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pH-responsive prodrug nanoparticles for anti-tumor drug delivery

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Abstract: *Endosomal pH-responsive micellar nanoparticles are prepared from an amphiphilic PEG-Schiff-DOX prodrug by the solution self-assembly. These nanoparticles have one outstanding advantage they can be stable in water for about 1 week if common conditions are provided. However, in a faintly acidic environment, it is unstable and easily disassembled. The concentration of DOX in the nanoparticle solutions could reach a high level of 265 μg/mL, along with an enormous drug loading capacity of 41.7%. The increase of intracellular DOX contents and the extension of circulation time are benefits of a pH-triggered drug release mechanism. CCK-8 found that these prepared nanoparticles possessed better antitumor ability than that of the free DOX against the Hela cells. These nanoparticles are expected to become a new DOX-supported dosage form for tumor research.*

Keywords: *Doxorubicin, pH-response, produrg, polymer nanoparticles*

1. Introduction

In the last few decades, much of the research on cancer therapy has focused on nanomedicines, for their merits in areas like increased drug solubility, enhanced therapeutic effects, and reduced side effects. Nanomedicines are better than small molecular anticancer drugs including long circulation time via efficiently evading the glomerular infiltration, enhanced pharmacokinetic abilities, and cancer accumulation using the typically enhanced permeation and retention effect [1-5]. There are various nanomedicines like micelles, prodrugs, vesicles, liposomes, prodrug-based nanoparticles, etc., in which the prodrug is known for its simple structure and progressive potential in clinical fields [6-9].

Doxorubicin (DOX), an antitumor antibiotic, as the anti-tumor spectrum is wide, has effects on various tumors, such as malignant lymphoma, acute leukemia, breast cancer, etc. Since DOX has good water solubility and extremely high flexibility, it has various side effects that make it very damaging to normal cells. So new dosage forms are developed to encapsulate doxorubicin while improving its drug delivery efficiency and reducing toxicity. Although there are much-published kinds of literature about DOX-encapsulated PEG prodrugs, they have their built-in disadvantages of lower drug encapsulation contents and incomplete drug release behaviors [10-14].

A necessary condition for the successful release of the drug within the cell is that the connection between the drugs and the polymers is divisible. On account of the abnormally unstable glutathione concentration and relatively low pH value within the tumors, environmentally sensitive bonds are used to fabricate stimuli-responsive polymeric DOX-linked prodrugs for tumor therapy. However, whether DOX is released intact in these systems is still a question. In addition, both DLC and drug release kinetics are important keys for drug delivery systems. Higher DLC can reduce cost and the possibility of toxic and side effects. On this basis, people have carried out a lot of exploration on new high DLC nanocarriers. Drug release kinetics is related to intracellular drug concentration and drug action time [15-17].

Here in this work, we tried to prepare an amphiphilic DOX-based polymeric conjugate of PEG-Schiff-DOX polymer, which is self-assembled into uniform micellar nanoparticles that are sensitive to acidic environments (Figure 1). The obtained results demonstrated that these prepared nanoparticles could be regarded as suitable drug carriers to pack DOX drugs. In the intracellular endosomes and lysosomal chambers with acidic environments, pH-induced cleavage of acetal bonds was the main reason for the decomposition of nanoparticles, thereby bringing about the quick release of loading DOX at a high rate of speed. While in the acidic environment, the hidden acetal linkages suffered, and finally the conjugated DOX was released. Uptake of HeLa cells by nanoparticles and their antitumor activity in vitro are also being investigated.

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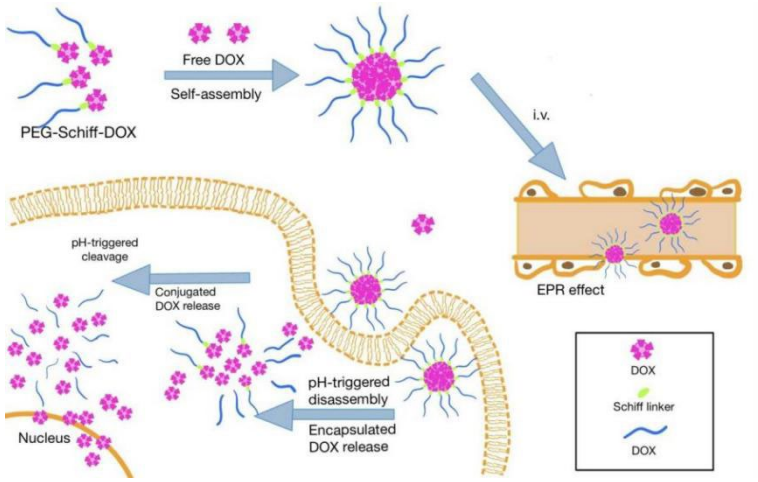

Figure 1: Schematic image of self-assembly and drug delivery of the PTX-loaded nanparticles.

2. Materials and Methods

2.1. Materials

Table 1: Experimental Materials

Name	Abbreviation	Manufacturer	Remark & Purity
Polyethylene glycol methyl ether	PEG-OH	Alfa Aesor	Mn = 750
4-carboxybenzaldehyde	-	J&K	98%
4-(Dimethylamino)-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_2SO_4		98%
Sodium bicarbonate	$NaHCO_3$		98%
n-Hexane	-		AR
Ether	-		AR

All the used cells came from the Chinese cell line resource infrastructure (Table 1).

2.2. Characterization and testing

Nuclear magnetic resonance spectroscopy is derived from Bruker 400 MHz spectrometer (internal reference tetramethylsilane, TMS). Dynamic light scattering spectroscopy comes from a laser scattering spectrometer with a multiτ 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 PEG-Schiff-DOX (PSD)

The composition of the pH-sensitive prodrug is carried out in Figure 2. 1 mmol of PEG-OH, 1.2 mmol of 4-carboxybenzaldehyde, 1.2 mmol of EDCI, and 0.3 mmol of DMAP were mixed to a 25 mL of bottom equipped with a magnetic stir bar. After adding 10 mL of dichloromethane to dissolve 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 solution, NaCl aqueous solution, bicarbonate solution, and water, and dried by the solid Na_2SO_4 after washing. The products were precipitated in ether to provide the white powders with a calculated yield of 89.1%.

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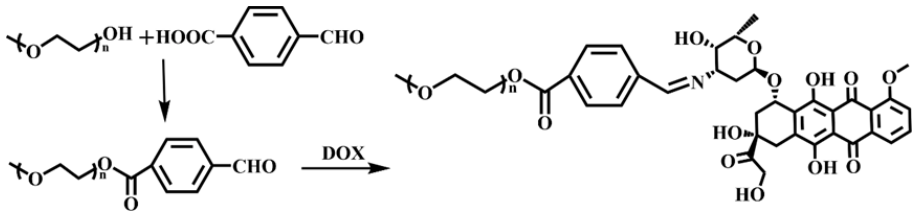

Figure 2: Synthetic approach of the PSD prodrug.

2.4. Synthesis of the PSD polymer

0.31 mmol of polyethylene glycol, 0.25 mmol of DOX and 1 mmol of TEA were dissolved in 20 mL of DMF solutions at normal temperature. Reflux the system under vigorously stirring for half a day, the product was extracted and dissolved in dichloromethane, washed by several times and further dried by solid Na_2SO_4 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 of 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 TEM and DLS measurements.

2.6. pH-triggered polymeric degradation in various pH solutions

We measured the change 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 TEM.

2.7. DOX release from the PSD nanoparticles in vitro

pH-induced DOX release: Add PEG-Schiff-DOX 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. A pH-triggered DOX release curve was obtained by measuring the UV/VIS absorbance (480 nm) of the solution. The experiment process was conducted 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 through seeding on a 200 μL of DMEM 96-well plate at 10% of FBS with 1×10^5 cells per well. Replace the cell medium by 90 μL of DMEM solution with 10% of FBS, then add 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 solution and place it on a plate. The optical density at 450nm was read by a microplate reader.

3. Results and discussion

3.1. Structural analysis

As shown in Figure 3, ^{1}H NMR spectra showed that the ratio of benzene cycle hydrogen in the PEG-CHO polymer corresponds to the typical methyl peaks at the end of PEG chains, indicating the formation of synthetic PEG-CHO polymer. After analyzing the ^{1}H NMR spectra of PEG-Schiff-DOX (Figure 3A) we can conclude the peaks of drug molecules are disordered and short (Figure 3B). The starting point for analysis is the area of the methyl peak could correspond to that of the PEG chains, meanwhile, it was considered the low field region was attributed to the total of 8H on the benzene ring peak. It is found that

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the area corresponds after integration. Particularly, the presence of peak e at 8.1 ppm represents the generation of pH-responsive Schiff base bonds (Figure 3C), thereby attesting to the successful preparation of the targeted polymer of PEG-Schiff DOX.

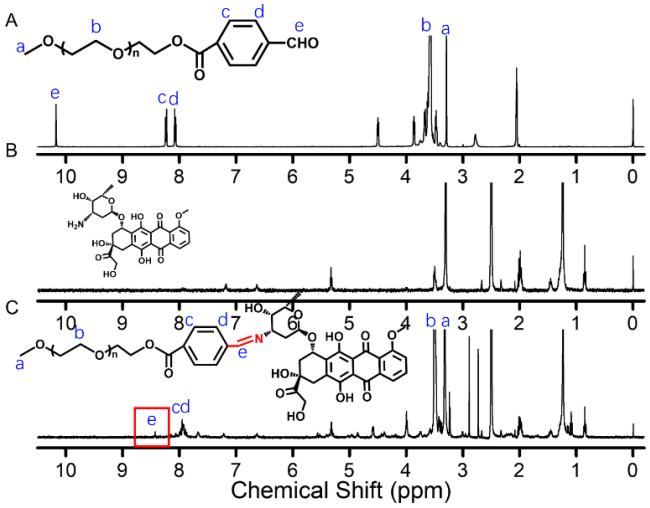

Figure 3: ^{1}H NMR spectra of polymeric PEG-CHO, DOX and PEG-Schiff-DOX

3.2. Morphology and size change in various conditions

The pH response of the nanomaterials is given by the particle size change after 24 h of shock at 37 °C in a PBS with a pH of 5.0, as shown in Figures 4 and 5. The form and size of the nanoparticles changed significantly before and after acid treatment along with the complete disintegration of the polymeric nanoparticles and the occurrence of narrow size distribution. In this case, the breakage of acetal linkages and disintegration of heterogeneous nanoparticles under the acidic condition led to the drug release.

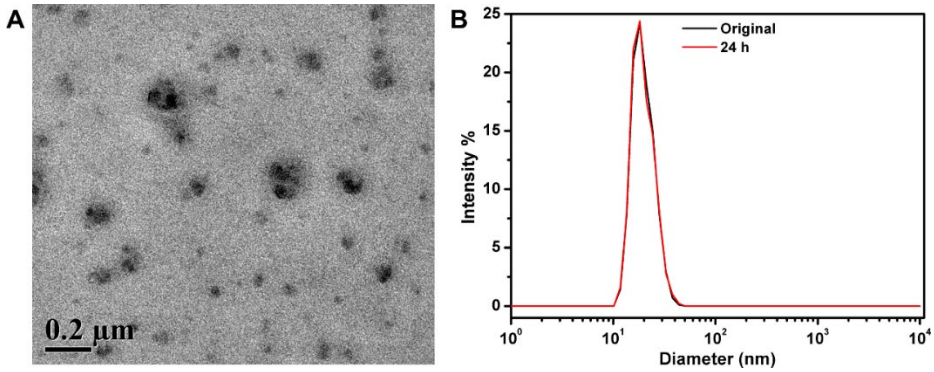

Figure 4: Self-assembled morphology and particle size of PEG-Schiff-DOX nanoparticles at pH=7.4

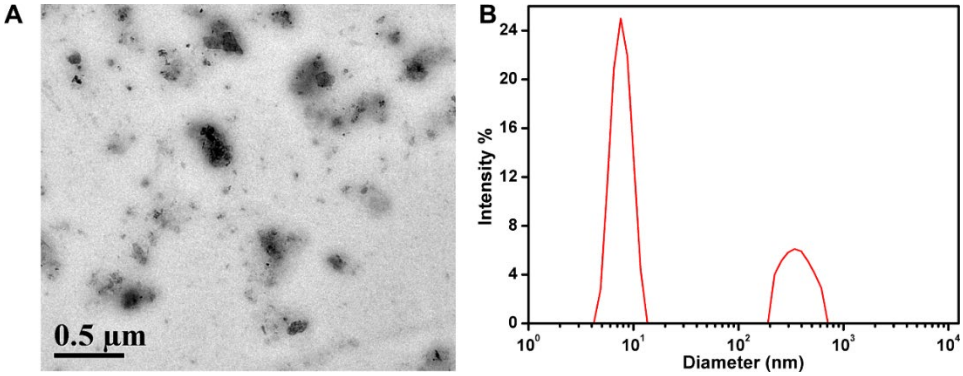

Figure 5: Self-assembled morphology and particle size of PEG-Schiff-DOX nanoparticles at pH=5.0

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3.3. Drug Release

As shown in Figure 6, according to the test results of the ultraviolet spectrometer, the drug-carrying nanoparticles can hardly release the DOX drugs at pH 7.4 solutions, and the maximum release amount was only 10.8% at 48 hours. However, in pH 5.0 solutions, the drug release content was markedly increased and the accumulated amount reached 92.8%. These results reflected that these polymeric prodrugs can be promised as potential carriers for effective tumor therapy.

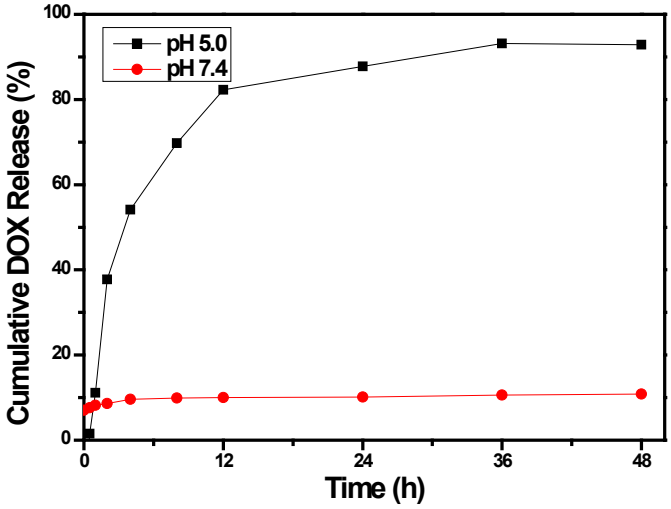

Figure 6: Drug release behavior of PEG-Schiff-DOX nanoparticles at various pH solutions in vitro

3.4. Antitumor activity

Based on the above structural and functional investigation, we believe that PEG-Schiff-DOX nanomaterials have excellent anti-tumor effects as shown in Figure 7, especially when the concentration of loaded DOX reaches 100 μg/mL, it will show a very high inhibitory effect on tumor growth.

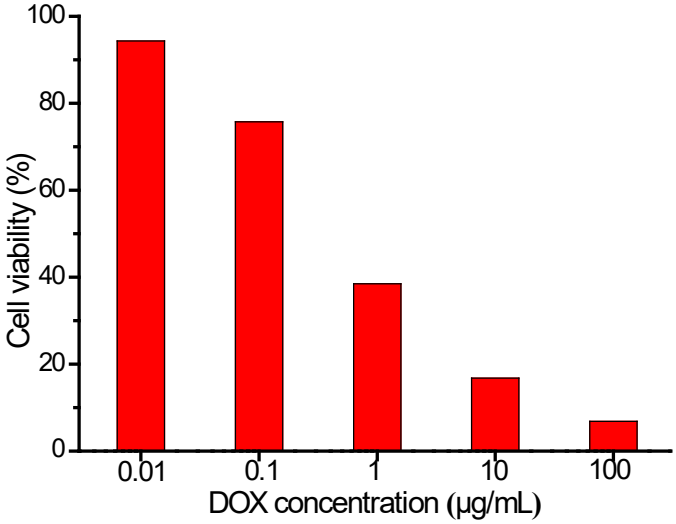

Figure 7: Evaluation of anti-tumor effects of PEG-Schiff-DOX nanomaterials

4. Conclusion

In summary, a typical PEG-Schiff-DOX polymer and its self-assembled nanoparticles with pH-responsive behavior were prepared in mild conditions. Using the pH-triggered release system, the drug action time can be extended and can respond to an acid environment in time, which has development potential to a certain extent. These polymeric drug-carries have many advanced advantages: 1) Well-

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defined chemical structure and easy preparation route; 2) High drug loading content; 3) Little or no drugs are released under neutral conditions, while DOX agents are released in the tumor microenvironment which is weakly acidic conditions to maintain drug efficacy and reduce side effects. Therefore, these polymer-based prodrug nanomedicines provide more opportunities for the development of advanced drug carries for effective cancer therapy.

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