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pH-sensitive doxorubicin-conjugated prodrug micelles with charge-conversion for cancer therapy

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ABSTRACT

Intelligent drug delivery systems with prolonged circulation time, reduced drug leakage in blood, target site-triggered drug release and endosomal escape are attractive and ideal for malignant tumor therapy. Herein, doxorubicin (DOX)-conjugated smart polymeric micelles based on 4-carboxy benzaldehyde-grafted poly (L-lysine)-block-poly (methacryloyloxyethyl phosphorylcholine) (PLL(CB/DOX)-b-PMPC) copolymer are prepared. DOX and electronegative 4-carboxy benzaldehyde are conjugated to the PLL block via an imine linkage and as a result, the drug loaded micelles exhibited the pH-triggered charge-conversion property and accelerated drug release at tumor pH. *In vitro* cytotoxicity studies of these DOX-loaded micelles exhibited great tumor inhibition against HeLa and 4T1 cells. Moreover, in mice models of breast cancer, these DOX-loaded micelles showed better anti-tumor efficacy and less organ toxicity than free drug. In summary, these polymeric micelles could be applied as potential nanocarriers for cancer therapy.

Statement of Significance

As a typical anti-cancer drug, Doxorubicin (DOX) exhibited remarkable tumor inhibition but was limited by its low drug utilization and strong toxicity to organs. To overcome these challenges, we developed a DOX-conjugated polymeric micelle as a nano drug carrier which was endowed with pH-sensitivity and charge-conversion function. The structure of micelles would quickly disintegrate with surface charge-conversion in acidic environment, which would contribute to the endosomal escape and accelerated drug release. These DOX-conjugated micelles would provide a promising platform for the efficient DOX delivery and better anti-cancer efficiency.

1. Introduction

Nano-scale polymeric micelles with anticarcinogen loading have been introduced as a robust strategy for cancer therapy over the past two decades, on account of their enhanced tumor inhibition and minimized adverse effects. Due to the enhanced permeability and retention (EPR) effect, nano-carrier with an appropriate size of <200 nm can accumulate in tumor tissue during circulation *in vivo* and effectively deliver water insoluble antitumor drugs to obtain better anticancer effect [1–3]. As an effective drug delivery system (DDS), desired polymeric micelles should possess several typical functions such as escaping from rapid renal clearance by reticuloendothelial system (RES), maintaining longevity in blood stream, rapid cargo delivering to the targeted location and degrading into ingredients without biotoxicity [4–6]. However, traditional polymeric micelles with drug loading via hydrophobic effect are limited by drug leakage during circulation. Meanwhile, nano drug carriers follow the receptor-mediated endocytosis, which cannot efficiently deliver payload intracellularly due to the entrapment by endosomal pathway [7–9].

To keep the micellar structure stable in blood stream and increase the drug-loading efficiency, prodrugs provide an attractive strategy to design efficient drug carriers with neglectable drug leakage and high drug-loading capacity [5,10–12]. Meanwhile, smart polymeric micelles which could response to the specific microenvironment of tumor tissue and tumor cell would be desirable [13,14]. Several approaches have been employed to introduce stimuli-responsive features to the polymeric micelles such as redox, pH and temperature responses, demonstrating the improved

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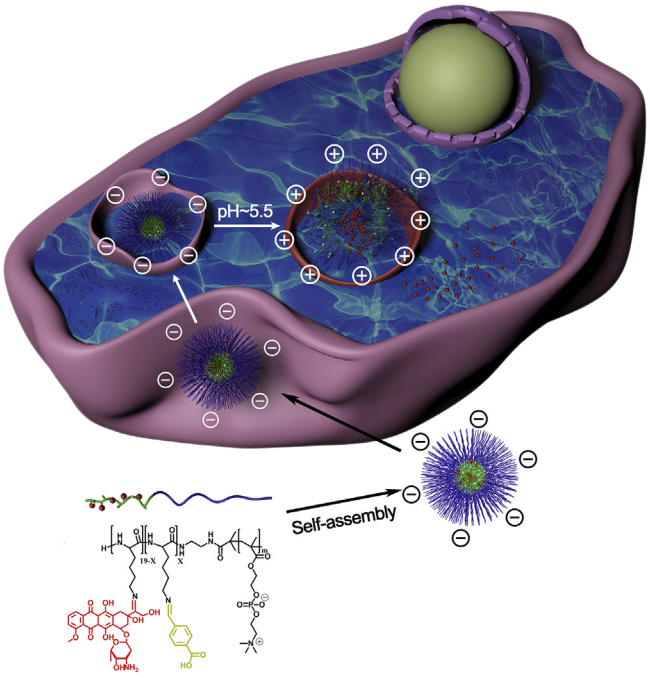
therapeutic effects and reduced side effects [1,13,15–18]. Among these stimuli, pH-responsiveness is frequently employed since tumor acidity at tumor tissues (pH < 6.8) and endosome/lysosome (pH < 5.5) are tremendously different from physiological pH (7.4) [18–20]. For instance, Kataoka et al. constructed pH-sensitive prodrug micelles conjugating DOX via hydrazone bonds which are stable at neutral pH but could quickly cleave at endosomal pH (∼ 5.5), leading to irruptive drug release[10]. Except hydrazone, a larger scale of pH-sensitive bonds has also been introduced for designing smart micelles conferring them rapid bond breakage at tumor pH (e.g., acetal, imine and orthoester) [11,21–24].

With pH-triggered structural disintegration and drug release, efficient prodrug micelles should also be able to escape from the lysosomal entrapment in endosomal pathway. Thus, "proton sponge", such as tertiary amine groups and histidine-rich molecules [25–27], was introduced to nano-carriers, leading to an extensive inflow of ions and water into the endosomal environment which subsequently resulted in the rupture of endosomal membrane and release of entrapped drugs [7]. Considering good hemocompatibility, the amino groups were first sheltered by micromolecules such as 2,3-dimethylmaleic anhydride or 4-carboxy benzaldehyde. At tumor acidic environment, the micromolecules were taken off and the amino groups were spontaneously exposed [28,29].

In this work, poly (2-methacryloyloxyethyl phosphorylcholine) (PMPC) was selected as the hydrophilic segment with zwitterionic phosphorylcholine for its outstanding biocompatibility, impressive biostability and fruitful resistance to protein adsorption [30–34], which were attributed to its high hydration by ionic solvation. On the other hand, poly (L-lysine) (PLL) was chosen as the functional segment, which was derived from natural α-amino acids and showed remarkable biodegradability *in vivo* by specific enzymes [35–39]. Hydrophobic DOX and 4-carboxy benzaldehyde were conjugated to PLL segment via the pH-sensitive imine linkage (expressed as PLL(CB/DOX)). PLL(CB/DOX)-b-PMPC copolymers could self-assemble into core-shell structural micelles in water which were stable in physiological environment. However, when transferred to acidic environment (pH ∼ 5.5), the imine linkage between the PLys and BA/DOX was broken with the exposure of electropositive amino and as a result, DOX was set free and released into cytoplasm as well as the charge-conversion (Scheme 1). Several *in vitro* and *in vivo* characteristics were performed and the indexes indicated a great pH-sensitive antitumor efficacy of these prodrug micelles, which would be potential nanocarriers for cancer therapy.



Scheme 1. Illustration of PLL(CB/DOX)-b-PMPC prodrug micelles with their pH-triggered charge-conversion and drug delivery.

2. Materials and methods

2.1. Materials

2-Methacryloyloxyethyl phosphorylcholine was purchased from Nanjing Natural Science and Technology Institute. 4-carboxy benzaldehyde, 2,2′-Dipyridyl (bpy), cuprous bromide (CuBr) and doxorubicin hydrochloride (DOX·HCl) were purchased from Chengdu Best Reagent Co., LTD (Chengdu, China). Dimethyl formamide (DMF) (from Chengdu KeLong Chemical Reagent Company (Chengdu, China)) were purified by distillation. All other reagents and solvents were purchased from Chengdu KeLong Chemical Reagent Company (Chengdu, China) and used without further purification.

2.2. Synthesis of poly (N6-carbobenzyloxy-L-lysine)-block-poly (methacryloyloxyethyl phosphorylcholine) (PLLs-b-PMPC) copolymer

Amphiphilic PLLs-b-PMPC copolymer was synthesized by atom transfer radical polymerization (ATRP). The macroinitiator poly (N6-carbobenzyloxy-L-lysine) (NH_2-PLLs-Br) was synthesized according to our previous report [40]. Typically, macroinitiator (1 g, 0.2 mmol) and MPC (1.5 g, 5 mmol) were dissolved in 40 mL of DMSO and methanol (1/1, v/v) in a schlenk flask. After three cycles of freeze-pumpthaw procedure, CuBr (28.8 mg, 0.2 mmol) and bpy (62.4 mg, 0.4 mmol) were added quickly into the flask under the protection of argon. The reaction was performed at 40 °C for 48 h with argon protection. The catalyst was removed by passing a neutral aluminum oxide column with the solvent of DMF/MeOH (1:2, v/v) as the eluent. The solution was concentrated by rotary evaporation and precipitated into excess cold ethyl ether. The final PLLs-b-PMPC copolymer was obtained by drying in vacuum at room temperature overnight.

2.3. Synthesis of 4-carboxy benzaldehyde-modified poly (lysine)-block-poly (methacryloyloxyethyl phosphorylcholine) [PLL(CB)-b-PMPC] copolymer

The deprotection of the carbobenzyloxy group was performed by treating the PLLs-b-PMPC (1.5 g, 0.11 mmol) with TFA (13 mL), HBr (0.8 mL) and acetic acid (1.2 mL) for 12 h at room temperature. The resulting solution was concentrated and dialyzed against deionized water (MWCO = 8000) for 48 h, followed by lyophilization to get deprotected PLL-b-PMPC.

PLL(CB)-b-PMPC copolymers were synthesized by a Schiff's based reaction. Particularly, PLL-b-PMPC (0.28 g, 0.02 mmol) and 4-carboxy benzaldehyde (40 mg, 0.27 mmol) were added in a dry flask with 3 mL of triethylamine and 20 mL of DMF and methanol (1/1, v/v) and allowed to react for 48 h at 35 °C. The product was purified by dialysis against deionized water (MWCO = 8000) for 24 h followed by freeze-drying.

2.4. Preparation of DOX-conjugated prodrug [PLL(CB/DOX)-b-PMPC] micelles

Hydrophobic DOX was first obtained by neutralizing DOX·HCl with triethylamine in DMF. Then 8 mg DOX (in 2 mL DMF) and