A biomimic pH-sensitive polymeric prodrug based on polycarbonate for intracellular drug delivery

Haibo Wang, Yangjun Chen(11129016), Qiao Jin* and Jian Ji*

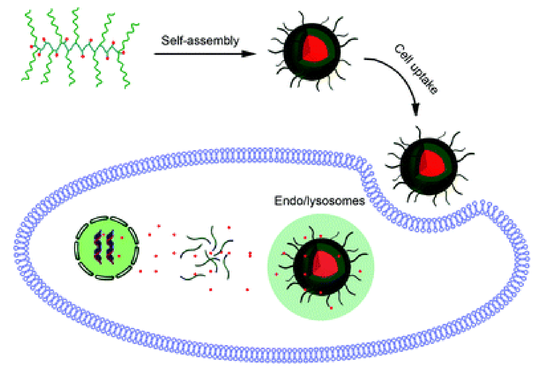
MOE Key Laboratory of Macromolecular Synthesis and Functionalization, Department of Polymer Science and Engineering, Zhejiang University, Hangzhou 310027, China.

*Contact to: Ji Jian, email: jijian@zju.edu.cn, Tel/Fax: +86-571-87953729

1. Introduction

Polymeric prodrug micelles have received extensive attention for cancer therapy due to the improved water solubility of drugs and enhanced drug bioavailability. One of the most suitable strategies is to design prodrugs which could respond to external stimuli. For example, hydrazone bond can maintain stability under physiological and blood conditions (pH 7.4) while easily cleaved at endosomal pH (5.0).[1] Moreover, development of DDS with biodegradable properties is of great importance. Polycarbonates are an important class of biodegradable and biocompatible polymers,[2] but few studies have explored polycarbonates for polymeric prodrugs. In addition, To exhibit long circulation time in blood, polymer micelles should avoid non-selective uptake by the reticuloendothelial system. Polymeric micelles with phosphorylcholine coating have excellent resistance to protein adsorption and cell adhesion, which could prevent polymer micelles from elimination.[3]

Based on these considerations, herein we developed a biodegradable pH-sensitive polymeric prodrug based on poly(5-methyl-5-allyloxycarbonyl-1,3-dioxan-2-one)-*graft*-12-acryloyloxy dodecyl phosphorylcholine (PMAC-*graft*-ADPC) containing hydrazone bond linked doxorubicin.



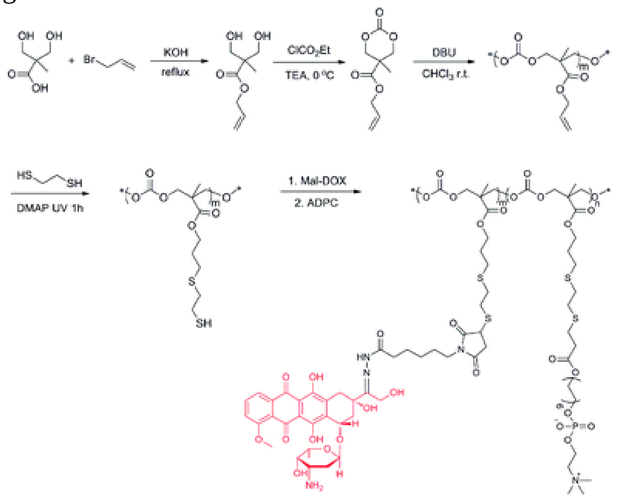
Scheme 1 *Illustration of the self-assembly of polymeric prodrug micelles and pH-responsive intracellular release in endosomal compartments.*

2. Method

5-Methyl-5-allyloxycarbonyl-1,3-dioxan-2-one (**MAC**), 12-acryloyloxy dodecyl phosphorylcholine (**ADPC**) and 6-maleimidocaproyl-doxorubicin (**Mal-DOX**) were synthesized according to the literature. **PMAC** was synthesized by ring-opening polymerization (ROP), then functionalized using 1,2-ethanedithiol to get **Thiol-PMAC**, afterward **PMAC-*graft*-(ADPC-*co*-Mal-DOX)** was synthesized by "click" ADPC and Mal-DOX onto Thiol-PMAC. Structures were verified by ^{1}H NMR and Mass spectra.

Prodrug micelles were prepared by dialysis and characterized by DLS and TEM.

Finally, in vitro degradation, *in vitro* drug release, cell uptake behavior and intracellular drug release were assessed.



Scheme 2 *Detailed synthetic route of PMAC-graft-(ADPC-co-Mal-DOX).*

3. Results and Discussion

^{1}H NMR spectra confirmed the successful synthesis of the following compounds.

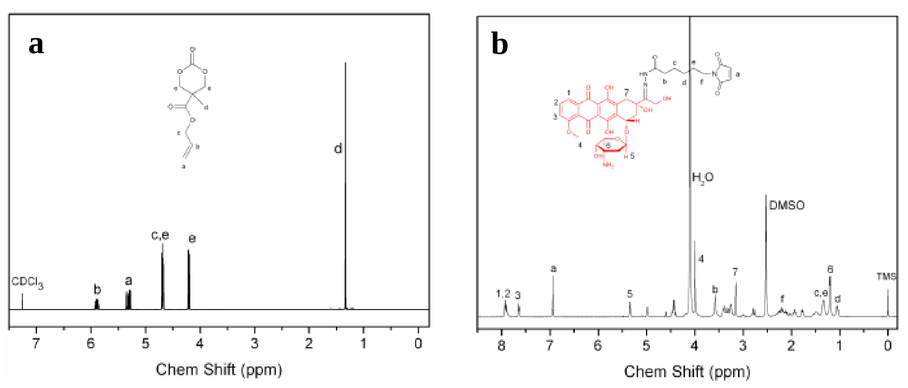


Fig. 1 *^{1}H NMR spectra of (a) MAC, (b) Mal-DOX, (c) PMAC-graft-(ADPC-co-Mal-DOX).*

Dynamic light scattering (DLS) and transmission electron microscope (TEM) further confirmed the formation of supramolecular micelles (Figure 2).

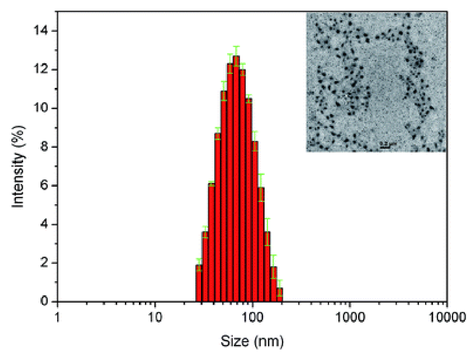


Fig. 2 *DLS plot and representative TEM image of the prodrug micelles.*

Size change caused by pH induced cleavage of DOX and degradation of the backbone was recorded by DLS.

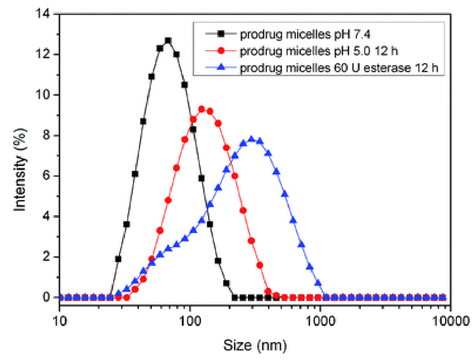


Fig. 3 *Change of size distributions of polymeric prodrug micelles at different pH values or treated by porcine esterase monitored by DLS.*

The degradation rate of PMAC was further measured by the weight loss of the polymer over predetermined time intervals.

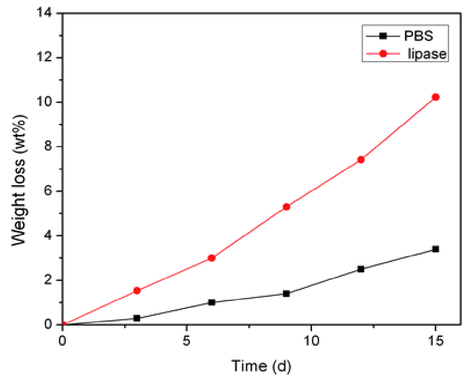


Fig. 4 *Mass loss of PMAC conditioned in lipase solution or PBS (pH = 7.4) at 37 ° C for different time periods.*

As shown in Figure 5, more DOX was released under acidic condition than that under physiological condition.

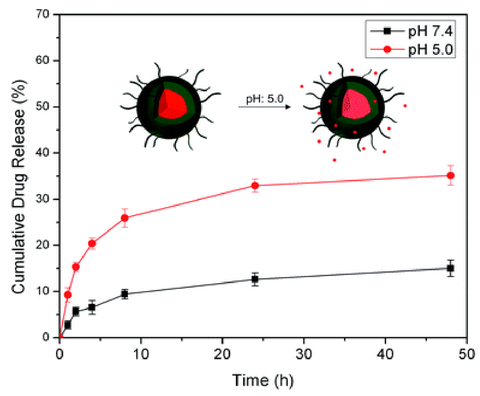


Fig. 5 *In vitro release of DOX from polymeric prodrug micelles in PBS under different pH conditions.*

Cellular uptake and intracellular release of DOX for cancer cells were studied by flow cytometry and fluorescence microscopy. Figure 6 indicated DOX could be cleaved from the prodrug and escape from the endo/lysosomes to nucleus rapidly.

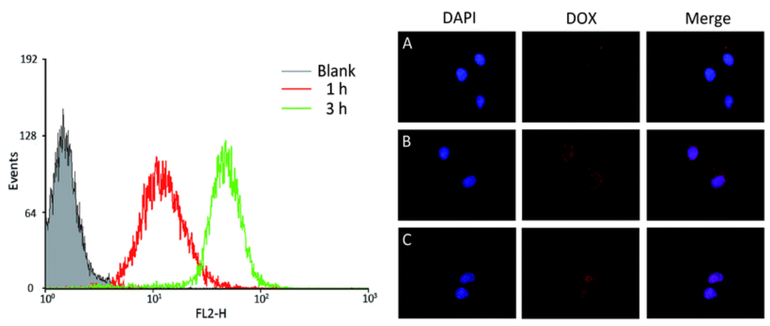


Fig. 6 Left: Flow cytometric profiles of HepG2 cells incubated with prodrug micelles for 1 h or 3 h; Right: Fluorescence microscopy images of HepG2 incubated with prodrug micelles or free DOX for different time periods. From left to right DAPI (blue), DOX (red) and merge of the two images. (A) Polymeric prodrug micelles, 1 h; (B) polymeric prodrug micelles, 3h; (C) free DOX, 1 h.

MTT assay results further demonstrated that the prodrug micelles could effectively inhibit proliferation of cancer cells with IC50 of 2.7 μg mL^{-1} and 3.1 μg mL^{-1} respectively evaluated by HepG2 cells and HeLa cells.

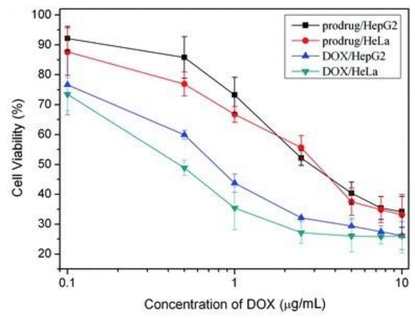


Fig. *7 cell cytotoxicity of HepG2 and HeLa incubated with various concentrations of polymeric prodrug micelles or free DOX for 48 h.*

4. Conclusion

A novel polymeric prodrug PMAC-*graft*-(ADPC-*co*-Mal-DOX) was synthesized via ROP and subsequent click chemistry. The Prodrug micelles were responsive to pH and esterase due to pH sensitive hydrazone bond and hydrolyzable polycarbonate backbone. When internalized by cancer cells through cell uptake, DOX could be cleaved and escape from the endosome to the nucleus rapidly, thus exhibited high cytotoxicity against HepG2 cells and HeLa cells. Hence, this polymeric prodrug could be an efficient and promising platform for cancer therapy.

5. References

1. Y. Bae et al., *Angew. Chem., Int. Ed.*, 2003, 42, 4640–4643
2. Z. Xie et al., *Biomacromolecules*, 2008, *9* (1), pp 376–380
3. H. Wang et al., *Polym. Chem.*, 2013, 4, 3012–3019

6. Acknowledgements

Financial support from the NSFC-21174126, National Science Fund for Distinguished Young Scholars (51025312), the National Basic Research Program of China (2011CB606203) and Research Fund for the Doctoral Program of Higher Education of China (20110101110037 and 20120101130013) and Open Project of State Key Laboratory of Supramolecular Structure and Materials (sklssm201316) are gratefully acknowledged.