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Facile construction of dual-bioresponsive biodegradable micelles with superior extracellular stability and activated intracellular drug release

Wei Chen [a,b], Fenghua Meng [a], Ru Cheng [a], Chao Deng [a], Jan Feijen [a,b,*], Zhiyuan Zhong [a,**]

[a] Biomedical Polymers Laboratory, Jiangsu Key Laboratory of Advanced Functional Polymer Design and Application, Department of Polymer Science and Engineering, College of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou 215123, PR China

[b] Department of Polymer Chemistry and Biomaterials, Faculty of Science and Technology, MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, P.O. Box 217, 7500 AE Enschede, The Netherlands

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ABSTRACT

It is still a major challenge for targeted cancer chemotherapy to design stable biodegradable micellar drug delivery systems which show a rapid and complete intracellular drug release. Here, reversibly core-crosslinked pH-responsive biodegradable micelles were developed based on poly(ethylene glycol)-poly(2,4,6-trimethoxybenzylidene-pentaerythritol carbonate-*co*-pyridyl disulfide carbonate) [PEG-P(TMBPEC-*co*-PDSC)] copolymers and investigated for intracellular doxorubicin (DOX) release. PEG-P(TMBPEC-*co*-PDSC) copolymers formed micelles with a small size of 58.6 nm were readily crosslinked by the addition of dithiothreitol (DTT). Notably, *in vitro* release studies showed that under physiological conditions only ca. 19.9% of DOX was released from the reversibly crosslinked micelles in 24 h at a low micelle concentration of 40 μg/mL. The release of DOX was accelerated at pH 5.0 or in the presence of 10 mM glutathione (GSH) at pH 7.4, in which 64.2% and 44.1% of DOX was released, respectively, in 24 h. The drug release was further boosted at pH 5.0 and 10 mM GSH, with 98.8% of DOX released in 12 h. Moreover, DOX release was also facilitated by a 4 h incubation at pH 5.0 followed by incubation at pH 7.4 with 10 mM GSH. Confocal microscopy indicated that DOX was delivered and released into the nuclei of RAW 264.7 cells following a 12 h incubation with DOX-loaded reversibly crosslinked micelles. MTT assays revealed that DOX-loaded reversibly crosslinked micelles had much higher antitumor activity than irreversibly crosslinked controls, with low IC_{50} values of 1.65 and 1.14 μg/mL for HeLa and RAW 264.7 cells, respectively, following a 48 h incubation. The blank crosslinked micelles had a low cytotoxicity of up to a concentration of 0.8 mg/mL. These reversibly crosslinked pH-sensitive biodegradable micelles with superior extracellular stability but activated intracellular drug release provide a novel platform for tumor-targeting drug delivery.

1. Introduction

Biodegradable polymeric micelles have been explored as one of the most promising drug delivery systems (DDSs) for targeted cancer chemotherapy, since they have overcome several problems that are associated with traditional hydrophobic anticancer drugs, such as low water solubility, nonspecific distribution and inefficient bioavailability in the body [1,2,3,4,5,6]. Furthermore, as drug carriers, polymeric micelles should be stable at low concentrations therefore achieving long *in vivo* circulation times and inhibiting premature drug release, and should have specific tumor targetability as well as fast and maximum drug release inside the target tumor cells [7,8,9,10]. The therapeutic efficacy of micellar drug-formulations would be drastically improved if these points could successfully be met.

In recent years, stimulus-responsive nanosystems that release payloads in response to an intrinsic biological signal have been designed and explored for enhanced cancer therapy [11,12,13,14]. For example, pH-sensitive polymers containing acid-labile groups such as ortho ester, hydrazone, cis-aconityl, and acetal have been widely studied for drug delivery applications, since the pH in the environment of tumor tissue is often 0.5–1.0 pH units lower than in normal tissue, whereas the pH in the intracellular endosomal/lysosomal compartments is as low as 4.0–6.5 [15,16]. Fréchet et al. reported that trimethoxybenzylidene acetals are rapidly hydrolysed at slightly acidic pH and that these entities can be incorporated in drug delivery systems for pH-triggered drug release [17,18]. We have designed poly(ethylene glycol)-poly(2,4,6-trimethoxybenzylidene-pentaerythritol carbonate) (PEG-PTMBPEC) block copolymers and prepared pH-responsive biodegradable micelles and polymersomes [19,20]. As compared to the

* Correspondence to: W. Chen, Biomedical Polymers Laboratory, Jiangsu Key Laboratory of Advanced Functional Polymer Design and Application, Department of Polymer Science and Engineering, College of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou 215123, PR China.
** Corresponding author.
E-mail addresses: j.feijen@utwente.nl (J. Feijen), zyzhong@suda.edu.cn (Z. Zhong).

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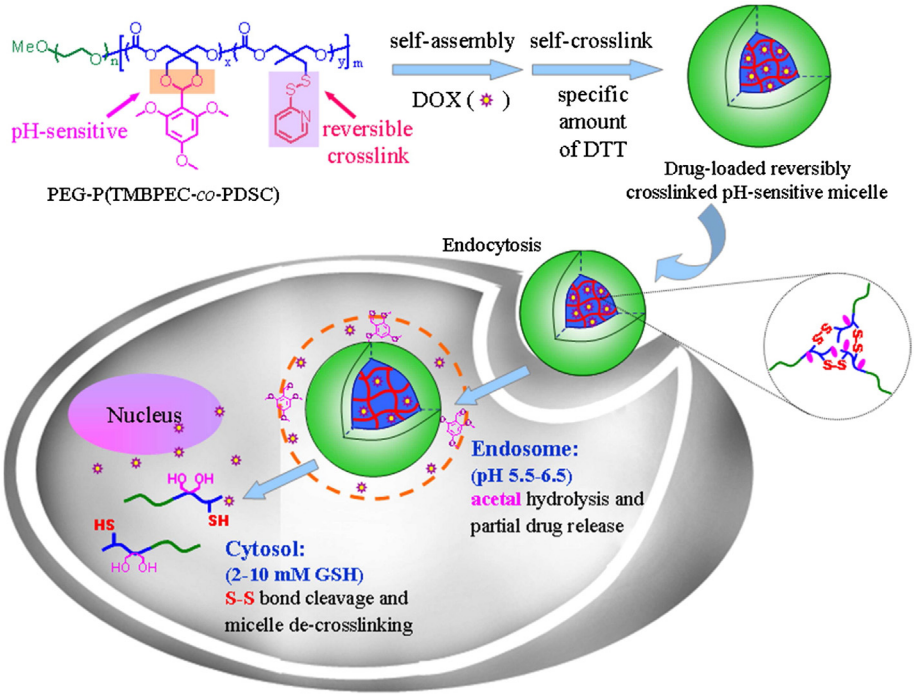
traditional aliphatic polyesters such as poly(ε-caprolactone) (PCL), polylactide (PLA), and lactide-glycolide copolymers (PLGA), PTMBPEC can be applied in nano-systems to induce fast swelling or even dissociation by hydrolysis of the acetal pendants at mildly acidic conditions. Furthermore, it has been demonstrated that hydroxy polycarbonates derived from pentaerythritol are prone to rapid degradation *in vitro* due to their highly hydrophilic nature [21]. Recently, we introduced disulfide bonds (S–S) into PEG-PTMBPEC copolymers (PEG-SS-PTMBPEC) to develop pH and reduction-sensitive micelles, which exhibit dually activated intracellular release behavior [22]. We also prepared pH-sensitive degradable chimeric polymersomes based on trimethoxybenzylidene acetals for high loading and triggered release of doxorubicin hydrochloride [23]. It should be noted, however, that these self-assembled polymeric nanocarriers, are often plagued by inadequate *in vivo* stability, which leads to premature burst drug release following i.v. injection, resulting in drug loss not only during storage, but also in the blood circulation causing increased side effects [24,25].

To improve the stability and to inhibit premature drug release, either the micellar core or the shell could be crosslinked. It has been demonstrated that crosslinked biodegradable micelles have advantageous properties like high drug loading efficiency, superior stability upon dilution, prolonged circulation time, and enhanced drug accumulation at the tumor site [26,27,28]. Very recently, we prepared photo-crosslinked pH-sensitive biodegradable micelles based on poly(ethylene glycol)-poly(2,4,6-trimethoxybenzylidene-pentaerythritol carbonate-*co*-acryloyl carbonate) [PEG-P(TMBPEC-*co*-AC)] copolymers, which had superior extracellular stability by UV crosslinking of AC units, and fast intracellular drug release through the acid-labile TMBPEC components [29]. These photo-crosslinked micelles provided with galactose units enhanced drug accumulation in the human hepatoma SMMC-7721 tumor and exerted more efficient antitumor activity, as compared to non-crosslinked micelles as well as non-targeted micelles [30]. It should be noted; however, that irreversible photo-crosslinking may induce incomplete drug release, and generate non-degradable polyacrylate components. In recent years, much attention has been given to the development of reversibly crosslinked polymeric micelles in that complete drug release can be obtained by de-crosslinking of the micelles [9,31]. In particular, crosslinking *via* disulfide bonds, which are prone to cleavage in the intracellular environment due to the presence of a high reduction potential in the cytoplasm as well as in the cell nucleus, is an attractive approach to construct reversibly crosslinked nanocarriers for triggered drug release [32,33]. A substantial number of reversibly crosslinked micelle systems using disulfide bonds have been prepared using cystamine and its derivatives as the reducible crosslinkers [34,35,36,37,38]. The reduction-sensitive reversibly crosslinked nanosystems can also be produced by oxidizing free thiol groups in the shell- or core-forming blocks [39,40]. We have developed disulfide-crosslinked micelles by conjugation of lipoic acid which can be self-crosslinked by ring-opening with a catalytic amount of DTT [41,42,43]. The self-crosslinking of pyridyl disulfide (PDS) units in polymers has emerged as another promising approach to develop reversibly crosslinked nanosystems. The PDS unit is highly reactive, but specific to thiols and provides disulfide-crosslinked structures by the thiol-disulfide exchange reaction under mild conditions [44,45,46,47]. For instance, Thayumanavan et al. developed reduction-sensitive self-crosslinked nanogels based on a random copolymer containing pendant oligoethyleneglycol and PDS side chains. The disulfide-crosslinked nanogels are of particular interest for intracellular protein release in that they on one hand possess superior colloidal stability and on the other hand are prone to rapid de-crosslinking and dissociation inside cells [48,49].

In this study, we report on the facile construction of reversibly crosslinked pH-responsive micelles from poly(ethylene glycol)-*b*-poly(mono-2,4,6-trimethoxy benzylidene-pentaerythritol carbonate-*co*-pyridyl disulfide carbonate) [PEG-P(TMBPEC-*co*-PDSC)] block copolymer, in which the core-forming TMBPEC units are acid-labile and PDS functionalities allow formation of reduction-sensitive crosslinks (Scheme 1). PEG-P(TMBPEC-*co*-PDSC) micelles were designed to be stabilized by disulfide crosslinking with minimal drug leakage during circulation while quickly and completely releasing payloads upon arrival inside the tumor cells. The synthesis, stability, *in vitro* drug release and tumor cell killing activity of DOX-loaded reversibly crosslinked PEG-(PTMBPEC-*co*-PDSC) micellar nanoparticles were investigated and the results were compared with those obtained using DOX-loaded irreversibly crosslinked PEG-P(TMBPEC-*co*-AC) micelles.



Scheme 1. Preparation of and activated intracellular drug release from reversibly crosslinked pH-sensitive biodegradable micelles.