Functional 2-methylene-1,3-dioxepane terpolymer: a versatile platform to construct biodegradable polymeric prodrugs for intracellular drug delivery

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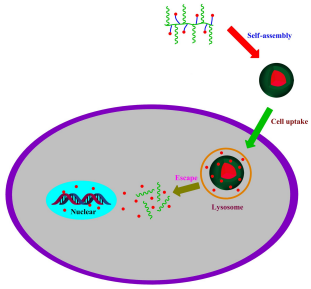
Introduction

Biodegradable polymers have been widely used in biomedical fields, such as tissue engineering, biomedical implants, and drug delivery.[1] Radical ring-opening polymerizations (RROPs) of cyclic ketene acetals, such as 2-methylene-1,3-dioxepane (MDO), have recently attracted significant interest for preparation of biodegradable aliphatic polyesters.[2]

Polymeric prodrug micelles have received extensive attention as drug nanocarriers.[3] Since the drug is covalently conjugated to the polymer, polymeric prodrugs are more stable than traditional physically encapsulated drugs during circulation. For the purposes of drug delivery vehicles, it is imperative for prodrug micelles to have good biodegradability properties because the low-molecular weight degradation products are expected to be metabolized or excreted, and not to cause long-term toxicity. In recent years, biodegradable polymers have been used to fabricate polymeric prodrug delivery systems.[4] In this research, we constructed biocompatible amphiphilic functional polyesters that can be used to covalently conjugate DOX. We hypothesize here that the facile introduction of functional polyester into polymeric prodrug systems will provide a novel approach to develop more sophisticated drug nanocarriers that might have great potential in biomedical applications.

Method

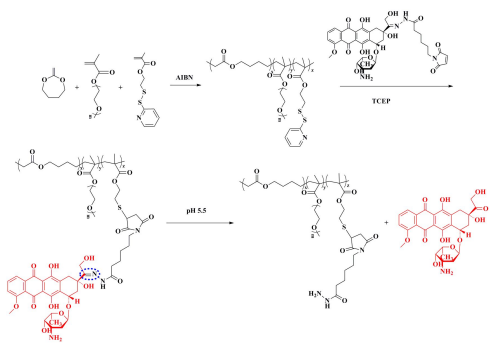
Drug loaded polymer micelles was first accumulated in tumor tissues via EPR effects and then entered into cells through cell uptake. Caused by the difference between cancer cells and physiological conditions, such as pH, drug can be easily released and enter into nucleus, thus prevent the growth of tumor cells.



Scheme 1. Schematic illustration of the self-assembly and drug release of the DOX conjugated polymer micells under endo-/lysosomal pH stimulus.

Results and Discussion

P(PEGMA-co-MDO-co-PDSMA) was synthesized via radical ring-opening polymerization (RROP). Then the DOX conjugated polymer was synthesized just by mixing P(PEGMA-co-MDO-co-PDSMA), tris (2 -carboxyethyl) phosphate (TECP) and Maleimide-containing end groups modified doxorubicin (MAL-DOX).



Scheme 2. Schematic illustration of the synthesis of DOX-conjugated P(PEGMA-co-MDO-co-PDSMA)

^{1}H NMR and GPC was used to show the successfully synthesized and potential of enzymatic degradation.

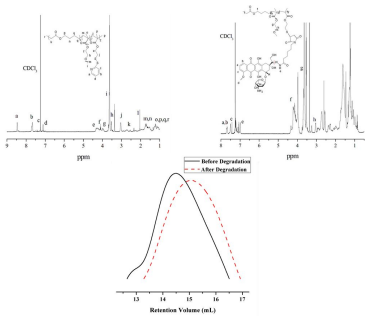


Figure 1. ^{1}H NMR spectra of P(PEGMA-co-MDO-co-PDSMA) in $CDCl_3$ and DOX conjugated polymer in $CDCl_3$ and GPC traces of the copolymer before and after enzymatic degradation

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

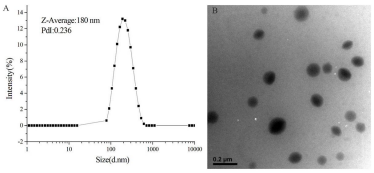


Figure 2. DLS plot(A) and representative TEM image(B) of the polymer micelles.

In vitro durg release was conducted at different pH values.The release rate was more faster at pH 5.5 than at pH 7.4.

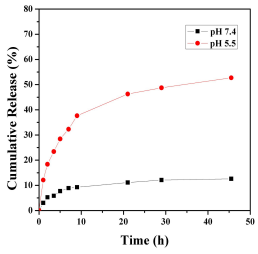


Figure 3. Percentage of cumulative release of DOX from DOX-conjugated polymer micells at pH 7.4 and 5.5.

It can be seen from Figure 4, DOX conjugated polymer micells were able to deliver and release DOX into the nuclei of cancer cells.

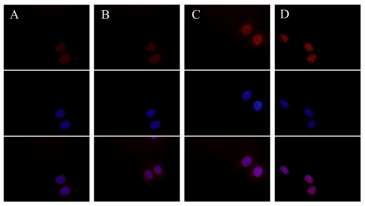


Figure 4. Fluorescence microscopy images of A549 cells incubated with the DOX-conjugated polymer micells and free DOX (10 mg/mL). From top to bottom: DOX (red), DAPI (blue) and a merge of the two images. (A) Prodrug, 2 h; (B) prodrug,4 h; (C) prodrug, 6 h; (D) free DOX, 6 h. The scale bars are 20 mm in all images

Flow cytometry analysis indicated that mean fluorescence intensities of cells treated with DOX conjugated polymer micelles were increased as the incubation time prolonged from 2 h to 6 h .

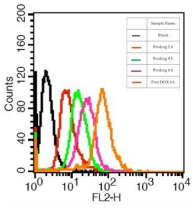


Figure 5. Flow cytometric profiles of A549 cells incubated with DOX-conjugated polymer micelles for different time intervals.

As shown in Figure 6, the DOX conjugated polymer micells can effective inhibition of A549 cells growth.

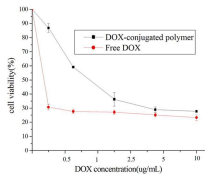


Figure 6. Cell viability of DOX-conjugated polymer micells and free DOX after 48 h incubation in A549 cells at different concentration.

Conclusions

In summary, the copolymer P(PEGMA-co-MDO-co-PDSMA) and corresponding DOX conjugated polymer were synthesized successfully according to ^{1}H NMR. MDO was a special material used here which was biodegradable and conjugated to PEGMA and PDSMA via ring-opening polymerization (ROP). This DOX conjugated amphiphilic copolymer can self-assemble into micells with DOX encapsuling into the hydrophobic core, as showed in DLS and TEM pictures.The DOX was conjugated to polymer chain via a hydra-zone bond which can cleavage upon Lysosomal/endosomes pH. Thus the DOX conjugated polymer micells can respond to lower pH and released DOX. This kind of drug loaded carriers has great potential for biomedical applications.

References

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