Construction of the biocompatible and pH-sensitive prodrug nanoparticles via the self-assembly for improving tumor therapy

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Abstract. Endosomal pH-responsive micellar nanoparticles were prepared by self-assembly of a macromolecular poly(ethylene glycol)-Schiff-Doxorubicin (PEG-Schiff-DOX) prodrug.. These nanoparticles exhibited excellent storage stability for over 1 week under normal conditions, but disassembled quickly in response to faintly acidic environment. According to the EPR effect, the surface pore diameter of tumor blood vessels can reach hundreds of nanometers, while the surface pore diameter of ordinary blood vessels does not exceed 20 nanometers. Therefore, the chemical assembly of the drug is designed to have a diameter of 20-200 nanometers, thereby greatly improving the selectivity of the drug for tumor cells. Additionally, benefitting from the difference in drug release mechanism and rate between encapsulated DOX and conjugated DOX, a programmed drug release behavior was observed, which may result in higher intracellular drug concentration and longer action time. CCK-8 assays showed that the nanoparticles demonstrated superior antitumor activity than free PTX against both HeLa cells. These nanomedicines, which utilize prodrugs, exhibit immense promise in the advancement of translational DOX formulations for cancer treatment.

Keywords: prodrugs, EPR effect, developing cancer therapy.

1. Introduction

Recently, polymeric nanomedicines are extensively investigated in tumor therapy because of the capacity on increasing the drug solubility and therapeutic efficacy as well as reducing side effects. Compared with small-molecule anticancer drugs, polymeric nanomedicines showed many advantages like the prolonged circulation via evading the glomerular filtration, promoted pharmacokinetic behavior and tumor accumulation through enhanced penetration and retention (EPR) effects [1-6]. Specially, the prodrug nanoparticles have attracted great attention on account of their clear structures and simple preparation steps for the truly clinical applications.

Doxorubicin, also known as hydroxyldaunorubicin, is a drug that acts on DNA and widely used in chemotherapy. Belonging to anthracycline antibiotics, its structure is similar to daunorubicin, and it can also intercalate DNA with it. Can be used to treat various cancers [7-10]. Doxorubicin is used to treat certain leukemias, Hodgkin's lymphoma, bladder cancer, etc. Due to its very strong hydrophilicity, the absorption rate of ordinary healthy cells is almost the same as that of tumor cells. Therefore, DOX has very large toxic and side effects on cells, and it does great harm to patients during chemotherapy. Consequently, there is a desire to create alternative DOX formulations that possess improved selective cytotoxicity and reduced adverse effects.

Due to the prominent limitations observed in the majority of literature concerning PEG-DOX prodrugs, such as comparatively lower drug loading capacity (DLC) and incomplete release, the development of innovative carriers with elevated drug content assumes paramount importance. To maintain the effective drug release behavior, it becomes crucial to disrupt the connection between the drug and the polymer within the tumor cell microenvironment. Stimuli-responsive polymer DOX prodrugs employing reduction-sensitive disulfides and pH-responsive acetal linkages are devised to respond to aberrant conditions found in tumor cells, such as elevated glutathione concentrations and acidic pH values. Moreover, DLC holds significant roles in drug delivery and pursuit of higher DLC not only translates to reduced costs, as less carrier material is required, but also decreases the risk of toxic side effects. Consequently, great efforts are dedicated to the exploration of new nanocarriers

with elevated DLC [11-13]. The kinetics of drug release is closely consistent with the intracellular concentration and the duration of drug action.

In this research, we formulated and synthesized a macromolecular drug conjugate called poly(ethylene glycol)-Schiff-Doxorubicin (PEG-Schiff-DOX). This conjugate has the ability to assemble into sphere nanoparticles that are sensitive to acidity when placed in water-based solutions. These nanoparticles exhibit remarkable storage stability, allowing them to maintain their initial structure for over a week. However, once exposure onto the acidic environment within the endosomal/lysosomal compartments, pH-tailored breakage of acetal could lead to the disaggregation of nanoparticles, and thus causing the rapid release of encapsulated DOX. Moreover, the subsequent exposure of the hidden acetal linkages to acid conditions could also accelerate the bound DOX to completely release. Here we investigated the preparation of DOX prodrugs, pH-responsive drug release, targeted uptake by tumors, and antitumor activity on HeLa cells.

2. Materials and methods

2.1 The experimental materials like PEG-OH, carboxybenzaldehyde, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI), 4-(dimethyl-amino)-pyridine (DMAP), anhydrous magnesium sulfate were basically purchased from the Chemical Works and Energy Chemical.

2.2 In this experiment, we have used the NMR from Bruker spectrometer, transmission electron microscopy (TEM) from JEM-2200FS microscope, dynamic light scattering (DLS) from Malvern Zetasizer Nano.

2.3 Synthesis of PEG-CHO: PEG-OH, EDCI, carboxybenzaldehyde and DMAP were dissolved in DCM solution. After continuous stirring for 24 h, the products can be obtained after the purifying by extraction and precipitation steps.

2.4 Preparation of the PEG-Schiff-DOX: PEG-CHO, DOX and TEA were dissolved in dried DMF solution. After continuous stirring for 24 h, the products can be obtained after the purifying by extraction and precipitation centrifugation steps (Figure 1)

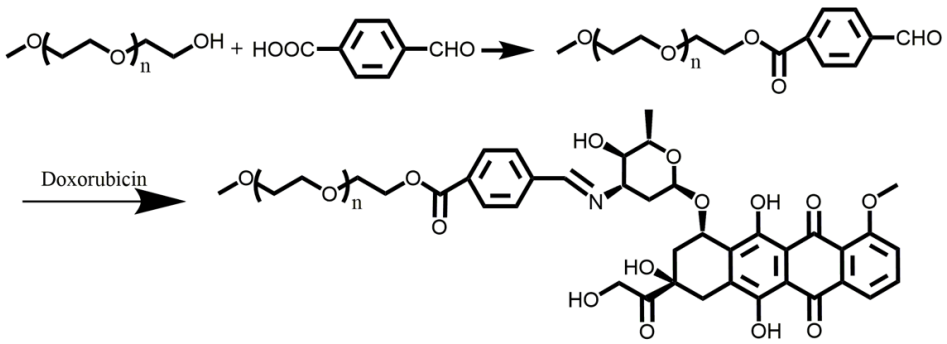

Fig. 1. The synthesis scheme of PEG-SCHIF-DOX

2.5 Formation of self-assembled nanoparticles: 5 mg of PEG-Schiff-DOX polymer was firstly dissolved in the 1 mL of DMF solution and dropwise into the 4 mL of H2O at a rate of 0.1 mL/min. Then the solution was stirred to obtain the colloidal dispersion and displacement for following 8 h and then purified by dialysis to yield the self-assembled nanoparticles.

2.6 pH-response: Briefly, a certain amount of phosphate buffered saline with pH 7.4 and pH 5.0 was added into above polymeric nanoparticles. After immersion for several times, the morphology and dimension changes could be recorded by TEM and DLS measurement.

2.7 In vitro drug release: The prepared PEG-Schiff-DOX spherical micelles were poured to the dialysis membrane tubes and incubated in pH 7.4 and pH 5.0 PBS solutions, and then UV-vis was used to detect the drug concentration in the specific time to assess the drug release behaviors.

2.8 Cytotoxicity: CCK-8 assay was used to assess the cell cytotoxicity of polymeric nanoparticles. After seeding the cells onto a plate with a density of 1×10^5, and the nanoparticle suspensions with various concentration were incubated for 24 h to respectively assess the cell survival numbers..

3. Results and discussion

3.1. Synthesis analysis

The 1H NMR spectrum of PEG-Schiff-DOX (Figure 2) showed the peak of the molecular drug with the relatively messy arrangement and methyl group of PEG peaks, wherein the methyl peak corresponds to the peak area of 3.6 ppm was the main chain of PEG, and the peaks in the low-field region come from a total of 8 Hs on the benzene and the Schiff base linkages, and the integrated areas were basically corresponded, indicating the successful preparation of polymeric PEG-Schiff-DOX prodrug.

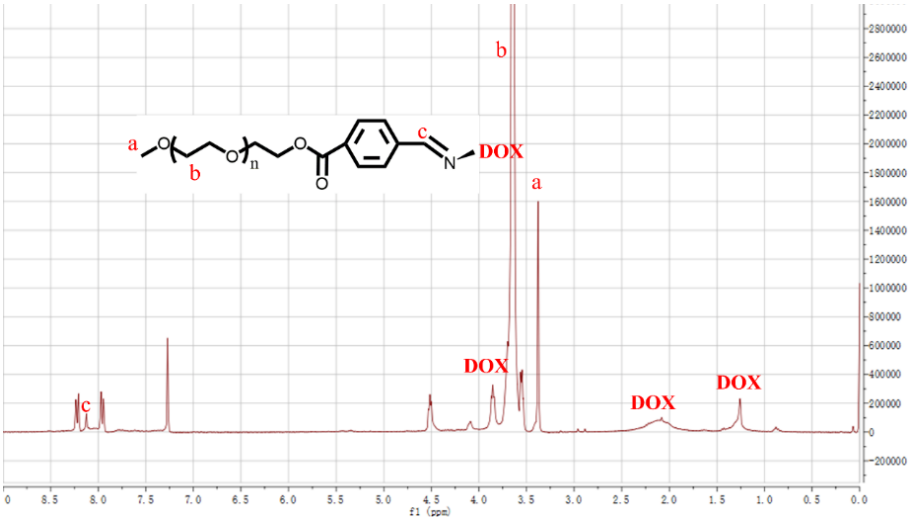

Fig. 2. The NMR spectrum of PEG-Schiff-DOX polymer

3.2. Self-assembly and stability of PEG-Schiff-DOX nanoparticles

PEG-Schiff-DOX can self-assemble into micelles with a dimension of ca. 200 nm in pH=7.4 solution (Figure 3A) while the destroyed formation with an amorphous structure is displayed at pH 5.0 solution as shown in Figure 3B. The pH-response of nanomedicine is given by the particle size change after shaking at 37 oC for two hours in a pH=5.0 buffer. Figure 3C and Figure 3D exhibited a significant change in the size after treatment in acidic conditions, and thus the nanoparticles were completely disassembled with the pH-responsive behaviors.

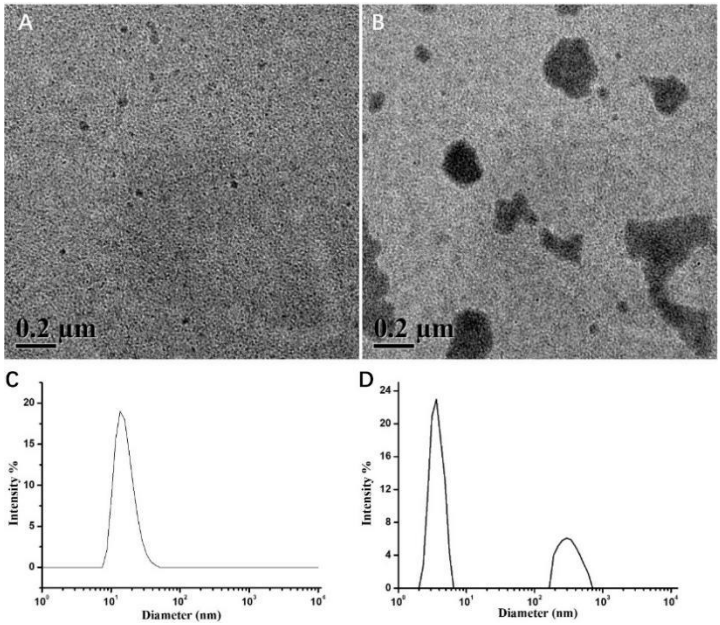

Fig. 3. The formation and size of PEG-Schiff-DOX nanoparticles at pH=7.4 and pH=5.0 solutions.

Through the in-situ loading method, PEG-Schiff-DOX nanoparticles can be directly wrapped into the four-arm polyethylene glycol hydrogel solution as shown in Figure 4 and Figure 5, providing a simple strategy to prepare the drug-loaded hydrogels.

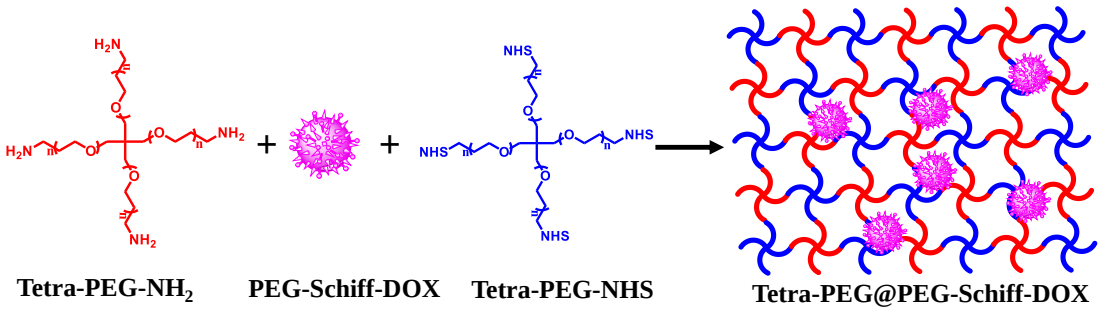

Fig. 4. The synthesis scheme of Tetra-PEG@PEG-SCHIF-DOX hydrogel

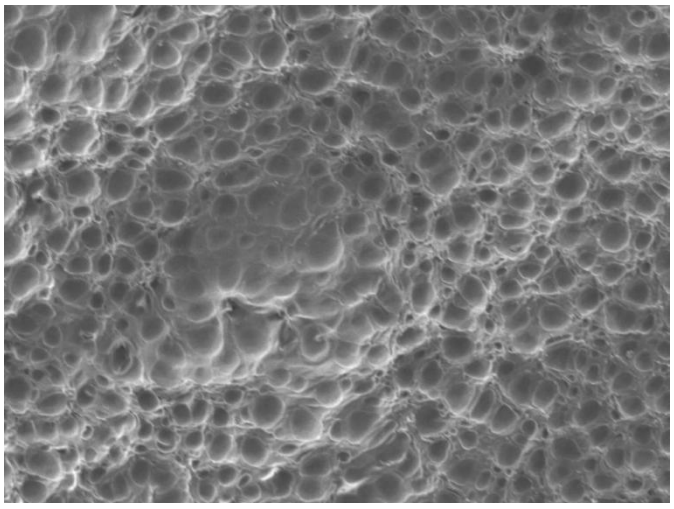

Fig 5. The morphology of tetra-PEG hydrogel loaded with PEG-Schiff-DOX

3.3. In vitro drug release

The test results of drug release behavior are more intuitive, which can be seen from the results of ultraviolet detection. As shown in Figure 6, the PEG-Schiff-DOX nanoparticles loaded with almost no drug release at pH = 7.4, and the release rate was significantly accelerated at pH = 5.0. These findings indicate that these nanoparticles have the potential to minimize the leakage in the neutral solution of blood circulation while enabling rapid drug release during the acidic tumors.

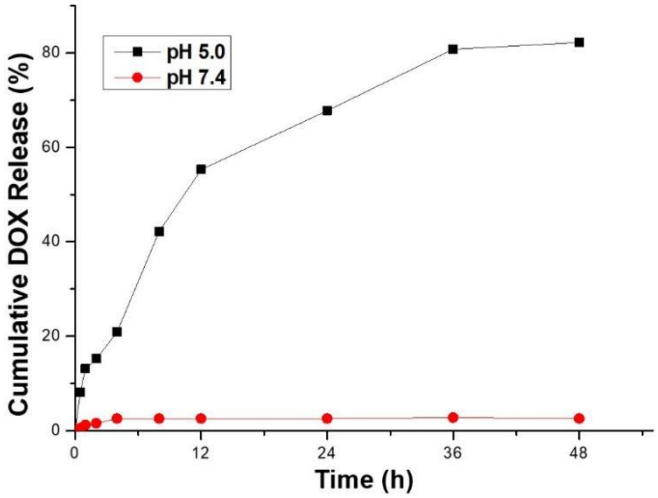

Fig 6. In vitro release curve of the gel loaded with PEG-Schiff-DOX nanomedicine

3.4. Anti-tumor ability evaluation

It can be concluded from Figure 6 and Figure 7 that PEG-Schiff-DOX nanomedicine has excellent anti-tumor effect, especially when the concentration of loaded DOX reaches 100 μg/mL, it shows a very high effect of inhibiting tumor growth, and the cell survival rate It is less than 8%, indicating that the prepared PEG-Schiff-DOX is a targeted agent that is expected to be applied in clinic.

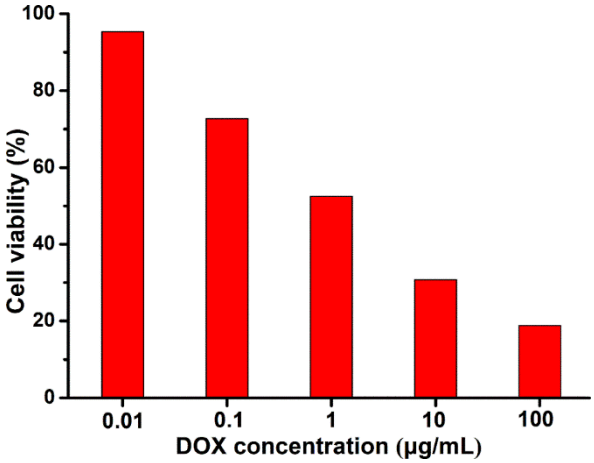

Fig 7. Evaluation of antitumor effect of PEG-Schiff-DOX nanomedicine

Taking consideration of the pH-responsive drug release, we believed that the tetra-PEG@PEG-Schiff-DOX hydrogel also possessed the high drug content and pH-triggered drug release for improving the tumor therapy, especially for the in-situ antitumor therapy of organs, which will be detailedly discussed in the future work.

4. Conclusion

In a word, we have successfully prepared an amphiphilic macromolecule known as PEG-Schiff-DOX and utilized it to create innovative micellar nanoparticles that respond to pH changes. These polymeric nanoparticles possessed the below notable advantages: 1) a clear structure and straightforward synthesis; 2) a high drug loading capacity; 3) excellent storage stability (maintained stable over 1 week); 4) minimal or negligible drug leakage of the original DOX release in non-neutral circulation and endosomal pH conditions, thereby preserving the therapeutic ability of DOX with low side effects; and 5) good tumor inhibition compared to pure small DOX drug. These prodrug-based nanoparticles present a beneficial avenue for the development of novel DOX formulations.

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