of the micelles. We further demonstrate potential improvements in therapeutic efficacy by studying the in vitro cytotoxicity of DOX-loaded micelles against different types of tumor cell lines.

2. Experimental section

2.1. Materials

ε-caprolactone was distilled over CaH_2 under reduced pressure. Copper bromide (CuBr) was washed with acetic acid, methanol and ether and then dried under vacuum. Ether and triethylamine were distilled under N_2. Millipore purified water (18.2 MΩ cm) was used in all experiments. The (3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) (MTT) assay kits are commercially available from Invitrogen. All other chemicals and reagents were commercially available and used as received. Poly(ε-caprolactone)-*b*-poly[triethylene glycol methacrylate-*co*-N-hydroxysuccinimide methacrylate] [PCL-*b*-P(TEGMA-*co*-NHSMA), P1-P5] and poly(ε-caprolactone)-*b*-poly[triethylene glycol methacrylate-*co*-N-methacryloyl caproic acid] [PCL-*b*-P(TEGMA-*co*-NMCA), P1-ACA-P3-ACA] were prepared according to the literatures [24,30–32].

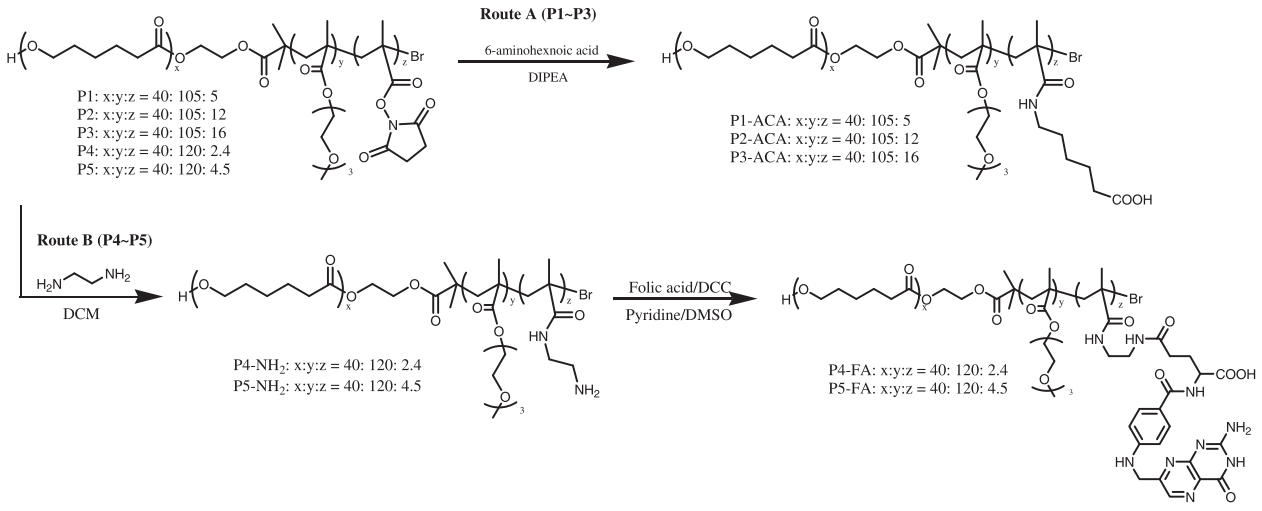
2.2. Synthesis of poly(ε-caprolactone)-b-poly[triethylene glycol methacrylate-co-N-(2-(methacrylamido)ethyl) folatic amide] [PCL-b-P(TEGMA-co-NMFA), P4-FA and P5-FA]

In a typical preparation of P5-FA, P5 (450 mg) and two equivalent amount of ethylene diamine relative to NHSMA of PCL-b-P(TEGMA-co-NHSMA) were weighed into a flask with dichloromethane, then N,N-diisopropylethylamine (DIPEA) was added under N_2, and allowed to react for 10 h. After removing solvents under reduced pressure, the reaction mixtures were purified by precipitating into diethyl ether twice to afford P5-amine (400 mg). ^{1}H NMR (400 mHz, $CDCl_3$, δ): 4.09 (br, $COOCH_2$), 3.67 (br, $COOCH_2CH_2OCH_2CH_2OCH_2CH_2OCH_3$), 3.57 (br, CH_2OCH_3), 3.38 (br, OCH_3), 0.75–2.09 (br, main chain CH_2, and CH_3).

In the second step, P5-amine (400 mg), folic acid and N,N'-dicyclohexylcarbodiimide (DCC) were dissolved in 1.4 mL DMSO (molar ratio of amine: folic acid: DCC = 1:1.5:1.8) in the presence of 0.55 mL pyridine. The mixture was stirred overnight in the dark at room temperature under nitrogen gas. Afterwards the mixture was dialyzed against DMSO to remove unreacted small molecules for three days and then lyophilized to get P5-FA (370 mg). $M_{n(GPC)}$ = 32.4 kDa, PDI = 1.68; $M_{n(NMR)}$ = 35.2 kDa, DP_{TEGMA} = 120, DP_{FA} = 4.5. $_1$H NMR (400 mHz, DMSO-d_6, δ): 6.4–8.5 (br, aromatic and amine protons of folic acid), 4.55 (br, CH_2NH), 4.40 (br, CONHCHCOOH), 4.09 (br, 2H, $COOCH_2$), 3.65 (br, $COOCH_2CH_2OCH_2CH_2OCH_2CH_2OCH_3$), 3.59 (br, CH_2OCH_3), 3.43 (br, 3H, OCH_3), 0.75–2.09 (br, main chain CH_2, and CH_3).

2.3. Preparation of polymeric micelles and encapsulation of doxorubicin

The empty or drug-loaded polymeric micelles were prepared by modifying reported protocols [33,34]. In brief, a solution of the



Scheme 1. Synthetic schemes for PCL-*b*-P(TEGMA-*co*-NMCA) (route A) and PCL-*b*-P(TEGMA-*co*-NMFA) (route B) through the post-functionalization of PCL-*b*-P(TEGMA-*co*-NHSMA).