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pH-Sensitive degradable polymersomes for triggered release of anticancer drugs: A comparative study with micelles

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ABSTRACT

pH-Sensitive degradable polymersomes and micelles were prepared based on diblock copolymer of poly (ethylene glycol) (PEG) and an acid-labile polycarbonate, poly(2,4,6-trimethoxybenzylidenepentaerythritol carbonate) (PTMBPEC). Polymersomes of PEG(1.9k)–PTMBPEC(6k) revealed average sizes of 100–200 nm. The acetals of polymersomes, similar to those of PEG(5k)–PTMBPEC(5.8k) micelles, though stable at pH 7.4 were prone to fast hydrolysis at mildly acidic pH of 4.0 and 5.0, with half lives of 0.5 and 3 d, respectively. The acetal hydrolysis resulted in significant size increase of polymersomes, to over 1000 nm in 24 h at pH 4.0. Drug encapsulation studies revealed that polymersomes were able to simultaneously load paclitaxel (PTX, hydrophobic) and doxorubicin hydrochloride (DOX·HCl, hydrophilic), whereas micelles loaded PTX only. Notably, polymersomes showed lower drug loading efficiencies for PTX than micelles (30.0–37.7% versus 61.4–65.2%). The *in vitro* release studies demonstrated that release of PTX and DOX·HCl from polymersomes was highly pH-dependent, *i.e.* significantly faster drug release at mildly acidic pH of 4.0 and 5.0 compared to physiological pH. Furthermore, much higher release rates were observed for PTX release from the polymersomes compared to that from the micelles under otherwise the same conditions. These pH-sensitive nano-sized degradable polymersomes hold great promise for combination therapy for cancers.

1. Introduction

In the past decade, polymersomes [1,2] have attracted rapidly growing interest due to their intriguing aggregation phenomena, cell and virus-mimicking dimensions and functions, as well as tremendous potential applications in medicine, pharmacy, and biotechnology [3–9]. As for liposomes, polymersomes have aqueous cores that are separated from the outside medium by the hydrophobic membranes, and can be used to deliver both hydrophilic and hydrophobic species such as anti-cancer drugs, therapeutic proteins and diagnostic probes. Unlike liposomes self-assembled from low molecular weight lipids, polymersomes are formed from macromolecular amphiphiles of various architectures including amphiphilic diblock [1,10,11], triblock [12,13], graft [14,15] and dendritic [16] copolymers. As a result, polymersomes usually display much higher stability as compared to liposomes.

Recently, stimuli-sensitive polymersomes have emerged as novel programmable delivery systems in which the release of drugs can be readily modulated by exerting an appropriate stimulus (e.g. temperature, pH, glucose, glutathione, etc.) [7,8,17]. The stimuli-responsive release may result in significantly enhanced therapeutic efficacy and minimal side effects. Among all applied stimuli, acidic pH as an internal stimulus is particularly appealing due to the mildly acidic pH encountered in tumor and inflammatory tissues as well as in the intracellular compartments such as endosomes and lysosomes of cells [18]. The existing tumoral pH variation has been considered as an ideal trigger for the selective release of anticancer drugs in tumor tissues and/or within tumor cells, accomplishing tumor-targeted drug delivery. For example, Armes and coworkers reported pH-sensitive polymersomes based on poly(2-(methacryloyloxy) ethyl phosphorylcholine)-*b*-poly(2-(diisopropylamino) ethyl methacrylate) for controlled release of DOX [19] and for DNA delivery [20]. Hubbell and coworkers reported that polymersomes based on PEG–PVP dissolve quickly when pH drops from 7.4 to 5.5 [21]. Deming and coworkers developed pH sensitive polymersomes based on polypeptides [22]. These polymersomes are, nevertheless, not readily degradable.

For drug delivery applications, usually degradable polymersomes are desired. Feijen and coworkers prepared biodegradable polymersomes from block copolymers based on poly(ethylene glycol) (PEG) and biodegradable polyesters or polycarbonates [11]. These biodegradable polymersomes were investigated as a basis for artificial cells for drug encapsulation and release [3]. Lee and coworkers reported biodegradable polymersomes of poly(2-hydroxyethyl aspartamide) grafted with lactic acid oligomers [15]. Discher and coworkers found that biodegradable polymersomes of PEG–PLA and PEG–PCL are self-porating, leading to controlled release of DOX [23]. Biodegradable polymersomes loaded with both PTX and DOX were reported to

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permeate and shrink tumor [24]. Recently, degradable polymersomes were applied as non-viral carriers for siRNA and antisense oligonucleotides [25], and mouse-anti-rat monoclonal antibody OX26 conjugated PEG–PCL polymersomes was investigated for peptide brain delivery [26]. It is worthy to note that none of these degradable polymersomes possess stimuli-sensitivity.

In this paper, we report on novel pH-sensitive nano-sized degradable polymersomes for triggered release of both hydrophilic and hydrophobic anticancer drugs (Scheme 1). These polymersomes were based on diblock copolymer of PEG and an acid-labile polycarbonate containing trimethoxybenzylidene acetals at the sides (PTMBPEC). Acid-labile acetals have been exploited by Frechet and coworkers for the development of rapidly pH-sensitive micelles, nanoparticles and hydrogels [27–33], and by Bulmus and coworkers for acid-labile polymeric core–shell particles and core-crosslinked micelles [34]. Trimethoxybenzylidene acetals are of particular interest because they show high sensitivity to mildly acidic pH and micelles containing trimethoxybenzylidene acetals exhibit low cytotoxicity [27–33]. Recently, we reported pH-responsive degradable micelles based on PEG–PTMBPEC for triggered release of hydrophobic anticancer drugs [35]. Interestingly, pH-sensitive degradable polymersomes developed in this study were able to release PTX and DOX·HCl in a controlled and pH-dependent manner, in which significantly faster drug release was observed at mildly acidic pH of 4.0 and 5.0 compared to physiological pH. Furthermore, release of PTX from polymersomes revealed a higher sensitivity towards mildly acidic pH compared to micelles. These pH-sensitive nano-sized degradable polymersomes hold great promise for combination therapy for cancers.

2. Materials and methods

2.1. Materials

Methoxy poly(ethylene glycol) (PEG, $M_n = 1900$ or 5000, Fluka) was dried by azeotropic distillation from anhydrous toluene. Dichloromethane (DCM) were dried under an argon atmosphere by refluxing over CaH_2 and distilled prior to use. Zinc bis[bis[trimethylsilyl]amide] (97%, Aldrich) was used as received. Doxorubicin hydrochloride (DOX·HCl) and paclitaxel (PTX) were obtained from Beijing Zhongshuo Pharmaceutical Technology Development Co. Ltd., and used as received. Mono-2,4,6-trimethoxybenzylidene-pentaerythritol carbonate (TMBPEC) was synthesized according to a previously reported procedure [35].

2.2. Synthesis of PEG–PTMBPEC diblock copolymers

PEG–PTMBPEC block copolymers were prepared by ring-opening polymerization of TMBPEC in DCM at 50 °C using PEG ($M_n = 1900$ or 5000) as an initiator and zinc bis[bis[trimethylsilyl]amide] as a catalyst, as reported previously. Typically, in a glove-box under a nitrogen atmosphere, to a solution of PEG ($M_n = 1900$, 0.2 g, 0.1 mmol) and TMBPEC (1.0 g, 2.94 mmol) in DCM (10 mL) was quickly added zinc bis[bis[trimethylsilyl]amide] (18 mg, 0.05 mmol). The reaction vessel was sealed and the polymerization was allowed to proceed at 50 °C under stirring for 8 d. The resulting PEG–PTMBPEC copolymer was isolated by twice precipitation from cold diethylether and dried in vacuo at r.t. Monomer conversion: 56%. ^{1}H NMR showed an M_n of 1900–6000. Similarly, using PEG 5000 as an initiator, PEG–PTMBPEC diblock copolymer with an M_n of 5000–5800 was prepared.

2.3. Preparation of polymersomes and micelles

The polymersomes and micelles were prepared by dropwise adding 4.0 mL of phosphate buffer (10 mM, pH 7.4) into 0.2 mL of dioxane solution containing 0.5 wt.% PEG–PTMBPEC block copolymer followed by dialysis overnight against phosphate buffer (10 mM, pH 7.4) with a molecular weight cut-off (MWCO) of 3500 at r.t. The final concentration of polymersomes and micelles was ca. 0.2 mg/mL.

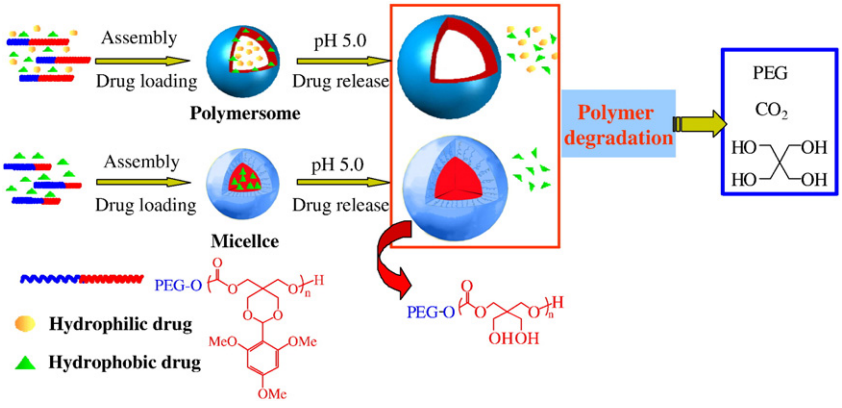
The critical aggregation concentration (CAC) was determined by using pyrene as a fluorescence probe. The concentration of block copolymer was varied from 2.0×10^{-5} to 0.2 mg/mL and the concentration of pyrene was fixed at 1.0 μM. The fluorescence spectra were recorded using FLS920 fluorescence spectrometer with the excitation wavelength of 330 nm. The emission fluorescence at 372 and 383 nm were monitored. The CMC was estimated as the cross-point when extrapolating the intensity ratio I_{372}/I_{383} at low and high concentration regions.

2.4. Determination of the pH-dependent hydrolysis rate of acetals in the polymersomes and micelles

The acetal hydrolysis was followed by UV/vis spectroscopy by measuring the absorbance at 290 nm, according to the previous reports by Frechet and coworkers. The polymersomes and micelles solutions (0.1 wt.%) were prepared and divided into three aliquots (2 mL). Their pH was adjusted to 4.0, 5.0 and 7.4, respectively, by addition of 50 μL of 4.0 M pH 4.0 and 5.0 acetate buffer or pH 7.4 phosphate buffer. The solutions were shaken at 37 °C. At desired time intervals, 80 μL aliquot was taken out and diluted with 3.5 mL phosphate buffer (0.1 M, pH 7.4). The absorbance at 290 nm was monitored.

2.5. Encapsulation and release of PTX

PTX was loaded into polymersomes and micelles by dropwise adding 8 mL of phosphate buffer (10 mM, pH 7.4) to 1 mL of dioxane



Scheme 1. Illustration of pH-sensitive degradable polymersomes based on PEG–PTMBPEC diblock copolymer for triggered release of both hydrophilic and hydrophobic anticancer drugs. In comparison, pH-sensitive degradable micelles are typically applied for release of hydrophobic drugs only.