

Feasible construction of a pH-responsive nanoparticle for smart drug delivery

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Abstract. Endosomal pH-responsive micellar nanoparticles have been prepared by amphiphilic poly (ethylene glycol)-Schiff base-Adriamycin (PEG-Schiff-DOX, PSD) prodrug, and wrapped DOX drugs in the hydrophobic core of the polymeric nanoparticles. Under normal conditions, these nanoparticles showed good stability over a storage period of 7 days but decomposed rapidly under the weakly acidic conditions. These nanoparticles possessed a high drug loading capacity and good cell compatibility that would be promising for the drug carries in the drug delivery system.

Keywords: amphiphilic poly (ethylene glycol); acetal; Adriamycin (DOX)

1. Introduction

Previously, the use of polymer nanomedicine has been more advanced in anticancer applications, including increase the potential solubility of the drug, improve drug treatment effectiveness, and reduce the side effects of medication [1–5]. The former shows many advantages over small molecule anticancer drugs, such as prolonged circulation time by avoiding glomerular filtration, improved pharmacokinetic ability, and by enhanced permeation and retention (EPR) effects [7–11]. Among variety of nanomedicine such as polymer nanoparticles, prodrugs, micelles, vesicles, nanogels, and liposomes [12–18]. Prodrug nanoparticles are of interest due to their clear, simple structure and great clinical potential.

Doxorubicin (DOX), an anti-tumor drug, acts on the chemical structure of DNA. DOX has a broad anti-tumor spectrum. DOX can inhibit nucleic acid synthesis (DNA and RNA synthesis), especially the strongest inhibitory effect on RNA, belonging to the non-specific drug cycle, and has killing act upon each growth cycle of tumor cells. On account of its extreme water solubility for the impaired EPR effects, it is necessary to develop alternative drugs to DOX that are more selectively cytotoxic and have fewer side effects.

To ensure successful intracellular drug release, it is important that the linkers between drugs and polymers must be cleavable in the tumor-cell environment. Due to the abnormal conditions such as higher glutathione concentration and lower pH within the tumor cells, redox-sensitive disulfide and acid-labile acetal bonds have been employed to fabricate stimuli-responsive polymer-DOX prodrugs [19–21]. In addition, DLC and drug release kinetics are also important for the drug delivery system. High DLC means that less carrier material is needed, so less cost and, in addition, less toxic side effects.

In this study, an amphipathic polymer-drug conjugate of poly (ethylene glycol)-Schiff-doxorubicin (PSD) has been designed and synthesized, which can self-assemble into the unstable micellar nanoparticles under acidic conditions. These nanoparticles have good stability and can be used for storage originating from the protective effect against the external PEG corona and its low critical aggregation concentration. As obtained by chemical coupling binding, these nanoparticles not only exhibit pH-responsive drug release capacity but also have very high DLC and DOX concentrations for the highly efficient tumor therapy. As shown in Figure 1, nanoparticles enter tumor tissue via EPR effect and then taken up by tumor cells transcytosis. In the endosomal/lysosomal compartment, acidic environment can initiate the acetal bond cleavage and cause the disintegration of the nanoparticle, rapid release of wrapped DOX, subsequently, the acetal bond is exposed to an acidic environment and breaks, releasing the encapsulated DOX completely. In this case, these DOX-conjugated

nanoparticles can cellular uptake into the cells and display the antitumor activity against HeLa cells in vitro.

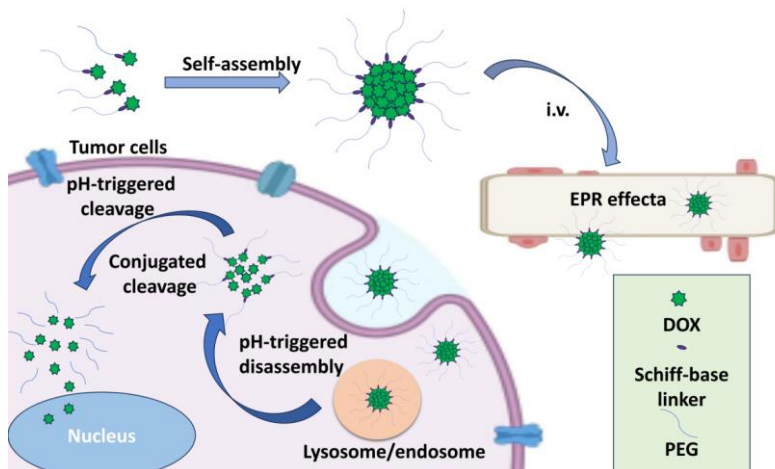


Fig. 1 Schematic illustration of the delivery behavior of the DOX-loaded polymeric micelles

2. Materials and Methods

2.1. Materials

Table 1. Main materials and its sources

Name	Abbreviation	Manufacturer	Remark & Purity
Polyethylene glycol methyl ether	PEG-OH	Alfa Aesor	Mn = 750
4-carboxybenzaldehyde	-	J&K	98%
4-(Dimethylamine)-pyridine	DMAP	Aldrich	99%
1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride	EDCI	Energy Chemistry	99%
Triethylamine	TEA	Beijing Chemical	99%
Hydrochloric acid	HCl	Beijing Zhongshuo Pharmaceutical Technology.	37%
Sodium sulfate	Na ₂ SO ₄		98%
Sodium bicarbonate	NaHCO ₃		98%
n-Hexane	-		AR
Ether	-		AR

All other reagents are purchased from Energy Chemistry and used directly without any further purification. All the used cells came from the Chinese cell line resource infrastructure.

2.2. Characterization and testing

Nuclear magnetic resonance (NMR) spectroscopy is derived from a Bruker 400 MHz spectrometer (internal reference tetramethyl silane, TMS). Transmission electron microscopy (TEM) images were obtained from the JEM microscope. Dynamic light scattering (DLS) spectroscopy comes from a laser scattering spectrometer with a mulita digital time correlator and requires the use of a cylindrical 22 mW single-phase helium-neon laser. Store the laser scattering cell in a vat with a thermostat index matched to the purified dust-free toluene. Fluorescent experiments use the Hitachi F4600 photoluminescence spectrometer, and the light source is a xenon lamp. All data were averaged over three doses.

2.3. Synthesis of PSD (PSD)

The composition of pH sensitive prodrug is carried out in Figure 2. 1 mmol of PEG-OH, 1.2 mmol of 4-carboxybenzaldehyde, 1.2 mmol EDCI and 0.3 mmol DMAP were mixed to a 50 mL of bottom equipped with a magnetic stir bar. After adding 10 mL of freshly dichloromethane to dissolve the reactive solids, the organic phase was collected by stirring the system, and the collected organic solution was washed three times using a solution divided into HCL aqueous solution, bicarbonate solution, NaCl solution and deionized water, and dried by the solid Na₂SO₄ after washing. The products were precipitated in ether to provide the white powders with a calculated yield of 89.1%.

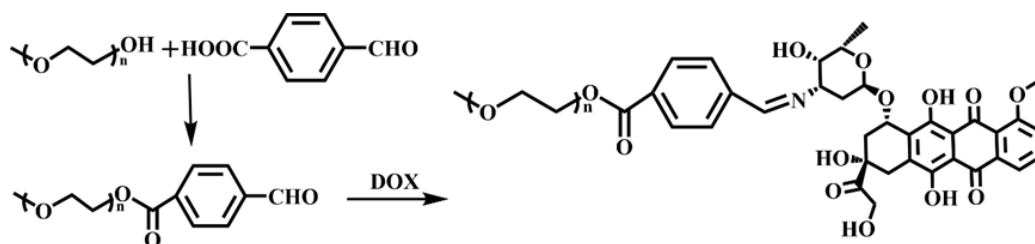


Fig 2. Synthetic approach of the PSD prodrug.

2.4. Synthesis of the PSD polymer

0.31 mmol polyethylene glycol, 0.25 mmol DOX and 1 mmol TEA were dissolved in 20 mL of DMF solutions at room temperature. Reflux the system under vigorously stirring for half a day, the product was extracted and dissolved in DCM, washed several times and further dried by solid Na₂SO₄ after washing. The final products were put into the cold ether to precipitate for several times to obtain red powders with a calculated yield of 62.4%.

2.5. Self-assembly of the polymeric PSD nanoparticles in mixed solutions

Briefly, 5 mg PSD was dissolved in the DMF solutions (1 mL), which was then added into the aqueous solutions (4 mL) via the slow dropwise rate. During self-assembly, the colloid was maintained for stirring of 2 h in solutions. Remove deionized water from the DMF solutions by the dialysis (MW cut-off, 2 kDa) process for 5 d. These PSD nanoparticles can be formed and characterized using the TEM and DLS measurements.

2.6. pH-triggered polymeric degradation in various pH solutions

We measured the shift in the size distribution of PSD nanoparticles in the PBS solutions at pH 7.4 to assess their stability and resistance adsorption at room temperature. After displacement into the various pH solutions (pH 5.0), the size distribution and variation were recorded using DLS measurement at the schedule time (e. g. 4 h). Similarly, the morphological formation and change were employed using the TEM images.

2.7. DOX release from the PSD nanoparticles in vitro

pH-induced DOX release: Add PSD nanoparticles to MW-cut-off, 1 kDa dialysis membrane tubes, and shake at 37 °C in 30 ml pH 5.0 and 7.4 in two PBS followed by a water bath. Measuring the UV/VIS absorbance of the solution at 480 nm yields a pH-triggered DOX release curve. The experiment process was conducted for three times, and the experiment results were obtained and averaged by the standard deviations.

2.8. CCK-8 assay.

Cytotoxicity of PSD nanoparticles by measuring Hela cells by CCK-8: seeded on a 200 μ L of DMEM 96-well plate at 10% FBS with 1×10^5 cells per well. Replace the cell medium by 90 μ L of fresh DMEM containing 10% FBS, then add various concentration of micellar suspension to the pH 7.4 PBS solution, respectively. Incubate for another 24 h, remove the medium from the plate,

immediately add fresh medium and CCK-8 kit solution and mix well and mix well, then incubate for 6 h in an incubator. Eventually, take 100 μL of solution and place it into a 96-well plate. Read the light density of each well at 450 nm with a microplate reader.

3. Results and Discussion

3.1. Structural analysis

The ^1H NMR spectra of PEG-CHO are shown in Figure 3. It can be seen that the ratio of phenyl cyclic hydrogen in the synthesized PEG-CHO corresponds to the methyl peak at the end of PEG, so it can be concluded that the synthesis of PEG-CHO is relatively successful. ^1H NMR spectra of PSD were analyzed (Figure 3A), and the peak shape of drug molecules was relatively small and chaotic (Figure 3B). The starting point of analysis was set as the methyl group at the end of PEG, and the area of the methyl peak basically corresponds to that of the main chain peak, and it was believed that the peak in the low field region came from the benzene ring and the 8 H on the Schiff base bond. Through integration, it is found that their areas correspond basically. In particular, the appearance of peak c at 8.1 ppm indicates the formation of Schiff base bonds (Figure 3C). In summary, it can be proved that the experiment successfully prepared PSD.

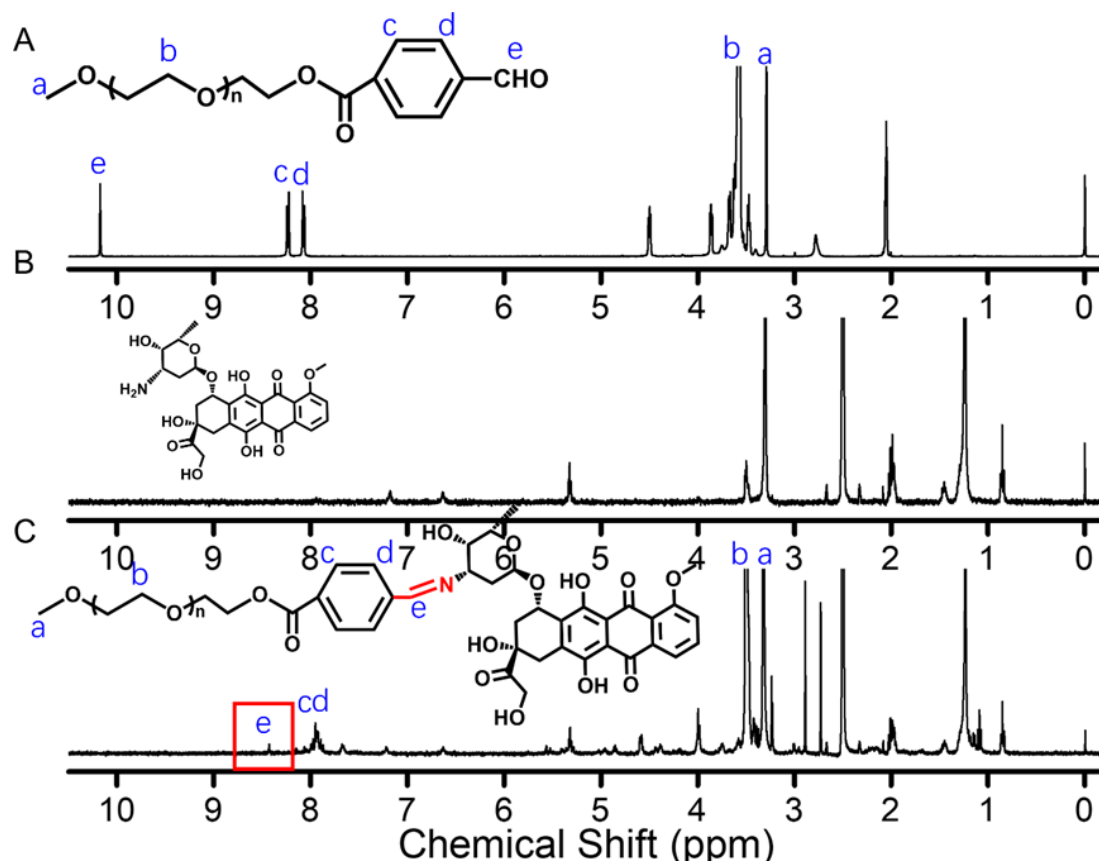


Fig 3. ^1H NMR spectra of PEG-CHO, DOX and PSD

3.2. Morphology and size variation at various pH solutions

The pH response of the nanomaterials is given by the particle size change after 24 hours of shock in a buffer of pH 5.0 at 37 $^{\circ}\text{C}$, as shown in Figures 4 and 5. By comparing the morphology and particle size of the nanoparticles before and after the acid treatment, we found that they changed significantly and some of the nanoparticles disintegrated completely. The particle size distribution of the particles became narrower, which implies that the heterogeneous nanoparticles disintegrated in the presence of acid, thus releasing the encapsulated drug.

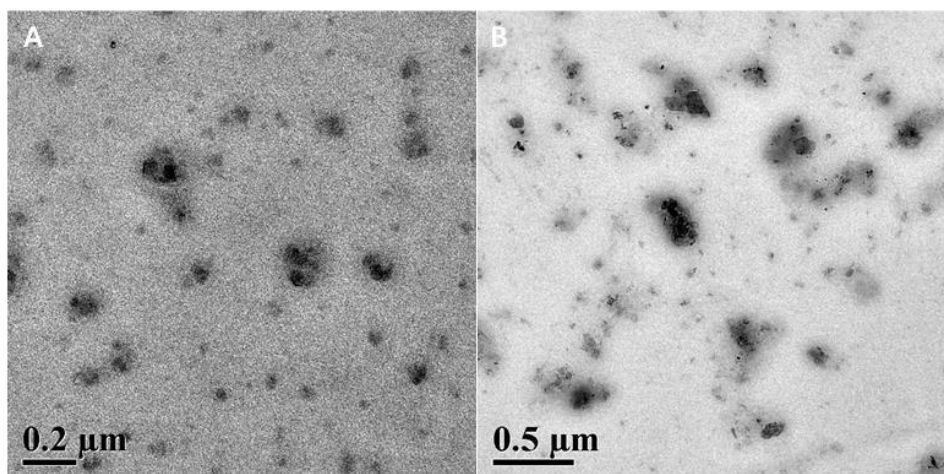


Fig 4. PSD self-assembled into drug-loaded nanoparticles with various morphologies at pH=7.4 and pH=5.0 solutions.

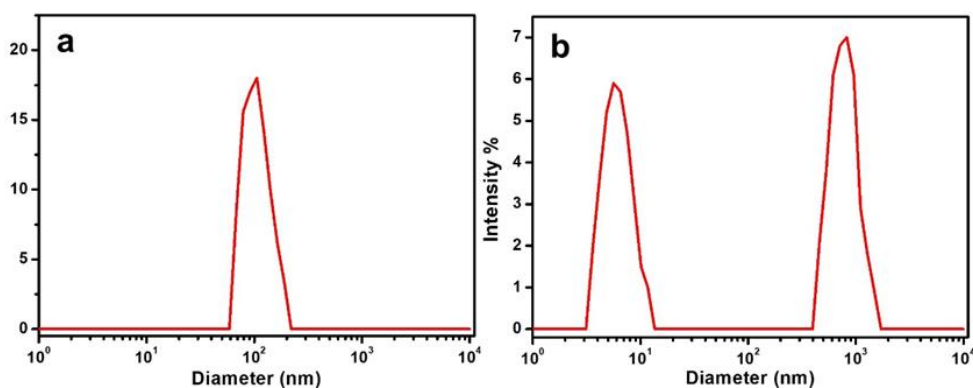


Fig 5. PSD self-assembled into drug-loaded nanoparticles various size at pH=7.4 and pH=5.0 .

3.3. Drug Release

The test results of gel-loaded drug are very intuitive. Through ultraviolet spectrometer detection, it can be seen that the drug-loaded nanoparticles release little to no drug when pH=7.4; At pH=5.0, both the release effect and the amount of release were significantly enhanced.

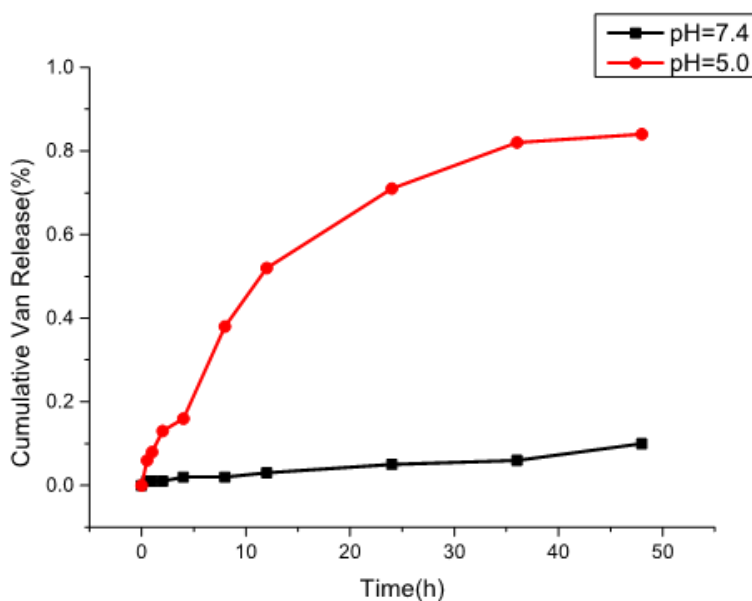


Fig 6. In vitro release curve of PSD drug-loaded nanoparticles

3.4. Antitumor activity

As can be seen from Figure 7, PSD nano-drugs have excellent anti-tumor effects, especially when the concentration of loaded DOX reaches 100 $\mu\text{g/mL}$, showing strong inhibition of tumor cell growth, with less than 15% of tumor cells surviving. The results indicated that PSD was a kind of targeted agent that could be used in clinic.

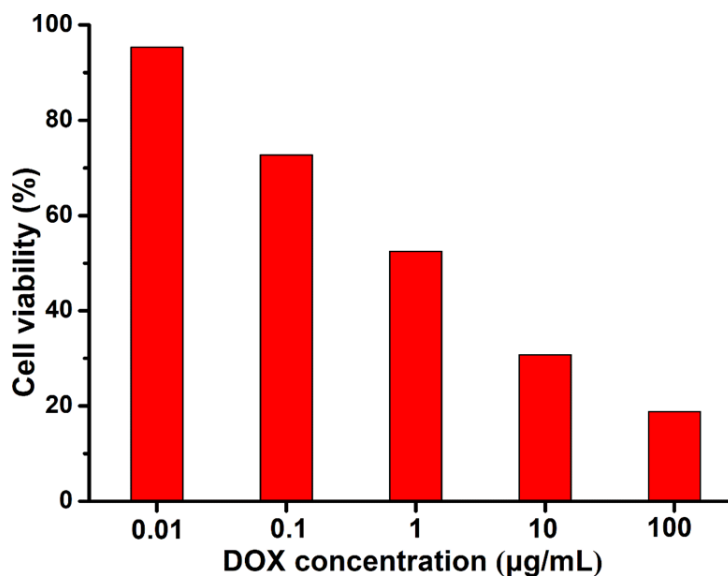


Fig 7. Evaluation of the antitumor effect of PSD nanodrugs

4. Conclusion

To summarize, an amphiphilic PSD prodrug has been designed and synthesized. And we developed a novel pH-responsive micellar nanoparticles using self-assembly of this prodrug and DOX. It probably has characteristics as followed: Clear structures, simple process to prepare; High concentration of drug and strong drug loading capacity; Good stability that can be used for storage (stable room temperature for more than 1 week); Negligible DOX leakage in neutral circulation with the responsive drug release for retaining the therapeutic activity; Greater tumor suppression capacity than free DOX. These features offer a promising option for developing the DOX formulations.

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