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Doxorubicin-loaded polypeptide nanorods based on electrostatic interactions for cancer therapy

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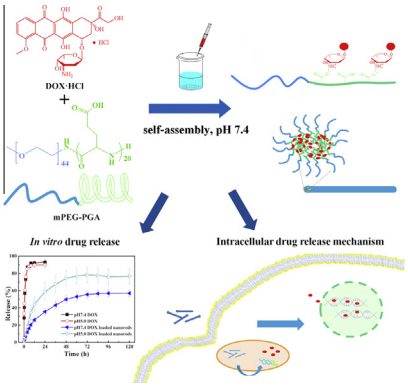
GRAPHICAL ABSTRACT



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ABSTRACT

An amphiphilic anionic polypeptide, methoxypolyethylene glycol–poly (glutamic acid) (mPEG–PGA), was synthesized, characterized and evaluated as a nanocarrier for the cationic anticancer drug doxorubicin hydrochloride (DOX·HCl). The complex self-assembled into nanorods in aqueous solutions via electrostatic interactions and exhibited a superior drug loading content (50.8%) and drug loading efficiency (90.2%). The average major axis of the drug-loaded nanorods was approximately 300 nm, as determined by transmission electron microscopy. An *in vitro* release assay showed that drug-loaded nanorods exhibited pH-sensitivity and sustained release. Haemolysis assays demonstrated that the polypeptide was haemocompatible, and the polypeptide drug carrier significantly reduced the haemolysis ratio of DOX·HCl. The pharmacokinetics study showed that DOX-loaded nanorods significantly prolonged the resident time in blood. An *in vitro* cytotoxicity study and cellular uptake assays demonstrated that the DOX-loaded nanorods resulted in higher cell proliferation inhibition and a higher level of tumour cell uptake in A549 cells than with free DOX·HCl. The prolonged circulation and enhanced antitumor efficacy of DOX-loaded nanorods shows promise for efficient cancer chemotherapy.

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1. Introduction

Cancer is a major public health problem in the 21st century. Despite the tremendous and remarkable advances in treatment strategies and novel therapies for cancer, the cure for cancer remains a Gordian knot [1,2]. Chemotherapy remains the main traditional treatment for cancer among because of its high efficiency. Unfortunately, its poor bioavailability, rapid blood/renal clearance, inability to achieve therapeutically effective drug concentration in the tumour, severe side effects, lack of selectivity and overwhelming toxicity limit the clinical application of chemotherapeutic drugs. Therefore, novel drug carries, especially polymer nanocarriers (such as nanoparticles [3–5], nanorods [6–9], nanotubes [10,11], nanofibres [12], and nanocages [13]) have been proposed as promising and reliable approaches to cancer treatment. Drug-loaded nanocarries are produced by physical entrapment and/or chemical conjugation [14–16]. Compared with traditional chemotherapies, nanosized drug carries have the following advantages: high drug-loading capacity, long circulation *in vivo*, enhanced permeation and retention (EPR) effect and enhanced tumour distribution [17,18].

Due to their biocompatibility, biodegradability and precise secondary conformations, polypeptide-based nano-assembly drug carries have garnered considerable attention as a potent platform for anticancer drug delivery [19]. The conformations of the polypeptide can be adjusted not only by the composition of amino acids but also by environmental factors, such as the temperature, pH value and ion intensity [20]. Polypeptides usually consist of hydrophilic poly(ethylene glycol) (PEG) blocks and hydrophobic derivatized polypeptide blocks. The mPEG coating can effectively inhibit rapid clearance by the renal and reticuloendothelial systems (RES) and prolong the circulation time *in vivo* [21,22]. These properties make the polypeptide highly desirable for biomedical applications [23,24].

Recently, methoxy-polyethylene glycol-b-polyglutamic acid (mPEG–PGA), a bottom-up synthetic di-block copolymer, has been widely used in biomedical fields [25]. The hydrophobic core serves as a reservoir for hydrophobic drugs, and ionic block polymers with good water solubility may also self-assemble into core–shell nanostructures via electrostatic interactions when loaded with oppositely charged molecules, such as DNA [26], plasmid [27] or platinum II [28].

Doxorubicin hydrochloride (DOX·HCl), a hydroxy derivative of a cytotoxic anthracycline antibiotic isolated from cultures of streptomyces peucetius, is the first-line treatment for a wide spectrum of cancers, such as breast carcinoma, ovarian carcinoma, and acute lymphoblastic leukaemia. However, the severe cardiotoxicity, myelosuppression, nephrotoxicity and development of multidrug resistance limit its therapeutic efficacy. In the present study, an mPEG–PGA block polypeptide was synthesized by a simple ring opening polymerization and loaded with DOX·HCl via electrostatic interactions [28,29]. The use of electrostatic interactions in this procedure avoids the use of organic solvents and ensures the safety and efficacy of the drug for patients [30]. Importantly, the prepared polypeptide-based nanocarriers can be loaded with more drug (approximately 50.8%) than traditional carriers (generally less than 20%) [31,32]. These carriers are ideal to deliver positively charged anticancer drugs to enhance drug bioavailability while reducing severe side effects by electrostatic interaction. In addition, the electrostatic interactions between the drug and polypeptide are especially susceptible to environmental acidity, which allows the development of pH-sensitive drug delivery systems [3,33–35]. In our study, the DOX-loaded nanorods were obtained by adjusting the stoichiometric ratios of the positively charged cationic amine group of DOX to the negatively charged pendant carboxyl moieties in the mPEG–PGA polymer. Drug-loaded nanocarries based on electrostatic interactions for cancer therapy have been previously studied. For example, Yu Zhang and Basar Bilgicer described micellar nanoparticle formation via electrostatic interactions for delivering multinuclear platinum(II) drugs [28]. Xuesi Chen and co-workers have also developed a polypeptide-based drug delivery system formed by anionic PEG–PGA and cationic DOX [29]. Nevertheless, to the best of our knowledge, our work is the first to increase drug loading in nanocarriers by a direct mixing reaction in an ultrasonic field and subsequent freeze-drying to obtain lyophilized powder without dialysis. Furthermore, the different structures of PEG–PGA block copolymers and the differences in the preparation process also lead to differences in the aggregation morphology, encapsulation efficiency and release behaviour.

In the present work, the molecular structures, physiochemical properties and morphology of the self-assembled synthesized polypeptide mPEG–PGA were assessed in an aqueous solution. The *in vitro* – *vivo* release profile, haemotoxic properties, *in vivo* antitumor efficacy and cellular uptake in MCF-7 cell lines and A549 cell lines were evaluated [29]. The *in vitro* release results demonstrated that the DOX-loaded nanorods were sensitive to pH and could prolong the circulation time. Their tumour cell uptake was high, and they enhanced tumour cell growth inhibition compared with free DOX·HCl. These findings indicate the potential of these nanocarriers for efficient cancer chemotherapy.

2. Experimental section

2.1. Materials and measurements

Methoxypolyethylene glycol ($mPEG_{44}$, molecular weight 1800–2200) was purchased from Aldrich and used without further purification. CH_2Cl_2 (Analytical reagent, AR) and pyridine (AR) were stored over calcium hydride (CaH_2, AR) and purified by vacuum distillation with CaH_2. 5-Benzyl-L-glutamate *N*-carboxyanhydride (BLG-NCA, purity ⩾ 98%) was supplied by Chengdu Enlai Biological Technology Co., Ltd and used as received. Trifluoroacetic acid (TFA, AR), trifluoromethane sulfonic acid (TfOH, AR), thioanisole (MPS, AR), $CDCl_3$–0.03%TMS (purity ⩾ 99.9%), DMSO-d_6–0.03%TMS (purity ⩾ 99.9%), and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, purity ⩾ 98%) were purchased from Aladdin and used as received. Doxorubicin (purity ⩾ 99%) hydrochloride was purchased from Dalian Meilun Biotech Co., Ltd. Daunorubicin (purity ⩾ 99%) was supplied by the Shanghai Institute for Food and Drug Control.

^{1}H NMR spectra were recorded on a Bruker Avance 400 spectrometer in $CDCl_3$–0.03%TMS or DMSO-d_6–0.03%TMS. FT-IR spectra were recorded on a Nicolet 6700 FT-IR spectrometer. Dynamic laser scattering (DLS) measurements were performed on a Beckman Coulter instrument at room temperature. Transmission electron microscopy (TEM) was performed on a JEM-100CX II transmission electron microscope with an accelerating voltage of 100 kV. The critical micelle concentration (CMC) was measured by fluorescence spectroscopy using pyrene as a probe on a F-7000 fluorescence spectrometer with an emission wavelength of 335 nm, and the excitation wavelength was recorded from 350 to 550 nm. UV–Vis spectra HPLC measurements were measured on a TU-1810 spectrophotometer and Agilent-1200, respectively. Circular dichroism spectra (CD spectra) were recorded from 180 to 400 nm at ambient temperature using a Chirascan spectropolarimeter from Applied PhotoPhysics. The baseline was taken from the pure water and subtracted from the spectra.