Biomimetic pseudopolyrotaxane prodrug micelles with high drug content for intracellular drug delivery

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Introduction

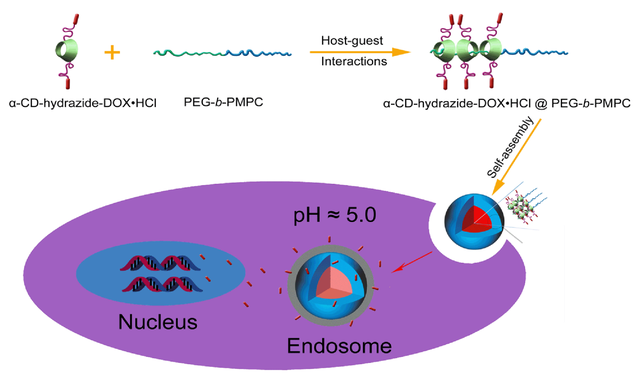
Prodrug micelles have been widely used for drug delivery and cancer therapy research.[1] However, up until now, most reported prodrug micelles are derived from amphiphilic block copolymers and the fabrication process usually requires sophisticated assembly techniques as well as the assistance of organic solvents.[1-4] Besides, most as-reported prodrug micelles usually involve a considerable amount of inert materials, which often reduces the drug proportion and causes an increased metabolic burden, in turn deteriorating the therapeutic efficacy.[1,2] Thus, in order to better preserve the bioactivity of payloads, further simplify the preparation process as well as increasing drug loading content, a facile and mild method to construct prodrug micelles with high drug content in water would be more preferable.

Inspired by the structure of (pseudo)polyrotaxanes formed from α-cyclodextrin (CD) and poly(ethylene glycol) (PEG),[5-7] we report here the preparation of stable pseudopolyrotaxane prodrug micelles with high drug content through simple mixing of two hydrophilic segments in aqueous medium. [8]

Method

Two hydrophilic segments [α-CD-hydrazide-DOX•HCl & poly(ethylene glycol)-*b*-poly(2-methacryloyloxyethyl phosphorylcholine) (PEG-*b*-PMPC) block copolymers] were firstly synthesized respectively. Structures were verified by ^{1}H NMR.

Then, prodrug micelles were prepared by simply mixing these two hydrophilic segments in water, which was characterized by 2D ^{1}H NOESY, DLS and TEM. Finally, *in vitro* drug release, cell uptake behavior and intracellular drug release were assessed.



Scheme 1. Schematic illustration for preparation of supramolecular prodrug micelles and control release of DOX under endo-/lysosomal pH stimulus.

Results and Discussion

^{1}H NMR spectra were used to characterize the structures of α-CD-hydrazide-DOX•HCl and PEG-*b*-PMPC. It can be seen from Figure 1, these two hydrophilic segments were synthesized successfully.

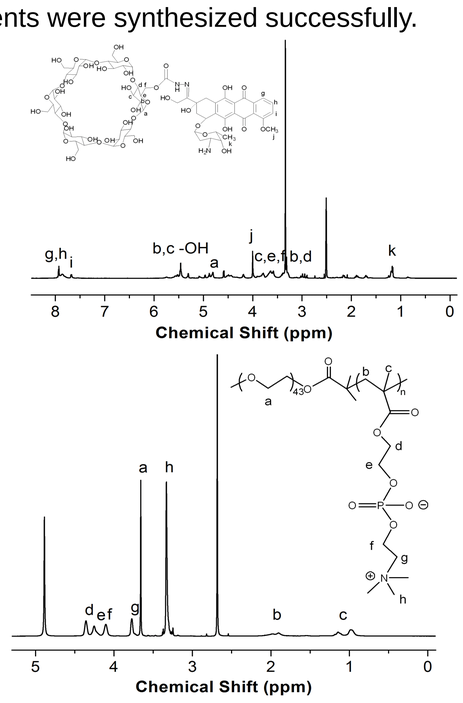


Figure 1. ^{1}H NMR spectra of α-CD-hydrazide-DOX HCl in DMSO-d_6 and PEG-b-PMPC in CD_3OD-d_4.

2D ^{1}H NOESY spectrum provided a direct evidence for the formation of host-guest inclusion complex in D_2O (Figure 2).

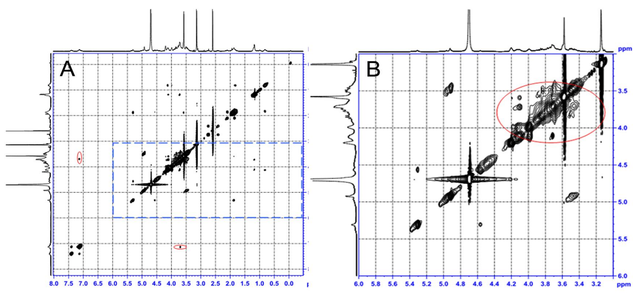


Figure 2. 2D ^{1}H NOESY spectrum of the mixture of PEG-b-PMPC and α-CD-hydrazide-DOX •HCl in D_2O at room temperature.

Dynamic light scattering (DLS) and transmission electron microscope (TEM) further confirmed the formation of supramolecular micelles (Figure 3).

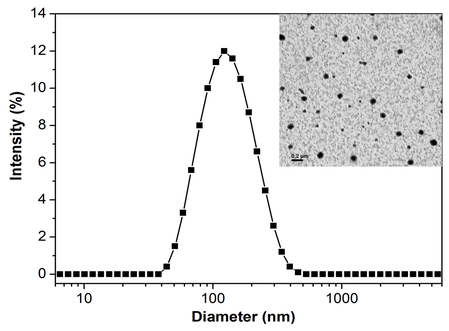


Figure 3. DLS plot and representative TEM image of the supramolecular micelles.

As shown in Figure 4, more DOX was released under acidic condition than that of under physiological condition.

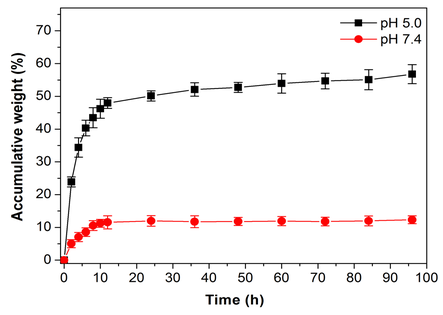


Figure 4. In vitro release of DOX from the supramolecular prodrug micelles in PBS under different pH conditions.

It can be seen from Figure 5, supramolecular prodrug micelles were able to deliver and release DOX into the nuclei of cancer cells.

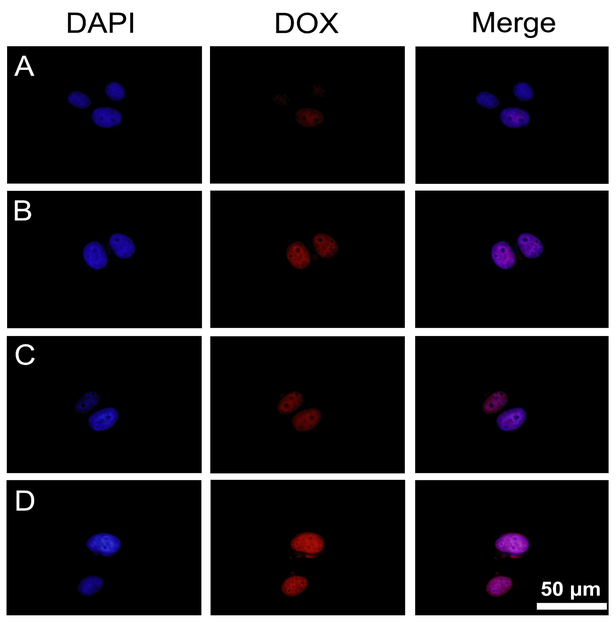


Figure 5. Fluorescence microscopy images of HepG2 incubated with the prodrug and free DOX (10 μg mL^{-1}). From left to right: DAPI (blue), DOX (red) and a merge of the two images. (A) prodrug, 1 h; (B) prodrug, 3 h; (C) free DOX, 1 h; (D) free DOX, 3 h.

Flow cytometry analysis indicated that mean fluorescence intensities of cells treated with prodrug micelles were increased as the incubation time prolonged from 1 h to 3 h (Figure 6A). Moreover, MTT assay results demonstrated that the as-prepared supramolecular micelles could effectively inhibit proliferation of cancer cells (Figure 6B).

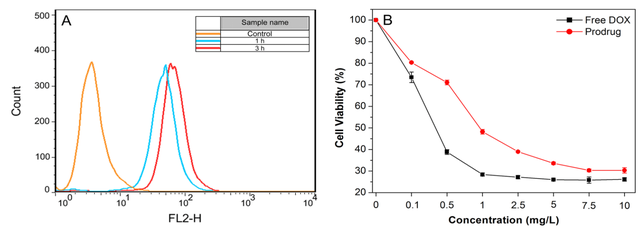


Figure 6. Flow cytometric profiles of HepG2 cells incubated with prodrug micelles (10 μg mL^{-1}) for 1 h or 3 h (A); Cell viability of HepG2 cells incubated with various concentrations of prodrug micelles for 48 h (B).

Conclusions

In summary, we have illustrated a facile strategy to construct biomimetic pseudopolyrotaxane prodrug micelles with high drug content by the host-guest interaction of PEG-*b*-PMPC and α-CD-hydrazide-DOX•HCl in water. The resultant micelles could respond to the endosomal pH, releasing DOX and inhibiting the growth of cancer cells, which implied their potentials in being applied as efficient platforms for cancer therapy. Moreover, it is expected that the strategy developed in this paper may offer a robust and versatile way for fabricating novel pseudopolyrotaxane-based drug delivery system with high loading content in water, which could potentially be instructive for multifunctional nanocarriers development.

References

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