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Smart pH-sensitive micelles based on redox degradable polymers as DOX/GNPs carriers for controlled drug release and CT imaging

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ABSTRACT

An amphiphilic copolymer poly(ε-caprolactone)-ss-poly(2-(dimethylamino) ethyl methacrylate), PCL-SS-PDMAEMA, was designed and synthesized using ROP and ARGET ATRP methods. Dual stimulus responsive micelles were prepared by the self-assembly of PCL-SS-PDMAEMA. PDMAEMA could respond to acid conditions with protonation, followed by enhanced hydrophilicity and swelling of the micellar shell. In addition, the cleavable joint disulfide bond between the core and shell was disrupted when exposed to an abundance of the reductant reductive glutathione GSH, leading to the disassembly of the micellar structure. The smart response behavior can be used for intracellular controlled drug release in tumor cells. In terms of "theranostics" with higher therapy effect, the tool for tumor imaging and diagnose through computed tomography (CT) was considered with the loading of gold nanoparticles (GNPs). GNPs with good distribution were prepared by means of *in situ* reduction by PDMAEMA block and stabilized by the micelles. Polymeric micelles were used to load the anticancer drug doxorubicin (DOX) in the hydrophobic core and GNPs in the hydrophilic PDMAEMA shell. Subsequently, the micellar theranostics platform combining chemotherapy and CT diagnose was obtained. The pH- or redox-triggered drug release profiles suggesting that the DOX/GNPs-loaded micelles facilitated controlled release in response to different simulated microenvironments. Cellular uptake study was carried out, indicating that the micelles could be fast internalized within several hours. MTT assay showing significant inhibition against HepG2 and MCF-7 cells for the DOX/GNPs-loaded micelles. Finally, the *in vitro* CT imaging assay indicated the good CT diagnosis potential of DOX/GNPs-loaded micelles. The micelle simultaneously loaded with DOX and GNPs represent a promising theranostics platform for efficient cancer chemotherapy and diagnosis.

1. Introduction

With the development of nanotechnology in the past decades, many kinds of inorganic and organic materials have been widely studied for biomedical application [1–4]. Recently, micellar systems have drawn much attentions for their unique properties and potential application in cancer chemotherapy, imagination and diagnosis [5–13]. Comprising a hydrophobic core and hydrophilic shell, the micellar architectures can maintain a stable self-assembled structure and enhance the solubility of hydrophobic anticancer drug in aqueous solution. Moreover, polymeric micelles with particle sizes less than 200 nm can easily take advantage of the enhanced permeation and retention (EPR) effect for passive targeted delivery and accumulation in solid tumors [14–17]. These advantages make polymeric micelles pretty drug delivery performance in cancer chemotherapy.

However, the micellar drug release at the tumor site is a typically slow and uncontrollable progress, followed by a failure in conquering the multidrug resistance (MDR) and decline in therapy efficiency [18–21]. To accelerate the intracellular drug release with high drug concentrations, many studies focused on the smart stimulus sensitive polymer carriers. To this end, multi-stimulus was used for smart drug delivery and release, such as pH [22–26], redox potential [27–31], light [32,33], enzyme [34,35], etc. Introducing these stimulus-responsive sites in amphiphilic copolymers could generate smart micellar systems for burst intracellular drug release in tumor cells, resulting in high intracellular drug concentrations. The intracellular tumor microenvironment showed obvious differences with normal cells, particularly in the pH values and concentration of reductive glutathione (GSH) [36,37].The pH values outside tumor cells appears acidic (6.5–6.8), and the pH value is even lower in endosomes and lysosomes (4.5–6.0), while

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the pH of normal tissue is typically 7.2–7.4, such as in the bloodstream [38,39]. In addition, the GSH concentration in tumor cells (approximately 2–10 mM) is much higher than that in the extracellular fluids (approximately 2–20 μM) [40–42]. The differences in pH value and GSH concentration between intracellular and extracellular conditions could be well used for designing and application of stimulus-sensitive micellar architectures, and to realize the fast release and accumulation of drugs. We have previously reported the pH sensitive behavior of the polymeric micelles containing PDEAEMA or PDMAEMA blocks, and resulted in a slow release rate of DOX at pH 7.4 but an overtly fast release at pH 5.0 [43–45]. Zhong's group recently reported the reduction of responsive biodegradable micelles based on poly(ethyleneglycol)-SS-poly(ε-caprolactone) (PEG-SS-PCL) copolymers efficiently released DOX in reductive conditions (10 mM reductive dithiothreitol), achieving significantly enhanced anticancer efficacy [46–48]. Studies on redox-sensitive micelles from Ji and Oh's groups similarly concluded that introducing an acid or redox-sensitive site in micellar architectures can realize fast intracellular drug release to overcome the MDR of tumor cells [49–53].

Compared with single-stimuli repsonsding, multi-stimulus responding owned more efficient potential to enhanced the intracellular drug release in tumor cells, and the combination of pH and redox stimulus in tumor cells would be a good strategy. Zhong's group obtained a pH/redox dual stimulus responsive copolymer PEG-SS-PTMBPEC [41]. The copolymer contained pH responsive site (acetal) at the side chain of core-forming block and redox sensitive group (disulfide) on the copolymer backbone. Their work made use of pH and redox dual stimulus to trigger the degradation (disassembly) of micelles, enhancing the intracellular drug release in tumor cells. The drug release content was up to 62.8% (21 h) at pH 5.0, while the release content at pH 5.0 in the presence of 10 mM GSH was significantly accelerated and as high as 94.2% (21 h). Lee's group manipulated the pH- and redox-sensitive performance within a micellar architecture based on the copolymer poly(β-amino ester)-grafted-disulfide methylene oxide poly(ethylene glycol), PAE-*g*-DSMPEG, which contains tertiary amine in the backbone and a disulfide bond in the side chain. When exposed to acid and high reductive dual stimulus, the micelle swelled and disassembled, resulting in fast drug release and high drug accumulation as well [54].

Currently, the term "theranostics" encompasses two distinct definitions, the therapeutics combined with diagnosis on a single platform [55–57]. In addition to chemotherapy, cancer diagnosis strategies, such as MRI, CT and ultrasonic imaging, were also introduced for accurate therapy to increase the treatment effect. Particularly, gold nanoparticles (GNPs) showed great potential for the unique physical, chemical and biological properties of these molecules [58,59], and has been used in biomedical fields with many applications [60–62]. However, GNPs tend to aggregate together to obtain a large surface area and increase surface activity. Moreover, the traditional methods for the synthesis of GNPs using reducing reagents ($NaBH_4$, etc.) and thio-functional stabilizers may lead to the generation of small molecular byproducts and relatively poor stability of Au-S bonds under light conditions and high temperature [63–65]. Recent reports have suggested that GNPs can be fabricated in one step using certain copolymers that act as both reducing agents and stabilizers without the drawbacks mentioned above [66–68]. Arm's group [69] synthesized the copolymer PDMAEMA-*b*-PMPC and observed that PDMAEMA block could reduce $AuCl_4^-$ to zero-valent gold *in situ* without any external reducing reagents in aqueous solution. The mechanism was that the tertiary amino groups on the PDMAEMA block reduced the metal salt of gold ($HAuCl_4$) from Au (III) to zero-valent Au (0) atom, then the Au atoms grew and gathered into GNPs. McCormick's group [70] also used the PDMAEMA block for the *in situ* reduction and formation of GNPs, resulting in polymeric vesicles based on the copolymer PDMAEMA-*b*-PNIPAM. All GNPs maintained good stability and did not aggregate.

For the purpose of cancer theranostics, our group obtained well-distributed GNPs by *in situ* redution with unimolecular micelles recently, and then the therapy drug DOX was loaded as well to build a theranostics platform. The unimolecular micelles owned relative high thermodynamic stability, pH controlled drug release and *in vivo* CT imaging capacity [71]. Combining the pH/redox dual stimulus for efficient drug release in Zhong's group and the theranostics consideration in our previous work by Lin, we designed a pH/redox dual sensitive multimolecular micelle for DOX and GNPs loading as a promising theranostics platform. In this work, we designed and synthesized an amphiphilic copolymer PCL-SS-PDMAEMA using ring opening polymerization (ROP) and continuous activators regenerated by electron transfer atom transfer radical polymerization (ARGET ATRP) in the present study. The pH reponsive structure (tertiary amine) was introduced to the side chain of the shell-forming block, and the redox-cleavable joint disulfide bond was located at the copolymer backbone. The copolymer could self-assemble and form micelles with PCL core and PDMAEMA shell. The PCL core acted as a container for hydrophobic drug molecules, such as DOX, and the PDMAEMA shell provided micellar architecture stability in an aqueous solution. The core-shell micelle owned good drug release in tumor cells with pH-triggered swelling of PDMAEMA shell and redox-triggered degradation of the backbone. Moreover, PDMAEMA also played an important role in the in situ reduction of $AuCl_4^-$ and stabilization of the formed GNPs (Scheme 1). Subsequently, the hydrophobic property and drug loading capacity of the micellar core were studied, and the self-assembly of the copolymer in aqueous solution were also investigated using DPD simulation. In addition, DOX/GNPs-loaded micelles were prepared, and their *in vitro* drug release profiles were confirmed in different physiological environments with various pH values and GSH concentrations. Furthermore, CLSM studies used to study the cellular uptake of DOX-loaded micelles using HepG2 cells as a model, and MTT assays also to evaluate the anti-tumor activities of these micelles. Finally, CT imaging in vitro based on the DOX/GNPs-loaded micelles was also performed.

2. Experimental methods

2.1. Synthesis of the macroinitiator PCL-SS-iBuBr

The dual functional initiator 2-hydroxyethyl-2′-(bromoisobutyryl) ethyl disulfide (HO-SS-iBuBr) was synthesized as described in **Supporting information**. The macroinitiator PCL-SS-iBuBr was synthesized by ring opening polymerization (ROP) of ε-CL initiated by HO-SS-iBuBr, in which $Sn(Oct)_2$ was as the catalyst. In detail, the initiator HO-SS-iBuBr (304 mg, 1 mmol) was added to a flame-dried Schlenk flask. Subsequently, measured amounts of the degassed monomer ε-CL and $Sn(Oct)_2$ (0.1 wt% of ε-CL) with anhydrous toluene (2 mL) was added, followed by evacuation and flushing three times with argon. Three 'freeze-pump-thaw' cycles were performed to remove any oxygen. Finally, the flask was heated to 130 °C for 48 h. The crude polymer was dissolved in THF, precipitated in a water/methanol (1:1, v/v) mixture, and then dried in vacuum (yield: 91%).

2.2. Synthesis of the amphiphilic copolymer PCL-SS-PDMAEMA

The macroinitiator PCL-SS-iBuBr (1.22 g, 0.2 mmol) and $CuBr_2$ (5 mg, 0.02 mmol) were placed in a flame-dried Schlenk flask, followed by evacuation and flushing three times with argon. HMTETA