Polymer Chemistry

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/polymers

Polymer Chemistry

RSCPublishing

COMMUNICATION

Facile Fabrication of Reduction-Responsive Nanocarriers for Controlled Drug Release†

Cite this: DOI: 10.1039/x0xx00000x

Rui Sun,‡[a] Qiaojie Luo,‡[b] Chen Gao,[a] Ying Wang,[a] Lilong Gao,[a] Hong Du,[a] Ying Huang,[b] Xiaodong Li,[b] Zhiquan Shen,[a] and Weipu Zhu*[a]

Received 00th January 2014,
Accepted 00th January 2014

DOI: 10.1039/x0xx00000x

www.rsc.org/polymers

An amphiphilic multiblock poly(ether-ester) containing multiple thiols was facilely synthesized by "one-pot" polycondensation of dihydroxyl poly(ethylene glycol), 1,4-butanediol and mercaptosuccinic acid, which could be used to fabricate reduction-responsive core-crosslinked micelles for controlled drug release.

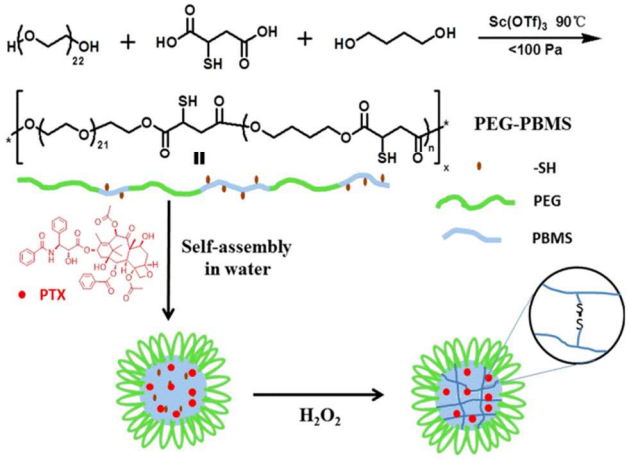
Recently, much attention has been attracted to polymeric micelles self-assembled from amphiphilic copolymers in the field of biomedical applications due to their unique characteristics such as core-shell structure, mesoscopic size range and prolonged blood circulation.1, 2 The hydrophobic cores can solubilize hydrophobic drugs and protect drug molecules from degradation, while the hydrophilic shells can prolong drug circulation time. Meanwhile, their nanoscopic size is effective on avoiding both rapid renal exclusion and recognition and uptake by the reticuloendothelial system (RES).3 Moreover, the small size of the drug carriers promotes drug penetrating through vascular pores and drug accumulating at targeted sites.4 However, polymeric micelle is a dynamic equilibrium and thermodynamic unstable system with a tendency to dissociate at low concentrations, especially upon intravenous administration.5 The instability may lead to poor drug capacity, premature drug release and low selective absorption by pathological tissues for drug loaded micellar carriers. Therefore, stabilization of polymeric micelles becomes a critical strategy to promote their performance as drug carriers. Nowadays, crosslinking of the cores or shells of polymeric micelles has emerged as a viable strategy for improving micellar stability by providing covalent linkages between polymer chains. This strategy has been employed to prepare core-crosslinked micelles,6, 7 shell-crosslinked micelles,8, 9 and also other nano-objects such as nanocages10, 11 and nanoporous films.12 Compared with non-crosslinked precursors, these crosslinked nano-objects can maintain stable structure under severe environments.

Recent studies have been directed to the development of stimuli-responsive micelles, from which the release of drug could be triggered by exerting an appropriate stimulus (e.g., temperature, pH, glutathione, etc.),13-15 after arrival at the targeted sites. Disulfide bonds are reduction-sensitive and readily cleavable by thiols, such as 1,4-dithiothreitol (DTT)16-18 and glutathione (GSH).19 It should be noted that the concentration of GSH in tumor cells (8-10 mM) is about 2-fold higher than that in normal cells (2-8 mM) and much higher than that in blood plasma (1-2 μM).20 This provides an excellent environment for targeted antitumor drug release. Therefore, design and preparation of reduction-sensitive crosslinked polymer micelles as antitumor drug carriers have attracted more and more attentions.21-23

As a U.S. Food and Drug Administration (FDA) approved hydrophilic polymer, poly(ethylene glycol) (PEG) has been widely investigated as a biocompatible polymer in both academia and industry for chemical and biological applications, such as drug delivery and tissue engineering.24-27 Aliphatic multifunctional poly(ether-ester)s containing PEG segments are attractive candidates for drug carriers due to their excellent biodegradability, biocompatibility and crosslinkability.28, 29 In most cases, PEG based multifunctional poly(ether-ester)s are prepared via the ring-opening polymerization of functional cyclic esters or carbonates in the presence of PEG as macroinitiator, which needs multi-step synthesis, including burdensome protections and deprotections. Alternatively, quite a few poly(ether-ester)s with various architectures have been synthesized facilely by polycondensation of hydrophilic PEG and hydrophobic molecules.30 Nevertheless, the high temperature of traditional polycondensation prevents it from introducing functional groups into the poly(ether-ester) backbone. Recently, our research group has developed a facile "one-pot" synthesis of PEG derivatives by polycondensation of diol PEG with functional diacids, such as malic acid,31, 32 mercaptosuccinic acid 33, 34 and maleic anhydride,35 in the presence of scandium trifluoromethanesulfonate [Sc(OTf)3] as catalyst. Water soluble PEG derivatives containing multi-hydroxys, multi-thiols or multi-enes could be successfully synthesized due to the chemoselectivity of Sc(OTf)3 catalyst and the relatively low polycondensation temperature.36, 37

In this work, 1,4-butanediol was used as hydrophobic comonomer to copolymerize with diol PEG and mercaptosuccinic acid by polycondensation in the presence of Sc(OTf)3 as catalyst under moderate temperature, resulting in an amphiphilic multiblock poly(ether-ester) containing hydrophilic PEG and hydrophobic poly(butylene mercaptosuccinate) (PBMS) segments. This amphiphilic multiblock copolymer (PEG-PBMS) could be facilely produced in a large scale, and used to prepare disulfide core-

crosslinked micelles as antitumor drug nanocarriers, which shows rapid response to reductive environment (Scheme 1).



Scheme 1 Synthesis, self-assembly and drug-loading of PEG-PBMS multiblock copolymer

The chemical composition of PEG-PBMS multiblock copolymer with pendent mercapto groups was characterized by proton nuclear magnetic resonance (1H NMR, 13C NMR) and Fourier transform infrared (FT-IR) spectroscopy. As shown in Fig. S1-2†, The characteristic proton signals of PEG and PBMS segments are presented and marked. All peaks can be well assigned with their chemical structures. Notably, the characteristic signal of mercaptoproton (Ha) was clearly detected at 2.38 ppm, demonstrating the stability of mercapto groups during the polycondensation process. The molar ratio of butylene units to PEG segments could be calculated by the integral of corresponding proton (Hb and Hf), which is 3.9: 1. This is close to the initial feed ratio (4.4: 1), indicating the similar activity of 1,4-butanediol and diol PEG in polycondensation with mercaptosuccinic acid. The PEG-PBMS multiblock copolymer was further confirmed by FT-IR as shown in Fig. S3†, in which the absorptions of the S–H stretch at 2540 cm-1 and the C=O stretch at 1750 cm-1 were clearly detected. Moreover, size exclusion chromatography (SEC) analysis shows a unimodal and symmetric peak with Mw of 12,200 g/mol, also suggesting the successful synthesis of PEG-PBMS multiblock copolymer (Fig. S4†).

Paclitaxel (PTX) was used to evaluate the drug-loading capacity of PEG-PBMS core-crosslinked (CCL) micelles. The initial weight ratio of PTX to copolymer was 1: 10. PEG-PBMS and PTX were dissolved in THF, and then distilled water was added dropwise to the solution under vigorous stirring to induce the hydrophobic PTX to incorporate into the hydrophobic micellar core. The micelles were finally prepared by dialysis method and crosslinked by H2O2. A PTX encapsulation efficiency of 51.6 % was obtained, and the actual PTX loading content was calculated to be 5.2 %. The hydrophobic antitumor drug PTX could be efficiently loaded into PEG-PBMS CCL micelles due to the hydrophobic interaction between PTX and PBMS blocks.

The sizes and morphologies of PEG-PBMS CCL micelles and PTX-loaded PEG-PBMS CCL micelles were studied by dynamic light scattering (DLS) and transmission electron microscopy (TEM) measurements as shown in Fig. 1. The diameter of the PEG-PBMS CCL micelles increased upon PTX incorporation, which is attributed to the hydrophobic interaction between PTX and PBMS blocks, resulting in expanded nanoparticles.38, 39 The presence of stable nanoparticles in non-selective solvent (DMF) strongly demonstrates the robust crosslinked structure of PEG-PBMS CCL micelles. Furthermore, the size of PEG-PBMS CCL micelles in non-selective solvent (DMF) is significantly larger than that in water. This may attribute to the considerable swelling of the crosslinked hydrophobic core of the CCL micelles in DMF. It is observed from the TEM images that the self-assembled micelles are well dispersed as individual nanoparticles with regularly spherical shapes. The diameter of PEG-PBMS CCL micelles in water is smaller than those of PTX-loaded PEG-PBMS CCL micelles in water and PEG- PBMS CCL micelles in DMF, which agrees with the DLS results quite well. It is notable that an obvious core-shell structure can be observed in Fig. 1 (D), in which the hydrophobic core is in dark color and the hydrophilic shell is in light color. The compact hydrophobic core was fully stained by OsO4, whereas the loose hydrophilic PEG shell cannot be fully stained leading to relatively light color. The difference in micelles size measured by DLS and TEM should be attributed to the fact that the former is the hydrodynamic diameter of micelles in solvent, whereas the latter reveals the morphology size of micelles in solid state.

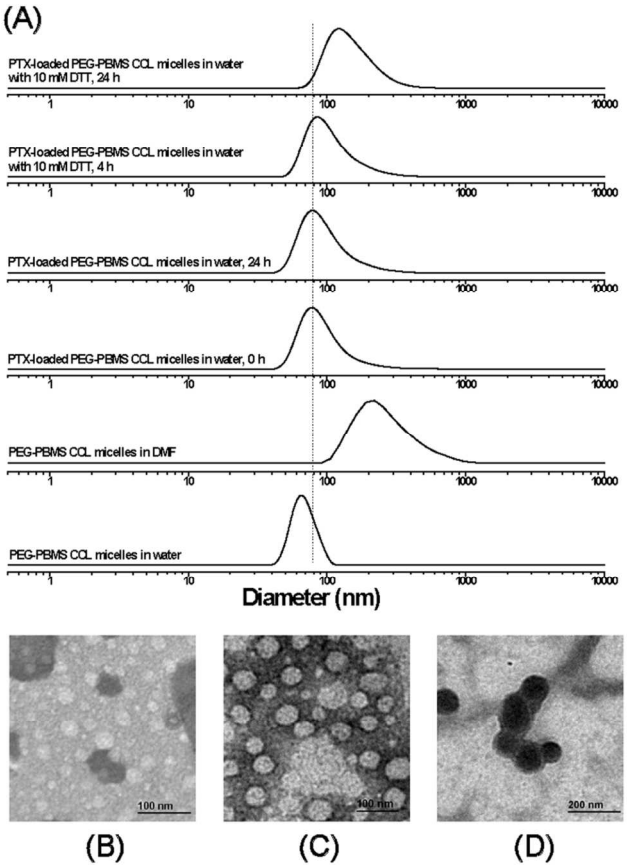


Fig. 1 Particle sizes and distributions of PEG-PBMS CCL micelles. (A) DLS results; (B) TEM image of PEG-PBMS CCL micelles in water; (C) TEM image of PTX-loaded PEG-PBMS CCL micelles in water; (D) TEM image of PEG-PBMS CCL micelles in DMF.

The stability of PEG-PBMS CCL micelles was also investigated by DLS. As Fig. 1 (A) shows, the diameter PEG-PBMS CCL micelles in water did not significantly change even after 24 hours, indicating good stability in water. On the other hand, the PEG-PBMS CCL micelles showed rapid response to reducing agent. The diameter of PEG-PBMS CCL micelles rapidly increased from 90 nm to 110 nm within 4 hours in the presence of 10 mM DTT. Moreover, larger aggregates with a diameter of 170 nm were obtained after 24 hours. This phenomenon suggests that the cleavage of disulfide bonds by DTT leads to unstable micelles with loose micellar structure.

The PTX release profiles from PEG-PBMS CCL micelles in phosphate buffer solution (PBS, pH = 7.4, containing 0.1 wt-% Tween 80) with or without 10 mM DTT were obtained using dialysis method. As shown in Fig. 2, PTX release from PEG-PBMS CCL micelles in PBS with 10 mM DTT was rapid and nearly 80 % of loaded PTX was released within 60 hours. In contrast, PTX release from the CCL micelles in PBS without DTT was much slower than that with DTT, and only about 20 % of loaded PTX was released in the test duration. Obviously, in the presence of a reducing agent (DTT, 10 mM), the hydrophobic drug molecules can be easily released from the core due to the cleavage of the disulfide bonds. However, it has been reported that disulfide bond was stable enough in simulative human blood plasma environment.40 Therefore, it is conceivable that PTX-loaded PEG-PBMS CCL micelles can be passively targeted to the tumor tissue through the EPR effect.41 This study demonstrated that PEG-PBMS CCL micelles could be potentially developed as efficient antitumor drug carriers.

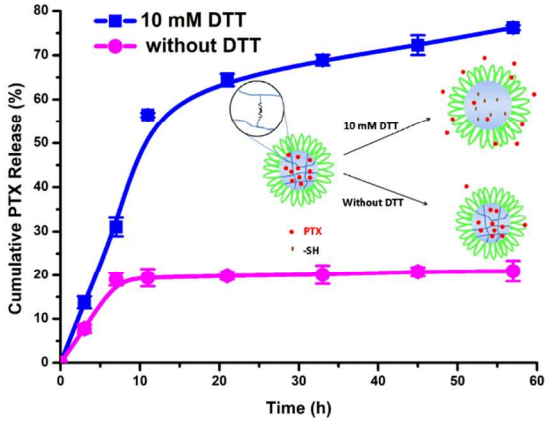


Fig. 2 PTX release kinetics from PEG-PBMS CCL micelles in 20 mM PBS (pH=7.4, containing 0.1 wt-% Tween 80) with and without 10 mM DTT at 37°C.

In order to investigate the cell uptake and internalization of drug-loaded CCL micelles into cells, Nile red (NR) was used to replace PTX as a fluorescence probe to prepare NR-loaded PEG-PBMS CCL micelles, which were incubated with HeLa cells. The cells treated with free NR were used as control. After incubating for 1 hour or 4 hours, the HeLa cells were monitored using confocal laser scanning microscopy (CLSM). As shown in Fig. 3, the NR fluorescence in the HeLa cells treated with free NR could be hardly observed even after incubation for 4 hours. However, strong NR fluorescence was observed around the nuclei of HeLa cells after only 1 hour incubation with NR-loaded PEG-PBMS CCL micelles. The NR fluorescence became stronger even in the cell nuclei at a longer incubation time of 4 hours. As a comparison, another HeLa cell line was pretreated with 0.5 mM buthionine sulfoximine (BSO), which is an inhibitor for the intracellular synthesis of GSH and will not affect the proliferation of Hela cells at the tested concentration.42-45 The fluorescence intensity of NR is much weaker in the HeLa cells pretreated with BSO than that in non-pretreated HeLa cells. All of the CLSM results indicate that PEG-PBMS CCL micelles could be used as efficient drug carriers to delivery hydrophobic drugs into cells and the loaded drug could be rapidly released in response to the intracellular reductive environment.

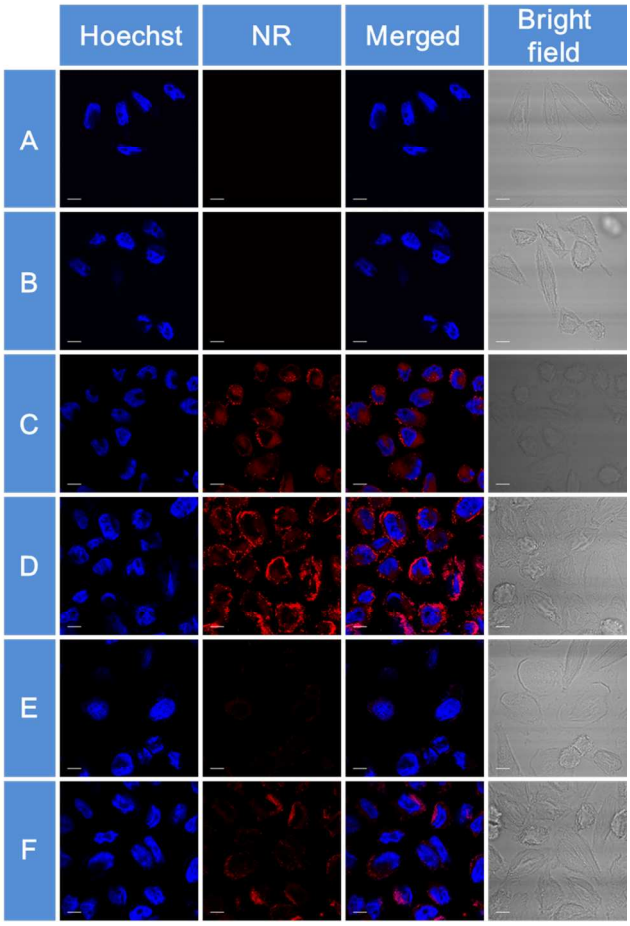


Fig. 3 CLSM images of HeLa cells after 1 or 4 h incubation with NR-loaded PEG-PBMS CCL micelles and free NR. For each panel, the images from left to right showed cell nuclei stained by Hoechst 33342 (blue), NR fluorescence in cells (red), overlays of two left images and bright field. The scale bars correspond to 10 μm in all the images. (A) free NR, non-pretreated HeLa cells, 1 hour; (B) free NR, non-pretreated HeLa cells, 4 hours; (C) NR-loaded PEG-PBMS CCL micelles, non-pretreated HeLa cells, 1 hour; (D) NR-loaded PEG-PBMS CCL micelles, non-pretreated HeLa cells, 4 hours; (E) NR-loaded PEG-PBMS CCL micelles, HeLa cells pretreated with BSO, 1 hour; (F) NR-loaded PEG-PBMS CCL micelles, HeLa cells pretreated with BSO, 4 hours.

Microplate reader was used for quantitative determination of cellular uptake of NR-loaded PEG-PBMS CCL micelles. As shown in Fig. S5†, after both 1 hour and 4 hours incubation, the HeLa cells incubated with NR-loaded CCL micelles showed much higher NR fluorescence intensity than those incubated with free NR. On the other hand, the HeLa cells pretreated with BSO exhibited weaker NR fluorescence intensity than non-pretreated cells under the same conditions. These data further confirm the GSH responsive release behavior of drug-loaded PEG-PBMS CCL micelles, which matches the CLSM results quite well.

To evaluate the cytotoxicity of the polymeric micelles, a HeLa cell line was exposed to PEG-PBMS multiblock copolymer and PEG-PBMS CCL micelles at a series of concentrations ranging from 0 to 1000 μg/mL for 48 hours by Cell Counting Kit-8 (CCK-8) assay. Compared with the control, the presence of all the samples tests did not significantly inhibit cell growth, which suggested that PEG-PBMS block copolymer and corresponding CCL micelles were almost nontoxic against HeLa cells. Even when the polymer

concentration was as high as 1 mg/mL, the relative cell viability still remained about 80 % after 48 hour incubation (Fig. S6†). The nontoxicity of PEG-PBMS copolymer and CCL micelles may arise from the biocompatibility of PEG and the intracellular reduction-degradable disulfide bonds.46 Furthermore, the cytotoxicities of the PTX-loaded PEG-PBMS CCL micelles were also monitored by CCK-8 assay in HeLa cells, which were pretreated with or without 0.5 mM buthionine sulfoximine (BSO), respectively. PTX stock solutions (free PTX) were used as controls. As shown in Fig. 4, both PTX-loaded CCL micelles and PTX stock solutions showed cytotoxicities. However, compared with free PTX, PTX-loaded micelles showed relatively lower cytotoxicities at the same concentrations. Notably, HeLa cells pretreated with BSO showed the highest cell viabilities. This phenomenon could be attributed to the fact that BSO could decrease the concentration of GSH in the cytoplasm, which makes the CCL micelles with disulfide bonds more stable and prevents PTX from burst releasing.

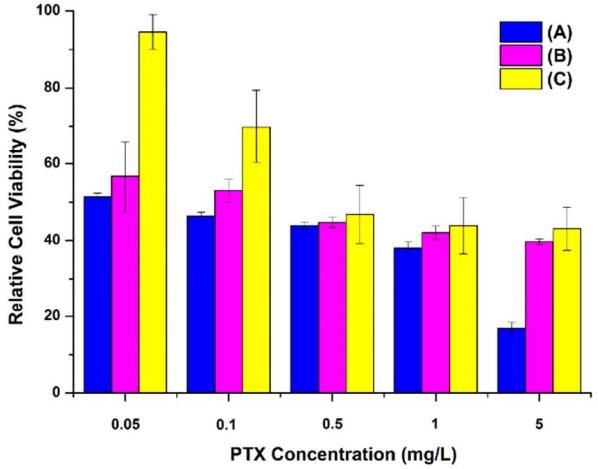


Fig. 4 Relative cell viabilities of free PTX and PTX-loaded PEG-PBMS CCL micelles against HeLa cells. (A) PTX stock solutions with non-pretreated HeLa cells; (B) PTX-loaded PEG-PBMS CCL micelles with non-pretreated HeLa cells; (C) PTX-loaded PEG-PBMS CCL micelles with HeLa cells pretreated by 0.5 mM BSO. Each group was tested in quintuplicate.

In conclusion, an amphiphilic multiblock poly(ether-ester) (PEG-PBMS) with pendent mercapto groups could be facilely synthesized by "one-pot" polycondensation using Sc(OTf)3 as catalyst in a large scale. This amphiphilic multiblock copolymer could self-assemble into regularly spherical core-shell micelles, and the inner hydrophobic core with pendent mercapto groups could be crosslinked through the oxidation of mercapto groups into disulfide bonds, resulting in reduction-sensitive micelles. After loading with PTX, the core-crosslinked micellar nanocarriers display an efficient cell-uptake and reduction-responsive drug release due to the nanoscale diameter and the splitting of disulfide bonds under reductive environment, which were evaluated by CLSM and in vitro drug release. The biocompatibility of the polymeric micelles was confirmed by CCK-8 assays. Meanwhile, the cytotoxicities of PTX-loaded PEG-PBMS CCL micelles are higher than that of free PTX. All the results suggest that this kind of polymeric CCL micelles can be a suitable and promising candidate as biodegradable and biocompatible nanocarriers for antitumor therapy.

Acknowledgment

The authors thank Mr. Dingcheng Zhu, Dr. Jianbin Tang and Prof. Youqing Shen for HPLC measurements. The financial supports from the National Natural Science Foundation of China (21274121 and 51173163), the Major State Basic Research Project (2011CB606001), the National Science-technology Support Plan project of China (2012BAI07B01), and the Fundamental Research Funds for the Central Universities (2012QNA7043) are gratefully appreciated.

Notes and references

[a] MOE Key Laboratory of Macromolecular Synthesis and Functionalization, Department of Polymer Science and Engineering, Zhejiang University, Hangzhou 310027, People's Republic of China.

[b] Department of Oral and Maxillofacial Surgery, Affiliated Stomatology Hospital, College of Medicine, Zhejiang University, Hangzhou 310006, P. R. China.

* Correspondence to: W. P. Zhu (E-mail: zhuwp@zju.edu.cn)

† Electronic Supplementary Information (ESI) available: Detailed experimental description, synthesis and cell-uptake experiments characterization. See DOI: 10.1039/c000000x/

‡ These authors contributed equally to this work.

1 R. Savic, L. B. Luo, A. Eisenberg and D. Maysinger, *Science*, 2003, **300**, 615-618.

2 L. Jabr-Milane, L. van Vlerken, H. Devalapally, D. Shenoy, S. Komareddy, M. Bhavsar and M. Amiji, *J. Control Release.*, 2008, **130**, 121-128.

3 H. B. Liu, S. Farrell and K. Uhrich, *J. Control Release.*, 2000, **68**, 167-174.

4 G. B. Jiang, D. O. Quan, K. R. Liao and H. H. Wang, *Mol. Pharm.*, 2006, **3**, 152-160.

5 S. R. Croy and G. S. Kwon, *Curr. Pharm. Design.*, 2006, **12**, 4669-4684.

6 Y. Kim, M. H. Pourgholami, D. L. Morris and M. H. Stenzel, *J. Mater. Chem.*, 2011, **21**, 12777-12783.

7 W. Chen, M. Zheng, F. H. Meng, R. Cheng, C. Deng, J. Feijen and Z. Y. Zhong, *Biomacromolecules*, 2013, **14**, 1214-1222.

8 J. Yue, R. Wang, S. Liu, S. H. Wu, Z. G. Xie, Y. B. Huang and X. B. Jing, *Soft Matter*, 2012, **8**, 7426-7435.

9 J. Zou, S. Y. Zhang, R. Shrestha, K. Seetho, C. L. Donley and K. L. Wooley, *Polym. Chem.*, 2012, **3**, 3146-3156.

10 L. Gao, K. Zhang, B. Peng, Y. Shi and Y. M. Chen, *J. Polym. Sci. Pol. Phys*, 2012, **50**, 323-327.

11 M. Elsabahy and K. L. Wooley, *J. Polym. Sci. Pol. Chem.*, 2012, **50**, 1869-1880.

12 G. J. Liu, J. F. Ding, T. Hashimoto, K. Kimishima, F. M. Winnik and S. Nigam, *Chem. Mater.*, 1999, **11**, 2233-2240.

13 S. Ganta, H. Devalapally, A. Shahiwala and M. Amiji, *J. Control Release.*, 2008, **126**, 187-204.

14 K. T. Oh, H. Q. Yin, E. S. Lee and Y. H. Bae, *J. Mater. Chem.*, 2007, **17**, 3987-4001.

15 C. J. F. Rijcken, O. Soga, W. E. Hennink and C. F. van Nostrum, *J. Control Release.*, 2007, **120**, 131-148.

16 Z. X. Zhou, Y. Q. Shen, J. B. Tang, M. H. Fan, E. A. Van Kirk, W. J. Murdoch and M. Radosz, *Adv. Funct. Mater.*, 2009, **19**, 3580-3589.

17 P. Sun, H. Du, L. L. Gao, W. P. Zhu, X. D. Li and Z. Q. Shen, *Acta Polym. Sin.*, 2012, 789-793.

18 Y. Wang, H. Du, L. L. Gao, H. G. Ni, X. D. Li, W. P. Zhu and Z. Q. Shen, *Polym. Chem.*, 2013, **4**, 1657-1663.

19 X. J. Cai, C. Y. Dong, H. Q. Dong, G. M. Wang, G. M. Pauletti, X. J. Pan, H. Y. Wen, I. Mehl, Y. Y. Li and D. L. Shi, *Biomacromolecules*, 2012, **13**, 1024-1034.
20 Z. B. Zheng, G. Z. Zhu, H. Tak, E. Joseph, J. L. Eiseman and D. J. Creighton, *Bioconjugate. Chem.*, 2005, **16**, 598-607.
21 J. Dai, S. D. Lin, D. Cheng, S. Y. Zou and X. T. Shuai, *Angew. Chem. Int. Edit.*, 2011, **50**, 9404-9408.
22 Y. P. Li, K. Xiao, J. T. Luo, W. W. Xiao, J. S. Lee, A. M. Gonik, J. Kato, T. A. Dong and K. S. Lam, *Biomaterials.*, 2011, **32**, 6633-6645.
23 Y. H. Tao, R. Liu, M. Q. Chen, C. Yang and X. Y. Liu, *J. Mater. Chem.*, 2012, **22**, 373-380.
24 P. F. Gou, W. P. Zhu and Z. Q. Shen, *Biomacromolecules*, 2010, **11**, 934-943.
25 F. M. Veronese and J. M. Harris, *Adv. Drug Deliver. Rev*, 2008, **60**, 1-2.
26 Z. Zarafshani, T. Obata and J. F. Lutz, *Biomacromolecules*, 2010, **11**, 2130-2135.
27 W. P. Zhu, M. J. Zhong, W. W. Li, H. C. Dong and K. Matyjaszewski, *Macromolecules.*, 2011, **44**, 1920-1926.
28 Q. Jin, S. Maji and S. Agarwal, *Polym. Chem.*, 2012, **3**, 2785-2793.
29 P. F. Gou, W. P. Zhu and Z. Q. Shen, *Polym. Chem.*, 2010, **1**, 1205-1214.
30 B. Xu, H. J. Dou, K. Tao, K. Sun, R. Lu and W. B. Shi, *J. Polym. Res.*, 2011, **18**, 131-137.
31 H. Du, L. L. Gao, W. P. Zhu and Z. Q. Shen, *Chinese. Chem. Lett.*, 2012, **23**, 879-882.
32 Y. Wang, Q. J. Luo, L. L. Gao, C. Gao, H. Du, G. Y. Zha, X. D. Li, Z. Q. Shen and W. P. Zhu, *Biomater. Sci.*, 2014, DOI:10.1039/C1034BM00065J.
33 L. L. Gao, Q. J. Luo, Y. Wang, H. Du, X. D. Li, Z. Q. Shen and W. P. Zhu, *RSC Adv.*, 2014, **4**, 4177-4180.
34 H. Du, G. Y. Zha, L. L. Gao, H. Wang, X. D. Li, Z. Q. Shen and W. P. Zhu, *Polym. Chem.*, 2014, DOI: 10.1039/C1034PY00030G.
35 W. P. Zhu, L. L. Gao, Q. J. Luo, C. Gao, G. Y. Zha, Z. Q. Shen and X. D. Li, *Polym. Chem.*, 2014, **5**, 2018-2026.
36 A. Takasu, Y. Shibata, Y. Narukawa and T. Hirabayashi, *Macromolecules.*, 2007, **40**, 151-153.
37 K. Yamamoto and A. Takasu, *Macromolecules.*, 2010, **43**, 8519-8523.
38 J. K. Kim, V. K. Garripelli, U. H. Jeong, J. S. Park, M. A. Repka and S. Jo, *Int. J. Pharm.*, 2010, **401**, 79-86.
39 Abdullah-Al-Nahain, H. Lee, Y. S. Lee, K. D. Lee and S. Y. Park, *Macromol. Biosci.*, 2011, **11**, 1264-1271.
40 F. Yang, J. Wang, G. Peng, S. C. Fu, S. Zhang and C. S. Liu, *J. Mater. Sci.-Mater. Med.*, 2012, **23**, 697-710.
41 Y. C. Wang, Y. Li, T. M. Sun, M. H. Xiong, J. A. Wu, Y. Y. Yang and J. Wang, *Macromol. Rapid. Comm.*, 2010, **31**, 1201-1206.
42 J. E. Liebmann, S. M. Hahn, J. A. Cook, C. Lipschultz, J. B. Mitchell and D. C. Kaufman, *Cancer Res.*, 1993, **53**, 2066-2070.
43 J. X. Ding, F. H. Shi, C. S. Xiao, L. Lin, L. Chen, C. L. He, X. L. Zhuang and X. S. Chen, *Polym. Chem.*, 2011, **2**, 2857-2864.
44 F. H. Shi, J. X. Ding, C. S. Xiao, X. L. Zhuang, C. L. He, L. Chen and X. S. Chen, *J. Mater. Chem.*, 2012, **22**, 14168-14179.
45 J. X. Ding, J. J. Chen, D. Li, C. S. Xiao, J. C. Zhang, C. L. He, X. L. Zhuang and X. S. Chen, *J. Mater. Chem. B*, 2013, **1**, 69-81.
46 Y. Wang, R. Zhang, N. Xu, F. S. Du, Y. L. Wang, Y. X. Tan, S. P. Ji, D. H. Liang and Z. C. Li, *Biomacromolecules*, 2011, **12**, 66-74.

Graphic abstract

Facile Fabrication of Reduction-Responsive Nanocarriers for Controlled Drug Release

Rui Sun,†[a] Qiaojie Luo,†[b] Chen Gao,[a] Ying Wang,[a] Lilong Gao,[a] Hong Du,[a] Ying Huang,[b] Xiaodong Li,[b] Zhiquan Shen,[a] and Weipu Zhu[a]*

[a] MOE Key Laboratory of Macromolecular Synthesis and Functionalization, Department of Polymer Science and Engineering, Zhejiang University, Hangzhou 310027, People's Republic of China

[b] Department of Oral and Maxillofacial Surgery, Affiliated Stomatology Hospital, College of Medicine, Zhejiang University, Hangzhou 310006, P. R. China

* Correspondence to: W. P. Zhu (E-mail: zhuwp@zju.edu.cn)

† These authors contributed equally to this work.

An amphiphilic multiblock poly(ether-ester) containing multiple thiols was facilely synthesized by “one-pot” polycondensation, and used to prepare reduction-responsive core-crosslinked micelles for controlled drug release.