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Direct formation of cationic polypeptide vesicle as potential carrier for drug and gene

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ABSTRACT

The pH-responsive polypeptide grafted with polycation was prepared through copper(I)-catalyzed "click chemistry". The amphiphilic polypeptide directly formed into cationic vesicle when it was dissolved in phosphate buffer solution (PBS). The hydrophilic DOX·HCl was loaded into the hollow core of vesicle. The in vitro release behavior of DOX·HCl from vesicle in PBS could be adjusted by the pH of release media. In vitro cell experiments demonstrated that the DOX·HCl loaded vesicle showed effective cellular proliferation inhibition. In addition, the preliminary gel retardation assay revealed that PLG-*g*-PAMA could efficiently bind to DNA, indicating its potential use as gene carrier. The more in-depth studies of PLG-*g*-PAMA vesicle for drug and gene co-delivery are in progress.

1. Introduction

Synthetic polypeptides are one of the most fascinated biocompatible and biodegradable materials, which have been widely studied for biomedical applications, such as drug and gene delivery [1,2], biosensors and diagnostics [3], etc. Recently, synthetic polypeptides have been employed to prepare polymeric vesicles [4,5]. Especially, the cationic polypeptide vesicles have received considerable attention for the ability of co-delivery conventional chemotherapeutic drug (e.g. doxorubicin (DOX) and paclitaxel (PTX)) and gene (e.g. plasmid DNA (pDNA) and small interfering ribonucleic acids (siRNAs)) [6,7]. These vesicles can load gene and hydrophobic active substances into the cationic membranes, and hydrophilic drugs into the hollow cores. The combination of two or more chemotherapeutic active substances with different mechanisms is a promising strategy for effective treatments of cancers through synergistic effects [8,9]. In the work, the cationic charged vesicle was prepared from the polypeptide with hydrophobic poly(L-glutamate) (PLG) backbone and pH-responsive hydrophilic poly(2-aminoethyl methacrylate hydrochloride) (PAMA) side chain. DOX·HCl, a hydrophilic anticancer drug, could be loaded into the hollow vesicle core. The gel retardation assay revealed that PLG-*g*-PAMA could efficiently bind to DNA. Therefore, the cationic polypeptide vesicle has the capability to co-delivery of drug and gene.

2. Experimental part

2.1. Materials

Poly(γ-propargyl-L-glutamate) (PPLG$_{40}$, $M_{n,\ NMR}=6800$, $M_{n,\ GPC}=10{,}500$, $M_w/M_n=1.05$) and *azide*-PAMA (N_3-PAMA$_{16}$, $M_{n,\ NMR}=2900$) were synthesized similarly as described in our previous works [4,7,10]. Doxorubicin hydrochloride (DOX·HCl) was purchased from Zhejiang Hisun Pharmaceutical Co., Ltd. All the other reagents and solvents were purchased from Sinopharm Chemical Reagent Co., Ltd. and used as obtained.

2.2. Synthesis of PLG-g-PAMA

PLG-*g*-PAMA was synthesized through the copper(I)-catalyzed "click chemistry" with "grafting onto" approach (Scheme 1), according to our previous work with slight modification [10]. In a typical procedure, PPLG (16.7 mg, 0.1 mmol propargyl pendants) and N_3-PAMA (288.5 mg, 0.1 mmol) were firstly dissolved in 10 mL of dimethyl sulfoxide (DMSO) with nitrogen bubbling. Then, 25.0 mg of $CuSO_4 \cdot 5H_2O$ and 99.0 mg of sodium ascorbate were added and the mixture was further bubbled with nitrogen for about 10 min. The reaction was carried out at 40 °C for 3 d. Thereafter, the reactive solution was dialyzed against deionized water for 3 d using a dialysis bag (MWCO 3500 Da). The final product PLG-*g*-PAMA was obtained as white solid by lyophilization (Yield: 83.2%, $M_{n,\ NMR}=122{,}200$).

2.3. Characterizations

Transmission electron microscopy (TEM) measurement was performed on a JEOL JEM-1011 transmission electron microscope with

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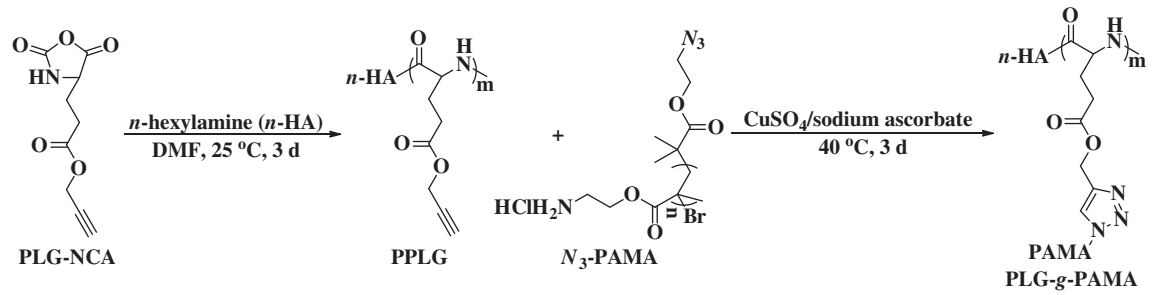

Scheme 1. Synthesis pathway of PLG-*g*-PAMA.

an accelerating voltage of 100 kV. The critical aggregation concentration (CAC) was measured by fluorescence spectroscopy using pyrene as a probe on a Perkin-Elmer LS50B luminescence spectrometer with $\lambda_{em} = 390$ nm. Dynamic laser scattering (DLS) measurements were performed on a WyattQELS instrument with a vertically polarized He–Ne laser (DAWN EOS, Wyatt Technology). The pK_a value of PLG-*g*-PAMA was measured by acid–base titration method.

2.4. In vitro DOX·HCl loading and release

PLG-*g*-PAMA (50.0 mg) and DOX·HCl (10.0 mg) were mixed in 5.0 mL of DMSO. The mixture was stirred at room temperature for 24 h, and then DMSO was removed by dialysis against deionized water for 24 h to obtain the DOX·HCl loaded vesicle. In vitro DOX·HCl release behaviors were investigated in PBS at pH 5.5, 6.8 or 7.4 as our previous work [7].

2.5. Cytotoxicity assay

The relative cytotoxicities of PLG-*g*-PAMA and DOX·HCl loaded PLG-*g*-PAMA vesicle were assessed with methyl thiazolyl tetrazolium (MTT) assay against Henrietta Lacks (HeLa) cells. Cell viability (%) was calculated based on the following equation: $(A_{sample}/A_{control}) \times 100$, where A_{sample} and $A_{control}$ were denoted as absorbencies of sample and control wells, respectively.

2.6. Gel retardation assay

PLG-*g*-PAMA/DNA and N_3-PAMA/DNA complex solutions (10 μL, 0.5 μg calf thymus DNA (ctDNA) or plasma DNA (pDNA, pGL3) contained) with defined PLG-*g*-PAMA/DNA and N_3-PAMA/DNA weight ratios (w/w) were prepared for gel retardation assay. After 10 min of incubation, the complex solutions were analyzed by 1% (w/v) agarose gel electrophoresis (120 V, 0.5 h).

3. Results and discussion

3.1. Synthesis and characterization of PLG-g-PAMA

PLG-*g*-PAMA was synthesized through copper(I)-catalyzed "click chemistry", an azide–alkyne cycloaddition. FTIR spectra confirmed the chemical structure of PLG-*g*-PAMA through the disappearance of the alkyne characteristic absorbance at 2130 cm^{-1} from PPLG and appearance of C–N broad band at 2108 cm^{-1} from PAMA (Fig. S1B

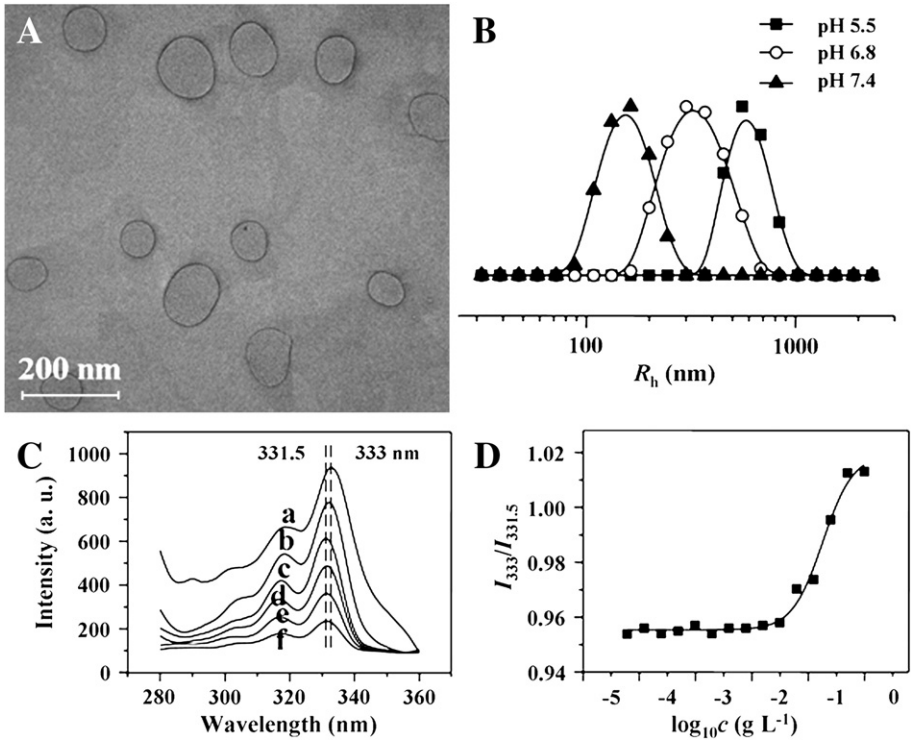

Fig. 1. Typical TEM micrograph (A), hydrodynamic radii (R_h) (B), excitation spectra of pyrene in aqueous solutions at different concentration (1.0 (a), 2.5×10^{-1} (b), 3.1×10^{-2} (c), 3.9×10^{-3} (d), 4.9×10^{-4} (e), 6.1×10^{-5} (f) g L^{-1}) (C), and the intensity ratio ($I_{333}/I_{331.5}$) as a function of concentration (D) of PLG-*g*-PAMA.