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# Reduction-responsive polypeptide nanogel delivers antitumor drug for improved efficacy and safety

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ABSTRACT

Chemotherapy plays an irreplaceable role in the treatment of various malignant tumors today. The traditional drug formulations lack of selectivity, cause serious damage to normal tissues, and can't achieve a desired therapeutic efficacy. For this situation, a facilely prepared reduction-responsive polypeptide nanogel was employed for targeting intracellular delivery of antitumor drug in this study. Doxorubicin (DOX) as a model drug was loaded into nanogel through a sequential dispersion and dialysis approach with a drug loading efficiency (DLE) of 56.8 wt.%. The loading nanogel, *i.e.*, NG/DOX, exhibited a medium hydrodynamic radius of 56.1 ± 3.5 nm, glutathione-accelerated DOX release, and efficient cellular uptake and proliferation inhibition. Moreover, NG/DOX exhibited upregulated intratumoral accumulation and improved antitumor efficacy toward HepG2 hepatoma-xenografted BALB/c nude mouse model compared with free drug. The enhanced tumor suppression of NG/DOX was further confirmed by the histopathological and immunohistochemical analyses. Furthermore, the excellent *in vivo* security of NG/DOX was systematically demonstrated by the variation detection of body weight, histopathological assay, levels of bone marrow cell micronucleus rate (BMMR) and white blood cells (WBCs), and detection of clinical parameters in corresponding organs and serum. With controllable large-scale preparation and fascinating properties *in vitro* and *in vivo*, the reduction-responsive polypeptide nanogel is revealed to exhibit great potential for on-demand intracellular delivery of antitumor drugs, and shows a good prospect for clinical chemotherapy.

**Statement of Significance**

The traditional drug formulations lack of selectivity, cause serious damage to normal tissues, and can't achieve a desired therapeutic effect. For this situation, a facilely prepared reduction-responsive polypeptide nanogel is employed for targeting intracellular delivery of antitumor drug in this study. The laden nanogel keeps structural integrity and less drug release in the circulatory system after intravenous injection, releases the payload triggered by the intracellular high concentration of GSH, and exhibits the excellent tumor inhibition and security *in vivo*. Furthermore, the other hydrophobic antitumor drugs can also be on-demand delivered by the smart nanogel. All of the above advantages confirm the bright prospect of reduction-responsive nanogel on the road of malignancy chemotherapy.



## 1. Introduction

Up to now, chemotherapy is still one of the most important approaches for the treatments of various malignancies [1–3]. Despite the continuous progress in the exploitation of various antitumor drugs, chemotherapy still has many serious flaws, such as severe side effects to normal tissues attributed to the free diffusion of antitumor drugs *in vivo* [4,5]. Nanotechnology is the commanding heights of science and technology in the 21st century, and it greatly promotes the development of pharmacy [6,7]. Recently, more and more researchers enable various polymeric nanoparticles, *e.g.*, micelles [8–10], vesicles [11–13], and nanogels [14,15], to controllably deliver a wide range of clinically used antineoplastic agents [16]. The smart drug delivery systems can extend the circulation time in blood, improve the selective intratumoral

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accumulation through the enhanced permeability and retention (EPR) effect, thus enhance the therapeutic effectiveness and minimize the systemic toxicity of drugs [17].

Given the different metabolic pathways and rates of tumor cells in comparison with normal ones, the microenvironments of tumor tissues show hypoxia, low sugar, and low pH [18]. More fascinatingly, the malignant and normal cells exhibit the acidic and reductive intracellular microenvironment [19]. That is, the differences in pH and glutathione (GSH) concentration between intracellular and extracellular spaces are significant [20,21]. In detail, the endosomal pH is 5.0–6.5, and 50–1000 times intracellular GSH concentration exists compared with the extracellular one [22,23]. Therefore, the stimuli-responsive polymeric nanoparticles attract more and more attention in the realm of smart antitumor drug delivery [16,24,25]. Among all the polymeric nanoparticles, nanogels demonstrate a good prospect for practical application benefited from the tunable and stable chemical and three-dimensional (3D) physical compositions, the ingenious stimuli-responsiveness toward outside microenvironment, and a high drug loading capability [26]. For preparing the drug-loaded nanogels, the small molecule antitumor drugs first disperse into the cores of nanogels, and then the outside free drugs are cleared away by dialysis. After intravenous injection into the body, the drug-loaded nanogels selectively gather into the lesion tissues through the EPR effect [27]. As soon as the laden nanogels are uptaken by the tumor cells through endocytosis, the payloads are instantaneously released out triggered by the intracellular microenvironment [15,28,29].

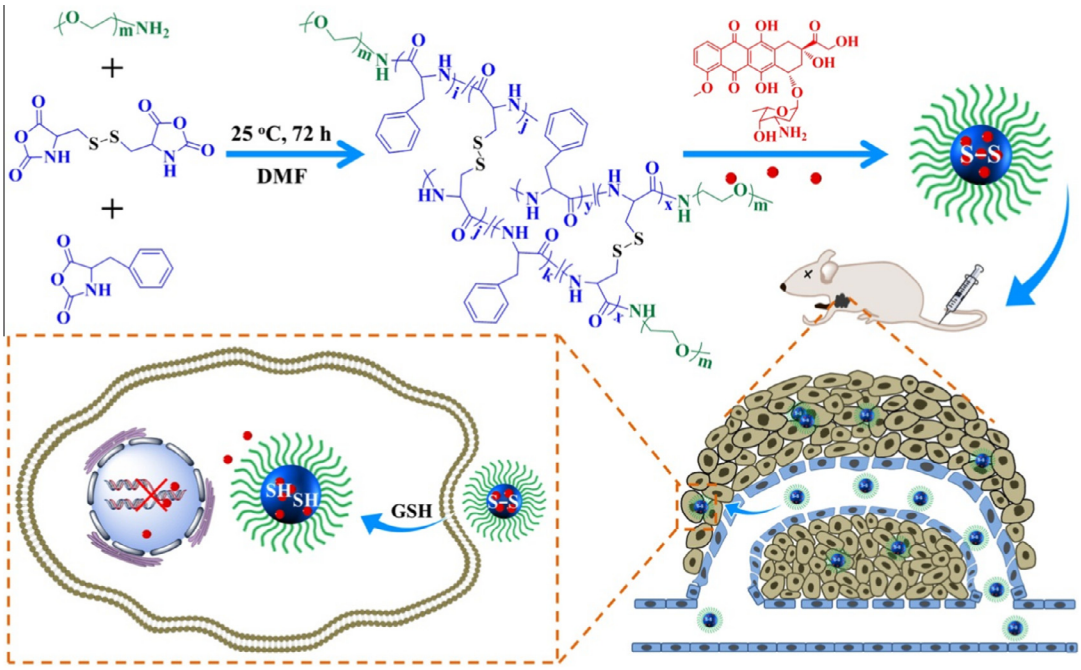
In the past three years, a series of intracellular microenvironment-responsive nanogels were designed and prepared for smart antitumor drug delivery in our group [3,15,29–33]. Especially, the reduction-responsive polypeptide nanogels with adjustable properties were facilely synthesized by the one-step ring-opening polymerization (ROP) of monofunctional and difunctional peptide *N*-carboxyanhydride (NCA) [3,29]. The simple and efficient synthesis process, tunable size and responsiveness, excellent biodegradability and biocompatibility, and high drug loading capability endow the above reduction-responsive polypeptide nanogels with fascinating potential for clinical applications. Nevertheless, the validation of the improved efficacy and security of drug-loaded nanogels *in vivo* have not yet been disclosed.

On the basis of preliminary studies, the reduction-responsive methoxy poly(ethylene glycol)–poly(L-phenylalanine-*co*-L-cystine) (mPEG–P(LP-*co*-LC)) nanogel was synthesized by the one-step ROP of LP NCA and LC NCA for the selective intracellular delivery of antitumor drug *in vitro* and *in vivo* in this work (Scheme 1). Typically, doxorubicin (DOX) was loaded into nanogel as a model clinically applied antitumor drug. The DOX-loaded nanogel (referred as NG/DOX) showed a reduction-dependent release behavior and an efficient capability for cellular proliferation inhibition *in vitro*. More importantly, the improved intratumoral accumulation, enhanced tumor growth suppression, and upregulated security *in vivo* of NG/DOX in comparison with free doxorubicin hydrochloride (DOX·HCl) demonstrated a good prospect of NG/DOX as a novel smart formulation for the clinical chemotherapy of malignancy.

## 2. Materials and methods

### 2.1. Materials

As shown in Scheme 1, the reduction-responsive $mPEG_{113}$–P($LP_{12}$-*co*-$LC_2$) nanogel was synthesized through the one-step ROP of LP NCA and LC NCA with amino-terminated mPEG ($mPEG-NH_2$) as a macroinitiator according to the protocol reported in our previous works [3,29]. The chemical structure of resultant nanogel was characterized by proton nuclear magnetic resonance ($^1$H NMR) and Fourier-transform infrared (FT-IR) spectra, which were shown in Supplementary Figs. S1 and S2, respectively. The subscript number represented the degree of polymerization (DP) of each component, which was calculated from elemental analysis. DOX·HCl was purchased from Beijing HuaFeng United Technology Co., Ltd. (Beijing, PR China). GSH (used for cell culture) was purchased from Aladdin Reagent Co., Ltd. (Shanghai, PR China). 4′,6-Diamidino-2-phenylindole (DAPI) and methyl thiazolyl tetrazolium (MTT) were all sourced in Sigma–Aldrich (Shanghai, PR




**Scheme 1.** Synthetic pathway for mPEG–P(LP-*co*-LC) nanogel, illustrations of DOX encapsulation by nanogel, and its circulation, intratumoral accumulation, endocytosis, and targeting intracellular DOX release after intravenous injection.

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