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PEG-DOX, Novel pH Responsive Prodrug Nanoparticles

Longyu Li[1*#], Bailun Liu[2#]

[1]Queen's University, 99 University Ave, Kingston, ON K7L 3N6, Canada

[2]Michigan State University, 450 Hannah Administration Building, East Lansing, MI, 48824

[#]These authors are contributed equally to this work

Abstract: pH responsive prodrug nanoparticles (PEG-DOX) were prepared by attaching free Doxorubicin (DOX) onto the amphipathic polyethylene glycol-aldehyde (PEG-CHO). The hydrophobic core of PEG-CHO enabled free DOX to be attached, while the hydrophilic outer layer of the carrier enabled the water solubility of the entire structure. This nanocarrier enabled a greater carrying capacity than free DOX, making its circulation time longer. The prodrug remained stable within neutral pH, ensuring its prolonged circulation time, but disassembled rapidly when reaching in the acidic environment of tumor tissues to release the free DOX. The newly designed nanocarriers have the potential to be applied clinically as a future DOX formulation in cancer chemotherapy.

Keywords: Nanoparticles, Ph-Sensitive, Doxorubicin, Water Solubility

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***Corresponding author:** Longyu Li, 16ll14@queensu.ca

1 Introduction

Doxorubicin, as a chemotherapeutic medication, is widely used clinically to treat a wide range of cancers including breast cancer, bladder cancer, Kaposi's sarcoma, lymphoma and acute lymphocytic leukemia[1]. Although DOX shows outstanding therapeutic effect, the disadvantages caused by its own structure cannot be completely avoided, such as poor water solubility, antibiotic nature, short half-life in human body (it would spend approximately 12 minutes to complete the first metabolizing phase), non-selectivity to tumor tissues and so on[2]. Thus, improved drug delivery methods should be developed to enhance its drug efficiency.

In order to achieve higher drug efficiency, smart drug delivery systems were developed in recent years. The study and utilization of nanoparticles as drug delivery media have been in rapid progress. One of the most well studied fields is to assemble nanoparticles with anticancer drugs for better performance and less toxicity[3-4]. Some of the advantages of utilizing nanoparticles in cancer therapy contained increasing water solubility by using hydrophilic outer layer and prolonging circulation time due to an increased stability et cetera[5]. Since the vessel wall of the tumor tissues caused by angiogenesis has larger gaps than that of the normal circulatory system, the nanoparticles could easily permeate the gaps[6-8]. Moreover, because the tumor tissues lack lymphatic circulation, the nanoparticles are difficult to be excreted, resulting in the accumulation of the particles at tumor tissues.

The smart drug delivery systems could react to different stimuli such as pH, heat, and light radiation[6,9]. Among them, pH-sensitive delivery systems have attracted great attention as tumor cells have weak acidic microenvironments from normal cells. However, there were several problems with respect to pH-sensitive nanoparticles. The first one is that compounds with imine and orthoester groups are not sufficiently stable at neutral pH, which meaning the structure of the nanocarrier could potentially break and release the drugs in circulation[3,10]. The other problem is that compounds with hydrazone and cis-aconityl groups showing high stablity in neutral environment, would form alkaline end products after degradation, leading to pH changing of the area[11-12]. Consequently, the ideal pH-sensitive nanocarriers should have the properties of being stable in neutral environment while being able to

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be degraded in weak acidic environment, and the end product is both nontoxic and easily metabolized[13-14].

In this study, we designed and synthesized novel pH responsive prodrug nanoparticles, named PEG-DOX. The prepared nanoparticles stable in relatively neutral environment such as blood circulation system, and could rapidly release the drug when reaching the relatively acidic environment such as tumor tissues. The suggested strategy not only increased the solubility of DOX, but also prolonged its circulation time. Moreover, this could also potentially reduce the amount of DOX used during the therapy, resulting in less drug resistance for tumor cells.

2 Materials and methods

2.1 Materials

4-Formylbenzoic acid, 4-dimethylaminopyridine (DMAP), dichloromethane (DCM), triethylamine (TEA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI), polyethylene glycol (PEG-OH), doxorubicin (DOX), dimethylformamide (DMF), ether, deionized water, HCl, NaCl, $NaHCO_3$, $MgSO_4$.

2.2 Aldehyde PEG (PEG-CHO) synthesis

4-Formylbenzoic acid (150 mg, 1 mmol), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI) (191.7 mg, 1 mmol) and 4-dimethylaminopyridine (DMAP) (61 mg, 0.5 mmol) were dissolved in the DCM. Then, polyethylene glycol (PEG-OH) was added in the system (375 mg, 67.5 mmol) under the protection of nitrogen gas. The entire system was stirred 25 hours within 37 Celsius degree's environment. After that the product should be washed three times by 1 M HCl, saturated $NaHCO_3$ solution and saturated NaCl solution. After the product was washed, the organic phase would be collected and be dried with $MgSO_4$ by the rotary evaporator.

2.3 PEG-DOX synthesis

The PEG-CHO (100 mg, 110 μmol), the deionized doxorubicin (50 mg, 90 μmol) and the TEA (70 μL, 500 μmol) were dissolved and vibrated overnight in 3 mL dehydrated dried DMF. The liquid part would be evaporated in the rotary evaporator and the solid product would be dissolved in DCM. The novel solution would then be extracted by saturated NaCl solution three times and the upper layer would be precipitated in the cold ether.

2.4 Drug release experiments of PEG-DOX nanoparticles

DOX release experiments of PEG-DOX nanoparticles were studied using a dialysis tube under shaking at 37 °C in PBS (pH 7.4) and acetate buffer (pH 5.0), respectively. Typically, 2 mL of PEG-DOX nanoparticle solution was dialyzed against 30 mL of release media. At pre-determined time pionts (0.5 h, 1 h, 1.5 h, 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, 24 h, 36 h and 48 h), 10 mL of release medium was withdrawn and renewed with an equal volume of fresh medium. Then the absorption at 480 nm of the release medium was measured by an UV-vis spectrophotometer, and the concentration of DOX in the release medium was obtained from absorption/ concentration equation of the standard curve. The cumulative release ratio of DOX was calculated according to the following formula:

$$\begin{cases} R_i = \dfrac{V_0 C_i}{m} \times 100\% \ (i = 1) \\ R_i = \dfrac{V_0 C_i + V_m \sum_1^{i-1} C_{i-1}}{m} \times 100\% \ (i \geq 2) \end{cases}$$

in which R_i is the cumulative release ratio of DOX (%), m is the total mass of DOX in the PEG-DOX nanoparticles (mg), V_0 and V_m are the volume of the total release medium and the exchanged medium respectively (mL), and C_i is the DOX concentration in the release medium withdrawn at the i^{th} time (mg/mL).

2.5 Results and discussion

The prepared PEG-DOX nanoparticles were synthesized following the synthetic route as illustrated in figure 1. The proposed synthetic method is simple and rapid, and the product was confirmed by its ^1H NMR spectrum. From figure 2, it can be seen that peaks at δ = 3.6 and 3.29 ppm attributed to PEG demonstrated successful conjugation of PEG. Peaks at δ = 7.9 – 8.5 ppm attributed to the protons of phenyls and double bond demonstrated successful conjugation of 4-formylbenzoic acid and DOX. The intermediate product, PEG-CHO, with high yield percent up to 90%, could self-assemble into acid-labile micellar nanoparticles, which serves as a nanocarrier to encapsulate free DOX. As shown in figure 3a, the obtained PEG-DOX nanoparticles were uniform with an average hydrodynamic size around 170 nm, and the morphology of these nanoparticles was spherical micelles.

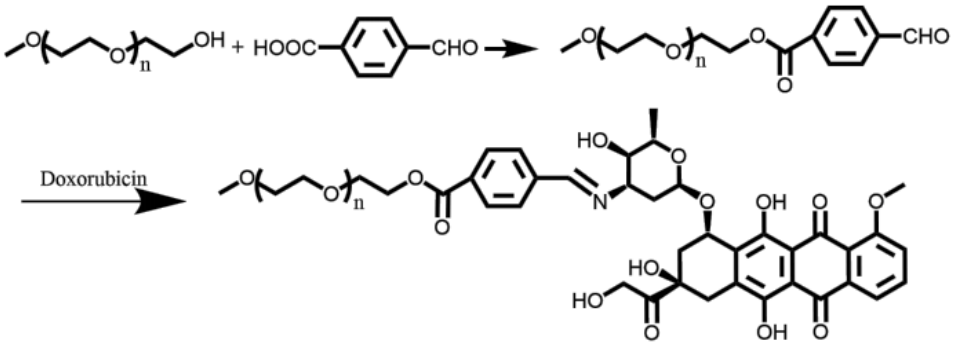

Figure 1. Synthetic route of PEG-DOX.

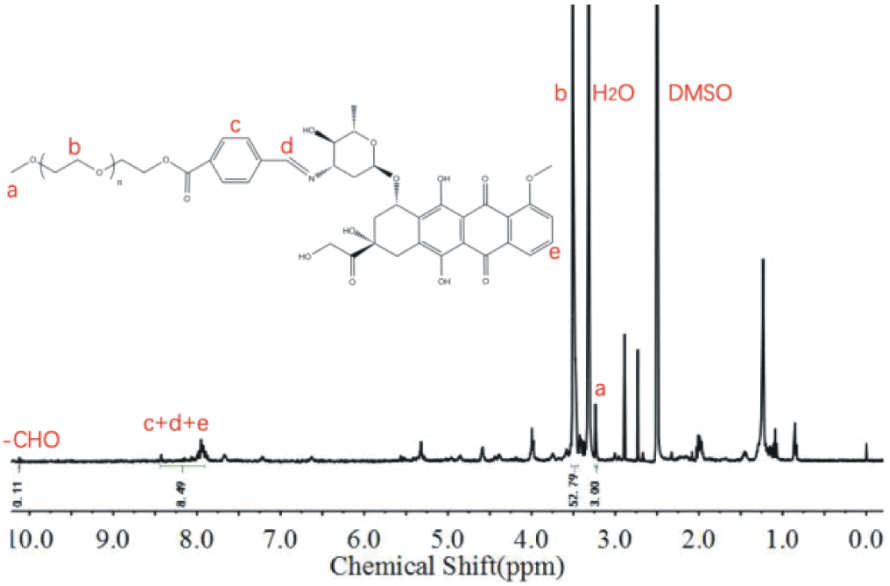

Figure 2. [1]H NMR spectrum of PEG-DOX.

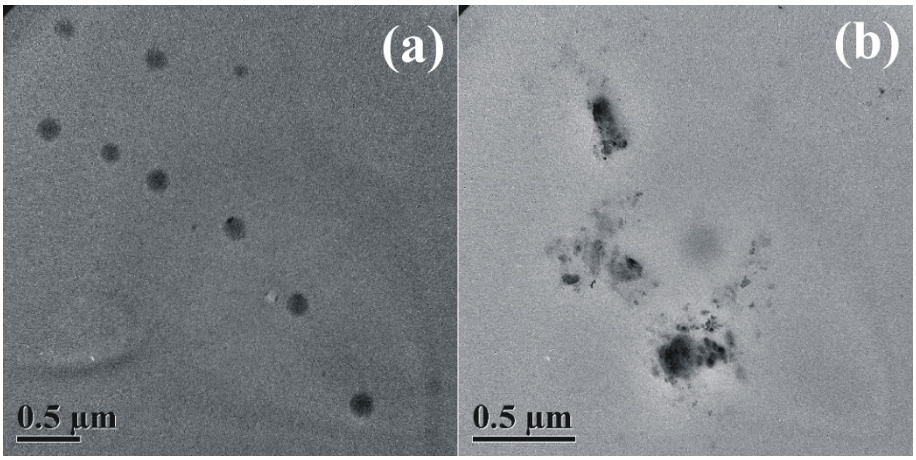

Figure 3. TEM images of PEG-DOX (a) and PEG-DOX after incubated at pH 5.0 for 48 h (b).

The acid-labile linker between the hydrophobic DOX and the hydrophilic PEG makes the nanoparticles susceptible to disassemble under faintly acidic condition. To investigate the pH-responsive degradation behaviors of PEG-DOX nanoparticles, dissolution experiments of the prodrug under different pH environments had been done. It is well known that pH value around tumor tissues would be lower than the normal health organs. Therefore, PBS with a pH of 7.4 and acetate buffer with a pH of 5.0 were introduced to mimic normal physiological situation and the tumor's regions body fluid. Dissolution curves were shown in figure 4. The results demonstrated that more free DOX molecules were released from PEG-DOX

nanoparticles under acetate buffer with a pH of 5.0 after incubated for 20 h suggesting the degradation of these nanoparticles under faintly acidic condition. And this was further confirmed by their TEM image (figure 3b), further confirming pH responsive degradation of the nanoparticles.

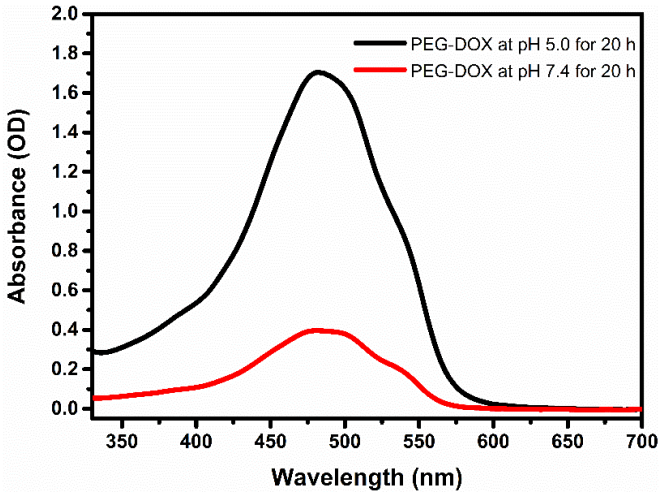

Figure 4. Absorbance spectra of the released DOX from PEG-DOX after incubated at pH 5.0 (black line) and at pH 7.4 (red line) for 20 h.

In the PEG-DOX prodrug nanoparticles, DOX was conjugated to PEG-CHO via an acid-labile linker, which will cleave to release DOX at the faintly acidic microenvironment of tumor tissues. To confirm the conjugated DOX can be released pristinely, we carried out drug release experiments in PBS with a pH of 7.4 and acetate buffer with a pH of 5.0 respectively, and the drug release profiles were summarized in figure 6. At pH 7.4, 20 % DOX was released from the PEG-DOX nanoparticles. While at pH 5.0, the accumulative release of DOX was dramatically accelerated, reaching 90 % (4.5 times that amount at pH 7.4). As discussed previously, the disassembly of the nanoparticles in response to acidic environment is due to the cleavage of the acid-labile linker. Benefitting from the combination of physical encapsulation and chemical conjugation, the PEG-DOX nanoparticles not only achieved high DOX concentration, but also exhibited a programed drug release behavior. The above results suggest that these nanoparticles will reduce drug leakage in the neutral environment of blood circulation and achieve fast drug release in the acidic environment of tumor tissues. Moreover, PEG-DOX prodrug nanoparticles provided a longer release period and prolonged the treatment time, resulting in enhanced therapeutic effect.

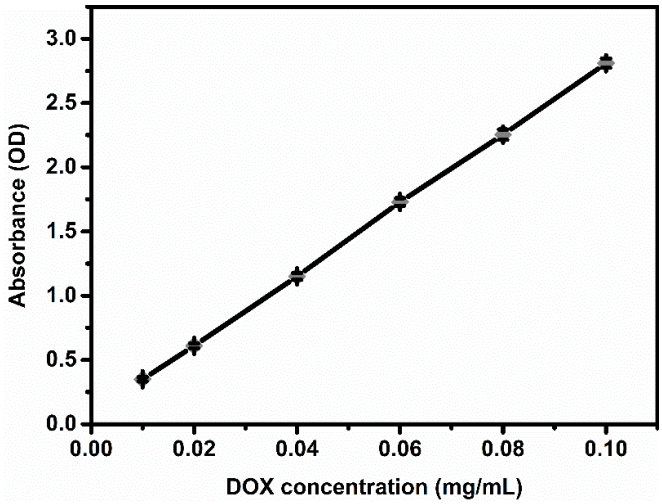

Figure 5. Standard curve of absorption (480 nm) vs concentration of free DOX.

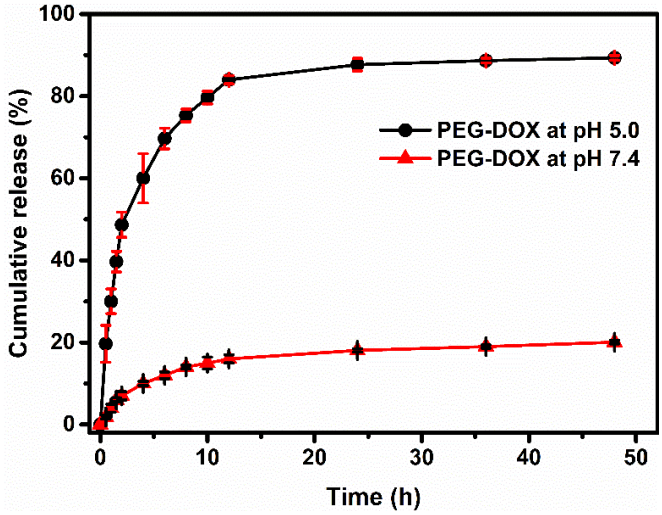

Figure 6. Drug release profile of PEG-DOX nanoparticles at pH 5.0 and pH 7.4.

3 Conclusion

In summary, we synthesized an amphipathic, pH sensitive PEG-DOX prodrug nanoparticles by attaching free DOX to PEG-CHO nanocarrier. There are several advantages with respect to this new prodrug. Firstly, the preparation process of the prodrug nanoparticles was relatively simple with well-defined structure. Secondly, the amphipathic properties of the nanocarrier enabled greater water solubility of DOX, enabling its accumulation in the blood circulation as well as at the tumor cells. Thirdly, it is stable in neutral environment, thus ensuring proper preservation of the drug in blood circulation. DOX was rapidly released when in the acidic environment of the tumor cells, making it more

effective in killing cancer cells than free DOX. Fourthly, the nanocarrier had an excellent DOX carrying capacity and the drug could accumulate at the cancer cells, resulting in a higher intracellular concentration. Thus, these advantages of the proposed prodrug nanoparticles provided a potential clinical option for future cancer treatment.

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