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Self-reinforced endocytoses of smart polypeptide nanogels for "on-demand" drug delivery

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ABSTRACT

The pH and reduction dual-responsive polypeptide nanogels with self-reinforced endocytoses were prepared through ring-opening polymerization of L-glutamate *N*-carboxyanhydrides, deprotection of benzyl group and subsequent quaternization reaction between γ-2-chloroethyl-L-glutamate unit in polypeptide block and 2,2′-dithiobis(*N*,*N*-dimethylethylamine). The nanogels were revealed to exhibit smart pH and reduction dual-responsiveness, and excellent biocompatibilities, which expressed great potential as antitumor drug nanocarriers. Doxorubicin (DOX) as a model antitumor drug was loaded into nanogels through dispersion. DOX-loaded nanogels displayed a stable core-cross-linked structure under normal physiological condition (pH 7.4), while rapidly releasing the payloads in the mimicking endosomal (pH 5.3), tumor tissular (pH 6.8) or intracellular reductive microenvironments (10.0 mM glutathione). Confocal fluorescence microscopy demonstrated that DOX-loaded nanogels could deliver DOX into HepG2 cells (a human hepatoma cell line) more efficiently than the parent DOX-loaded micelle and free DOX. The enhanced cellular internalizations of DOX-loaded nanogels were more significant under tumor tissular acidic condition (pH 6.8) ascribed to the quaternary ammonium groups in the cores. In addition, DOX-loaded nanogels exhibited improved *in vitro* and *in vivo* antitumor activities, and *in vivo* securities compared with DOX-loaded micelle and free DOX. These excellent features of the smart nanogels with quaternary ammonium groups were endowed with a bright prospect for intracellular targeting antitumor drug delivery.

1. Introduction

In the past decades, multifarious polymeric nanogels have emerged as one kind of the most fascinating nanocarriers for the controlled delivery of small molecular antitumor drugs, such as doxorubicin (DOX) [1–3], paclitaxel (PTX) [4] and docetaxel [5], to improve the therapeutic efficacies and eliminate the organism adverse effects. Recently, the stimuli-responsive nanogels, which sensitively respond to the tumor tissular or intracellular microenvironmental triggers including pH [6–8], redox [1,9,10], enzymes [11,12] *etc.*, have attracted tremendous attentions. As soon as the targeted lesion sites are reached, the smart nanogels can specifically and rapidly release the payloads triggered by the tissular or intracellular stimuli, leading to maximal chemotherapeutic efficacies with slight side effects [13].

In all current intelligent nanogels, the enormous variations of pH and redox potential are the most common stimuli to induce the antitumor drugs to abruptly release from cargos [2,13,14]. Taking the microenvironments of both pathological tissues and intracellular compartments into account, the pH and reduction dual-responsive nanogels are promising for controlled antitumor drug delivery [15–17]. As compared with the normal physiological conditions (pH ~ 7.2–7.4), the microcircumstances of solid tumors present acidic (pH ~ 6.8–7.2) due to the Warburg effect [18,19], that is, the mildly acidic conditions should be ascribed to an excess of lactic acid produced by the incomplete conversion of glucose to energy in malignant cells. The intracellular compartments also render various acidic conditions. Once accumulated in tumor tissues, the nanogels will be internalized into cellular clathrin-coated vesicles that fuse to form endosomes (pH ~ 5.0–6.5) and eventually lysosomes (pH ~ 4.0–5.0) [13,20]. In addition, the intracellular reductive glutathione (GSH) concentration (~0.5–10.0 mM), especially in tumor cells, is reported to be around three orders of magnitude higher than that in extracellular fluids (~10.0–40.0 μM) [21].

In view of the marked alterations of pH and GSH concentration among blood circular, tumor tissular and intracellular conditions, the pH and reduction dual-responsive nanogels containing both "titratable" groups (*e.g.* amino and carboxyl groups) and reductive cleavage linkages (*e.g.* disulfide and diselenide bonds) begin to be exploited as unique "on-demand" drug delivery platforms for a

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variety of antitumor molecules. For instance, Chen's group has developed two kinds of disulfide core-cross-linked poly(ethylene glycol)-polypeptides nanogels with ionizable cores for efficient DOX or DOX derivatives loading and intracellular delivery [2,13]. Wooley and co-workers have exploited the pH and reduction dual-responsive PTX-loaded polymeric nanogels with an enzymatically and hydrolytically degradable poly(lactic acid) core and a disulfide cross-linked poly(acrylic acid) and poly(oligoethylene glycol) containing corona [12].

Two requisites should be followed to fabricate the nanogels as ideal nanocarriers, that is, 1) "on-demand" drug delivery induced by the stimuli in the lesion sites, and 2) well biocompatible and opportunely biodegradable matrices [22,23]. Recently, several environment-friendly and organism-friendly synthetic polymers, such as aliphatic polyesters and polypeptides, have been applied to construct promising nanogels [22,24–26]. Among those, synthetic polypeptides are one kind of the most important potential biomaterials, which have precise secondary conformations and have been widely studied for various biomedical applications, such as drug and gene delivery and tissue engineering [27–32].

Presently, the pH and reduction dual-responsive polypeptide nanogels were fabricated through a two-step approach. First, methoxy poly(ethylene glycol)-*b*-poly(L-glutamic acid-*co*-γ-2-chloroethyl-L-glutamate) (mPEG-*b*-P(LGA-*co*-CELG)) was synthesized by the ring-opening polymerization (ROP) of γ-benzyl-L-glutamate *N*-carboxyanhydride (BLG NCA) and γ-2-chloroethyl-L-glutamate *N*-carboxyanhydride (CELG NCA) with mPEG-based macroinitiator (mPEG-NH_2), and then elimination of protecting benzyl groups. Subsequently, the nanogels with quaternary ammonium groups were prepared through a quaternization reaction between chlorine in CELG unit and nitrogen from cross-linker. The quaternary ammonium groups, which exhibited excellent activity to penetrate the outer membranes of many cell types, endowed the nanogels with significant self-reinforced endocytoses [15,33,34]. The pH and reduction dual-responsiveness, and excellent biocompatibilities of nanogels were demonstrated. DOX was loaded into nanogels, and DOX-loaded nanogels exhibited accelerated DOX release in the endosomal (pH 5.3), tumor tissular (pH 6.8) and intracellular reductive microenvironments (10.0 mM GSH). The enhanced cellular internalizations of nanogels as well as the increased DOX release in the cytoplasms resulted in the efficient intracellular DOX delivery. Furthermore, the *in vitro* and *in vivo* superior antitumor efficacies, and *in vivo* securities of DOX-loaded nanogels were systemically confirmed.

2. Materials and methods

2.1. Preparations of polypeptide nanogels

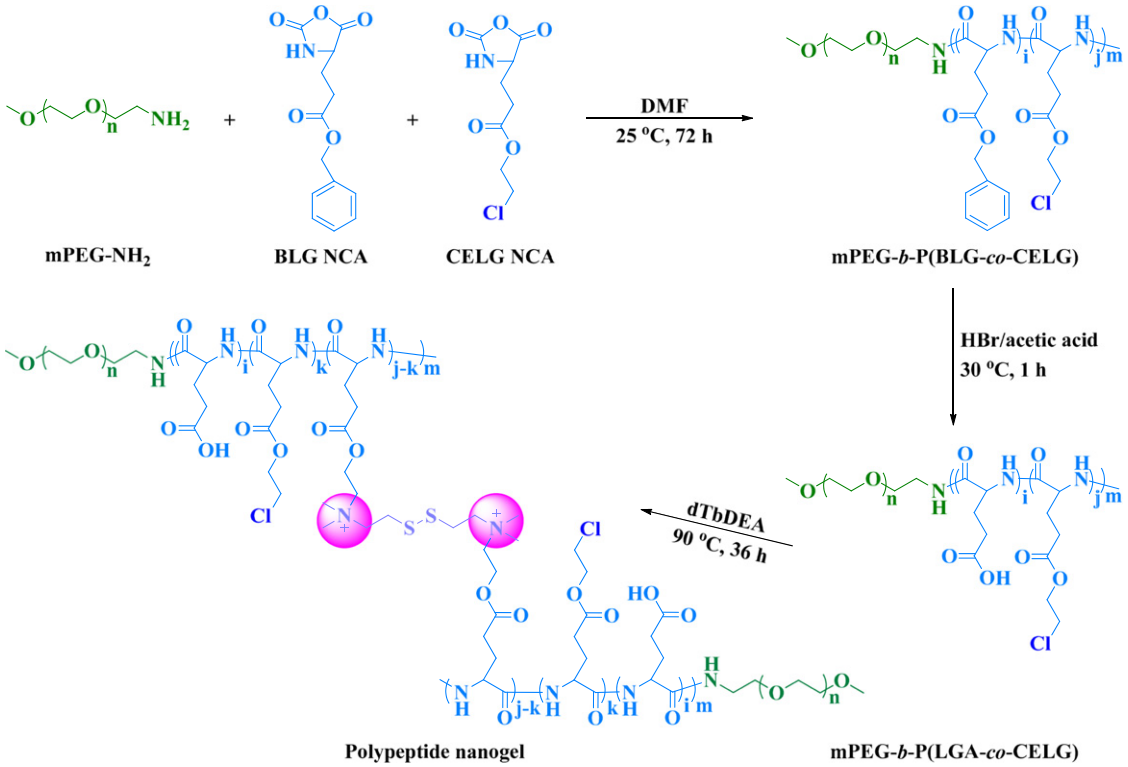
As shown in Scheme 1 and depicted in Supporting information, mPEG-*b*-P(LGA-*co*-CELG) was first synthesized by the ROP of BLG NCA and CELG NCA with mPEG-NH_2 as macroinitiator, and then deprotection of benzyl group [35]. Subsequently, the polypeptide nanogels were prepared through quaternization reaction between CELG unit and 2,2′-dithiobis(*N*,*N*-dimethylethylamine) (dTbDEA).

2.2. Characterizations

The characterizations of chemical structures and compositions of copolymers and nanogels, and the properties and biocompatibilities of micelle and nanogels were systemically performed and detailed in Supporting information.

2.3. DOX loading and release

DOX-loaded micelle and nanogels were prepared by a dialysis technique. Typically, mPEG-*b*-P(LGA-*co*-CELG) or nanogel (20.0 mg), doxorubicin hydrochloride (DOX·HCl) (4.0 mg) and triethylamine (0.72 mg) were mixed in 4.0 mL of *N*,*N*-dimethylformamide (DMF). The solution was allowed to stand at 25 °C for 2 h. And then, 2.0 mL



Scheme 1. Synthesis pathway for mPEG-*b*-P(LGA-*co*-CELG) and preparation approach of polypeptide nanogel.