Journal of Applied Polymer Science

Synthesis of Biomimetic Hyperbranched Zwitterionic Polymers as Targeting Drug Delivery Carriers

Xiaohong Wang,[1,2]* Xinke Sun,[1,2]* Guohua Jiang,[1,2] Rijing Wang,[1,2] Ruanbing Hu,[1,2] Xiaoguang Xi,[1,2] Yang Zhou,[2] Sheng Wang,[1,2] Tao Wang[1,2]

[1]Key Laboratory of Advanced Textile Materials and Manufacturing Technology (Ministry of Education), Zhejiang Sci-Tech University, Hangzhou 310018, China

[2]Department of Materials Engineering, College of Materials and Textiles, Zhejiang Sci-Tech University, Hangzhou 310018, China

Correspondence to: G. Jiang (E-mail: polymer_jiang@hotmail.com)

*These authors contributed equally to this research work.

ABSTRACT: Novel biomimetic hyperbranched copolymers that synthesized by polymerization of zwitterionic monomer (CBB) on the surface of a hyperbranched poly(3-ethyl-3-(hydroxymethyl)oxetane) (HBPO) core and used as a drug delivery carrier have been investigated by analysis of protein-adsorption-resistance, cytotoxicity and cell type-specific targeting properties. The as-synthesized biomimetic hyperbranched copolymers showed low toxicity, favorable protein resistant properties and were ultrastable in 100% fetal bovine serum. Folic acid and rodiamin-B were conjugated to the surface of synthesized micelles to endow it with target drug delivery and fluorescence activity, respectively. Intracellular uptake and *in vitro* cytotoxicity of HBPO-poly(carboxybetaine) micelles were investigated. Doxorubicin was used as a model drug for Hela cells during the experiment. All results show that the biomimetic hyperbranched copolymer is a candidate carrier for target drug delivery. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

KEYWORDS: biocompatibility; biomaterials; dendrimers; hyperbranched polymers; macrocycles

Received 2 May 2012; accepted 20 August 2012; published online
DOI: 10.1002/app.38497

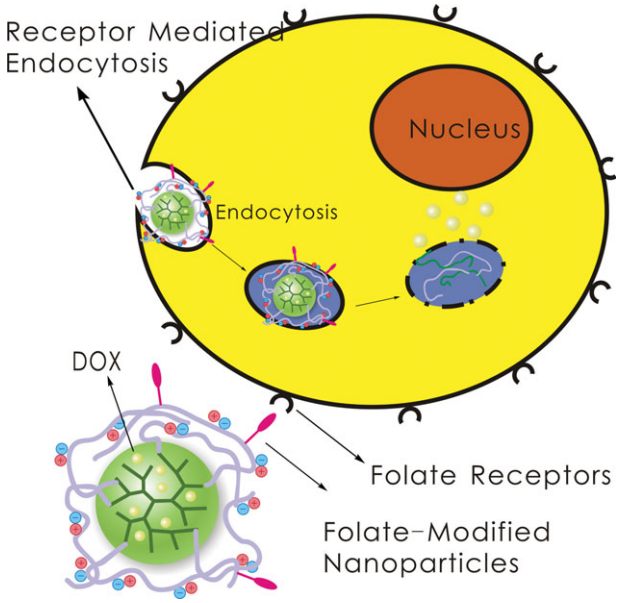
INTRODUCTION

Polymeric drug carriers have been intensively studied in the pharmaceutical field because they can be prepared at the nanoscale level and are relatively straightforward to prepare, have high-drug-loading capacities, offer controlled drug release, and enable surface modifications to be made and different functionalities to be introduced.[1] Hyperbranched polymers have three-dimensional structures and unique properties that are different from those of linear polymers.[2] Various types of hyperbranched polymers have high therapeutic drug-loading property and are capable of releasing a payload at the target site. They have shown the great potential to work as carriers for both imaging and therapy.[2–5] They can be synthesized in a one-pot reaction, avoiding multiple reaction-purification.[2–4] For example, in our previous work, multiarm star amphiphilic hyperbranched copolymers with poly(2-(dimehylamino) ethyl methacrylate) shell and hyperbranched poly(3-ethyl-3-(hydroxymethyl) oxetane) (HBPO) core were synthesized. The hyperbranched copolymers were further modified by succinic anhydride to obtain the novel pH- and thermosensitive-hyperbranched copolymer. The indomethacin-loaded micelles displayed a rapid drug release at an alkaline pH.[6] Liu and coworkers[7] reported affibody-attached hyperbranched conjugated polyelectrolyte can be utilized as a reliable fluorescent probe for target cellular imaging. However, the low stability and protein resistant properties of hyperbranched polymer carriers are still the main limitations for the application in biological system.

Most of hyperbranched polymers are lack of biocompatibility, and polyethylene glycol (PEG) has been generally used to enhance their biocompatibility. However, it turns out that PEG decomposes in presence of oxygen and transition-metal ions. Protein and cell-repellant polymer are desirable in surface modification of biomaterials and biosensors and in ultrafiltration and marine antifouling technologies.[8] In typical zwitterionic polymers, the zwitterionic groups are usually located in pendant groups rather than the backbone of the macromolecular chains. They contain both anion and cation in the same monomeric unit and show excellent stability against complex media such as

Additional Supporting Information may be found in the online version of this article.

Applied Polymer SCIENCE



Scheme 1. Receptor-mediated endocytosis of drug loaded HBPO-PCB-FOL. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

undiluted blood serum or plasma.[9] Among them, poly(carboxybetaine) (PCB) is a kind of zwitterionic copolymer in which the structure of caboxybetaine (CB) is similar to that of glycine-betaine, which is one of the compatible solutes vital to the osmotic regulation of living organisms. Estimates of glycine-betaine intake by humans range from 0.1 to 2.5 g per day, thus, it can be considered as biomimetic materials.[8]

Doxorubicin (DOX), known as an efficient anticancer drug, shows its great ability of inhibiting the growth of cancer cells.[10,11] However, the inability of targeting tumor cells and the multi drug resistance effect make it less attractive for use.[12,13] A series of nanosized DOX formulation such as polymer conjugates, liposomes, and polymeric micelles have been developed to overcome these disadvantages of DOX. These formulations are consequently an enhanced therapeutic efficacy.[14] Conversely, drug-loaded biomimetic hyperbranched copolymer without target drug delivery serves harm to normal cells by endocytosis during blood circulation. It is crucial to modify biomimetic hyperbranched copolymers with target function to enhance its application in drug delivery. Folic acid has shown great promise as a targeting ligand for a number of *in vivo* applications. Although folic acid is internalized by the low-affinity reduced folate carrier expressed by nearly all cells ($K_m \approx 10^{-6}$M), it has greater affinity ($K_d \approx 10^{-10}$M) toward the folate receptor, which is overexpressed in a number of tumors and cancer cell lines[15–17], as shown in Scheme 1. Folate-modified nanoparticles can be endocytosed more efficiently by some tumor cells. Then, drug-loaded micelles were able to release drug in the cell and destroyed the nucleus of cells.

In our previous work, biomimetic hyperbranched copolymers (HBPO-PCB) with HBPO in the core and zwitterionic monomer (CBB) on the surface were successfully synthesized and characterized, for example, morphology, cytotoxicity, drug loading and release, and nonspecifical protein resistant ability. The biomimetic hyperbranched copolymers showed low cytotoxicity, favorable protein resistant properties, and ultrastability in 100% fetal bovine serum (FBS). The loading content of a model drug, indomenthacin, was determined by UV-vis analysis to be 22.68 wt % and the drug release rate depends greatly on the pH and protein concentration of the solution.[18] Here, we successfully synthesized biomimetic hyperbranched copolymer HBPO-PCB and focus on the targeted delivery of its pharmaceuticals to folate-receptor-positive cancer to investigate *in vivo* application of biomimetic hyperbranched copolymer modified with folic acid (HBPO-PCB-Fol).

RESULTS AND DISCUSSION

HBPO was first reacted with DMP by esterification to form HBPO-DMP as the reversible addition-fragmentation chain transfer (RAFT) macro-CTAs.[19,20] Then, the star copolymers of HBPO-PCBB were synthesized via a RAFT polymerization. Biomimetic HBPO-PCB was obtained after hydrolyzation of HBPO-PCBB in DI water. Figure 1(a) shows the ^{1}H NMR spectrum of synthesized HBPO-PCB. The peak at 3.20–3.30 ppm is

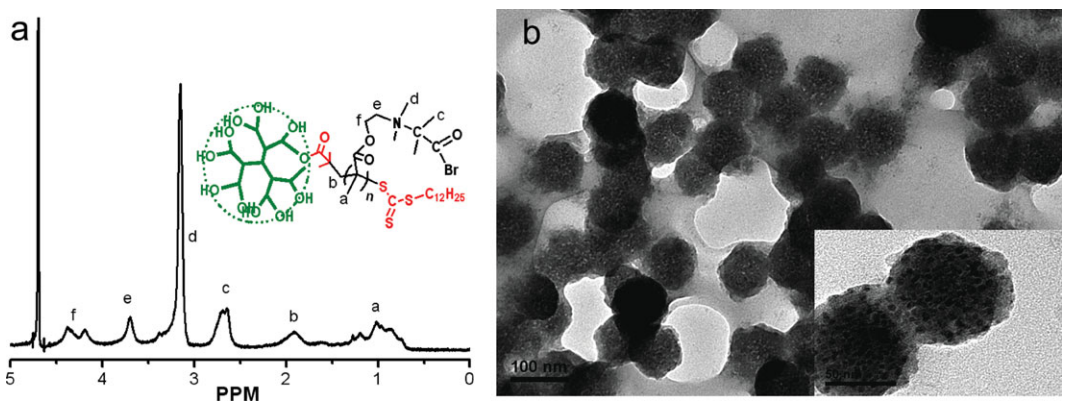


Figure 1. ^{1}H NMR spectrum of HBPO-PCB in D2O (a) and TEM image of HPBO-PCB micelles (b) in PBS at pH 7.0. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

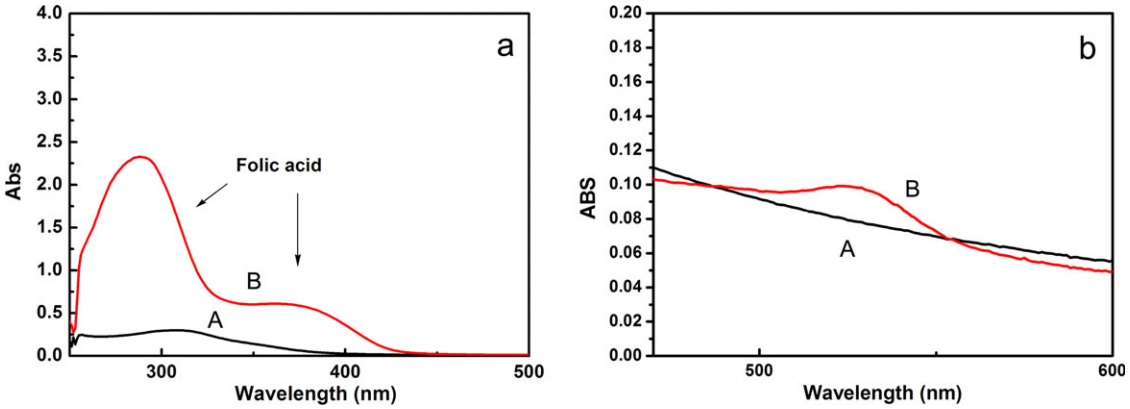


Figure 2. UV/vis absorbance spectra of HBPO-PCB (A) and HBPO-PCB-FOL(B) in PBS solutions with concentration at 1 mg/mL (a), and HBPO-PCB (A) and HBPO-PCB-RB(B) in PBS solutions with concentration at 1 mg/mL (b). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

caused by methyl of (N—CH_3) groups, indicating successful conjugation of CBB to HBPO. The morphology of HBPO-PCB was investigated by TEM. It is well-known that amphiphilic copolymers with hydrophilic arms tend to self-assemble into large compound micelles.[21] Figure 1(b) shows the transmission electron microscopy (TEM) images of the HBPO-PCB micelles in phosphate buffered saline (PBS) at pH 7.0 which indicates that the self-assembled micelles are well dispersed with spherical shape and the diameter of the HBPO-PCB micelles is around 70 nm.

For investigating the target and fluorescence properties of drug carriers, folic acid and rhodamine B have been conjugated onto the surface of HBPO-PCB via DCC/NHS chemistry. Figure 2(a) reveals the UV/vis absorbance spectra of HBPO-PCB (A) and HBPO-PCB-FOL (B) solution at the concentration of 1 mg/mL. Comparing with curve A, curve B shows characteristic peaks of folic acid at 280 nm and 360 nm,[22,23] indicating successful conjugation of folic acid to the surface of HBPO-PCB. Figure 2(b) shows the UV/vis absorbance spectra of HBPO-PCB (A) and HBPO-PCB-RB (B) solutions. Characteristic peak at 530 nm belonging to rhodamine B in curve B implies successful conjugation of rhodamine B with HBPO-PCB.[24,25]

Inspired by the delicate composition and structure of most outer-cell membranes, the protein-adsorption-resistance property of zwitterionic substances has been gradually recognized and utilized in creating biomimetic surface/interface.[26,27] In this study, the surface of hyperbranched HBPO was grafted with zwitterionic PCB to gain the protein resistant property. As shown in Figure 3(a), the diameter of HBPO-PCB in 10% FBS is much smaller than that in PBS solution, which can be caused by the protein resistant properties of the zwitterionic PCB on the biomimetic HBPO-PCB. Zwitterionic PCB shell of HBPO-PCB dehydrated and collapsed onto the surface of the micelles when HBPO-PCB was added to a solution containing protein, resulting in decrease of diameter. Stability of biomimetic HBPO-PCB against 100% FBS solution was investigated by measuring the diameter change of the micelles as shown in Figure 3(b). HBPO-PCB micelles almost retained their original size even after incubating for 30 h in 100% FBS, due to its great stability against protein solution. Thus supports its potential stability when applied *in vivo*.

We examined the effect of various concentrations of HBPO-PCB and HBPO-PCB-FOL on the cell viability of Hela cells by

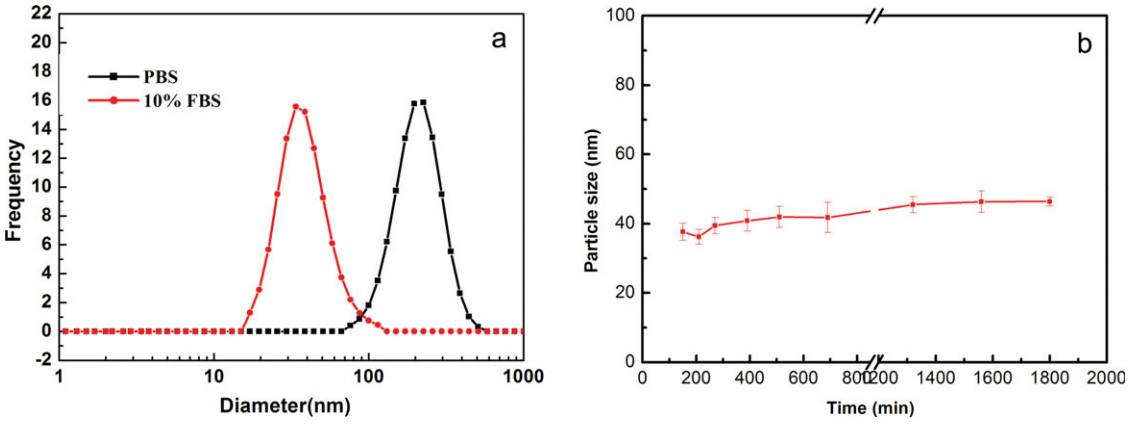


Figure 3. DLS graphs of HBPO-PCB in PBS and 10% FBS (a), and the stability of HBPO-PCB in 100% FBS (b). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

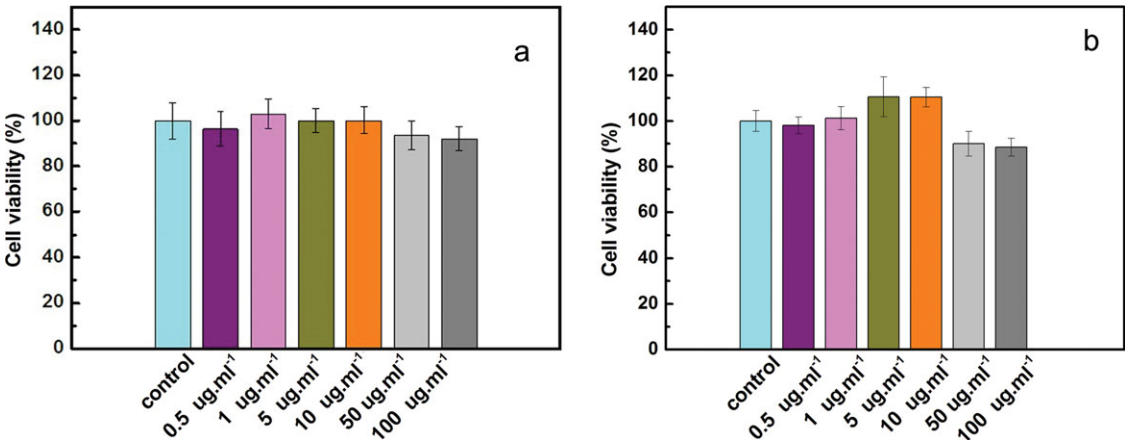


Figure 4. Viability of Hela cells against HBPO-PCB (a) and HBPO-PCB-FOL (b) micelles for 24 h by MTT assay. Data are presented as the average standard deviation ($n = 5$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

using MTT assay. Figure 4 shows Hela cell viability after incubation against DMEM containing different concentrations of HBPO-PCB and HBPO-PCB-FOL. It can be seen that the viability of Hela cells can still be kept more than 80% even when the concentration of HBPO-PCB and HBPO-PCB-FOL has been increased to be 100 μg/mL. This result confirms good biocompatibility and potential application in target drug delivery against cells.

Rhodamine B has been used to investigate uptake of synthesized biomimetic hyperbranched copolymer by Hela cells.[28] In current work, Hela cells were incubated in culture fluid containing HBPO-PCB-RB, which possesses fluorescence activity before detected by fluorescence microscope. As shown in Figure 5, Hela cells gain fluorescence effects obviously after 6 h of incubation, implying the successful uptake of HBPO-PCB-RB. The ability of entering into cancer cells makes it a potential carrier for drug delivery.

To examine the benefit of folic acid-conjugated HBPO-PCB for cell type-specific targeting, the effective cytotoxicity against Hela cells was investigated in the presence of either folic acid-conjugated HBPO-PCB or free HBPO-PCB in serum-containing cell media. It is well-known that DOX is highly toxic to both endothelial cells and cardiomyocytes, which accounts for some of the adverse side effects associated with its systemic administration.[29] The results shown in Figure 6 suggest that the blank polymeric micelles showed no cytotoxicity due to the high cell viability. In contrast, the DOX loaded HBPO-PCB micelles exhibits obvious cytotoxicity against Hela cells. As shown in Figure 6(a), after 24 h incubation with 1, 5, 25, 50, and 100 μg/mL drug concentrations, the cell viabilities of DOX loaded HBPO-PCB micelles are 98.17, 102.32, 80.11, 37.09, and 9.22%, respectively. It implies that the higher concentration of the DOX-loaded HBPO-PCB-FOL micelles, the higher cytotoxicity against Hela cells can be found. After 24h incubation with 1, 5, 25, 50, and 100 μg/mL drug concentrations, the cell viabilities are 93.45, 87.41, 44.79, 12.32, and 9.25%, respectively [Figure 6(b)]. This confirms that synthesized zwitterionic hyperbranched copolymer possesses excellent delivery property for DOX.

Furthermore, using folic acid-conjugated HBPO-PCB as carriers, lower cell viabilities can be obtained which implied good target drug delivery property of HBPO-PCB-FOL. Figure 7 is a summary diagram of cytotoxicity of HBPO-PCB, HBPO-PCB-FOL, HBPO-PCB/DOX, HBPO-PCB-FOL/DOX, and pure DOX to Hela cells. The content of DOX is 1 μg/mL in A group and 2 μg/mL in B group. It can be seen from the figure that HBPO-

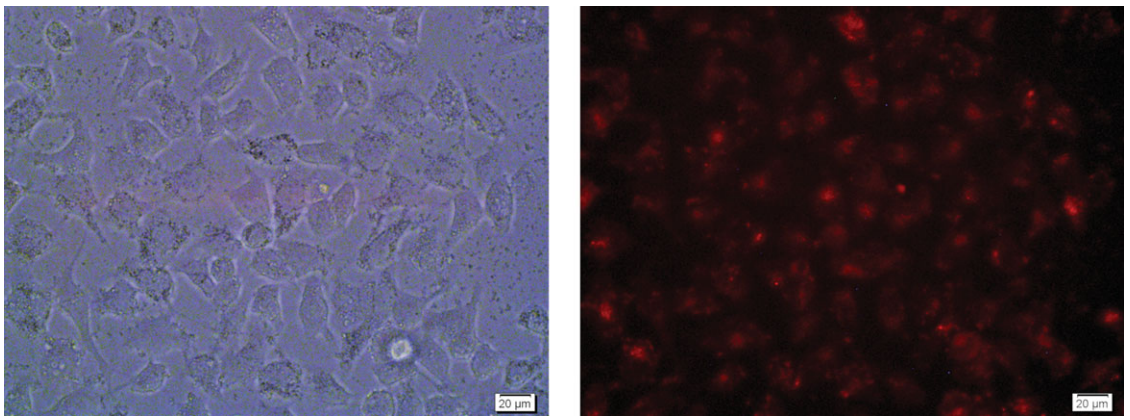


Figure 5. Fluorescence images of Hela cells incubated with HBPO-PCB-RB for 6 h.

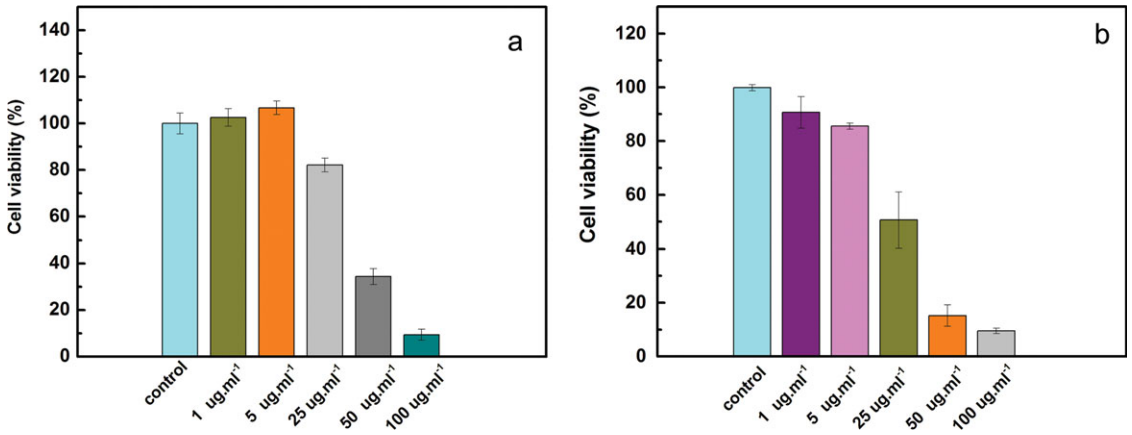


Figure 6. Viability of Hela cells against DOX loaded HBPO-PCB (a) and DOX loaded HBPO-PCB-FOL (b) after 24 h incubation. Data are presented as the average standard deviation ($n = 5$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

PCB-FOL/DOX micelles do better in inhibiting the growth of Hela cells than HBPO-PCB/DOX micelles, implying good target drug delivery property of HBPO-PCB-FOL.

CONCLUSIONS

In summary, this article discusses the using of a novel biomimetic hyperbranched copolymer (HBPO-PCB) as drug delivery carriers. HBPO-PCB was synthesized by a RAFT polymerization using HBPO-DMP as the RAFT macro-CTAs. The as-prepared polymers self-assembled micelles with HBPO as core and PCB as shell. Those micelles possess unimodal size distribution and homogeneous spherical shape. The blank polymeric micelles showed no cytotoxicity due to the high cell viability and good protein-adsorption-resistance property. *In vitro* cytotoxicity assay of DOX-loaded micelles against Hela cells showed that DOX could be efficiently released from the micelles to inhibit the proliferation of cancer cells.

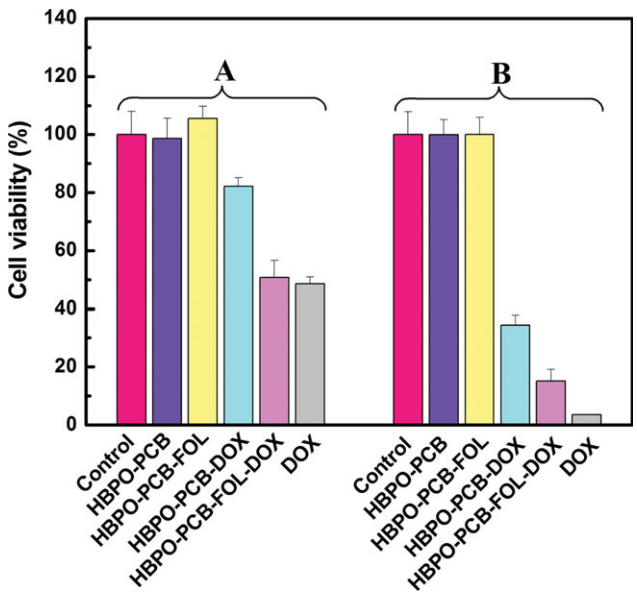


Figure 7. Summary diagram of cytotoxicity of HBPO-PCB, HBPO-PCB-FOL, HBPO-PCB/DOX, HBPO-PCB-FOL/DOX, and pure DOX to Hela cells. The content of DOX is 1 μg/mL in A group and 2 μg/mL in B group. Data are presented as the average standard deviation ($n = 5$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

ACKNOWLEDGMENTS

This work was financially supported by the National Natural Science Foundation of China (20604024), Natural Science Foundation of Zhejiang Province (Y4100045), Qianjiang Talents Project of Zhejiang Province (2010R10023), Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry (1001603-C), Scientific Research Foundation of Zhejiang Sci-Tech University (0901808-Y), Key Bidding project of Zhejiang Provincial Key Lab of Fiber Materials and Manufacturing Technology, Zhejiang Sci-Tech University (S2010002), and Program for Changjiang Scholars and Innovative Research Team in University (PCSIRT: 0654). The authors would like to thank Prof. Juming Yao for the assistance during the MTT measurement.

REFERENCES

1. Kim, J. H.; Oh, Y. T.; Lee, K. S.; Yun, J. M.; Park B. T.; Oh, K. T. *Macromol. Res.* **2011,** *19*, 453.
2. Liu, B.; Kazlauciunas, A.; Guthrie, J. T.; Perrier, S. *Macromolecules* **2005,** *38*, 2131.
3. Jiang, G. H.; Wang, Y.; Sun, X. K.; Shen, J. *J. Polym. Chem.* **2010,** *1*, 616.
4. Kim, C. K.; Ghosh, P.; Pagliuca, C.; Zhu, Z. J.; Menichetti, S.; Rotello, V. M. *J. Am. Chem. Soc.* **2009,** *131*, 1360.
5. Xia, W.; Jiang, G.; Chen, W. *J. Appl. Polym. Sci.* **2008,** *109*, 2089.
6. Sun, X.; Jiang, G.; Wang, Y.; Xu, Y. *Colloid Polym. Sci.* **2011,** *289*, 677.
7. Pu, K.-Y.; Shi, J.; Cai, L.; Li, K.; Liu, B. *Biomacromolecules* **2011,** *12*, 2966.
8. Krishnan, S.; Weinman, C. J.; Ober, C. K. *J. Mater. Chem.* **2008,** *18*, 3405.
9. Xuan, F.; Liu, J. *Polym. Int.* **2009,** *58*, 1350.

10. Laginha, K. M.; Verwoert, S.; Charrois, G. J. R.; Allen, T. M. *Clin. Cancer Res.* **2005,** *11*, 6944.

11. Frederick, C. A.; Williams, L. D.; Ughetto, G.; van der Marel, G. A.; van Boom, J. H.; Rich, A.; Wang, A. H. *J. Biochemistry* **1990,** *29*, 2538.

12. Marchi, N.; Hallene, K. L.; Kight, K. M.; Cucullo, L.; Moddel, G.; Bingaman, W.; Dini, G.; Vezzani, A.; Janigro, D. *BMC Med.* **2004,** *2*, 37.

13. Lee, Y.; Park, S. Y.; Mok, H.; Park, T. G. *Bioconjugate Chem.* **2008,** *19*, 525.

14. Batrakova, E. V.; Kelly, D. L.; Li, S.; Li, Y.; Yang, Z.; Xiao, L.; Alakhova, D. Y.; Sherman, S.; Alakhov, V. Y.; Kabanov, A. V. *Mol. Pharm.* **2006,** *3*, 113.

15. Danielle, S. W.; Benoit, S. S.; Andrew, D. *Biomacromolecules* **2011,** *12*, 2708.

16. Weitman, S. D.; Frazier, K. M.; Kamen, B. A. *J. Neuro-Oncol.* **1994,** *21*, 107.

17. Ross, J. F.; Chaudhuri, P. K.; Ratnam, M. *Cancer* **1994,** *73*, 2432.

18. Jiang, G. H.; Sun, X. K.; Ma, Y. S.; Cao, J.; Wang, Y.; Wang, R. J.; Wang, X. H.; Wang, S. *Soft Materials.* **2011**, ID: LSFM-2011-0116.R1. DOI:10.1080/1539445X.2011.635742.

19. Jiang, G. H.; Wang, L.; Chen, W. X. *Eur. Polym. J.* **2006,** *42*, 3333.

20. Lai, J. T.; Filla, D.; Shea, R. *Macromolecules* **2002,** *35*, 6754.

21. Liu, J. Y.; Pang, Y.; Huang, W.; Zhu, Z. Y.; Zhu, X. Y.; Zhou, Y. F.; Yan D. Y. *Biomacromolecules* **2011,** *12*, 2407.

22. Chen, T.-J.; Cheng, T.-H.; Hung, Y.-C.; Lin, K.-T.; Liu, G.-C.; Wang, Y.-M. *J. Biomed. Mater. Res. Part A* **2007,** 87A, 165.

23. Xiao, R.; Wang, W.; Pan, L.; Zhu, R.; Yu, Y.; Li, H.; Liu, H.; Wang, S.-L. *J. Mater. Sci.* **2011,** *46*, 2635.

24. Gan, Z. H.; Ju, J. H.; Zhang, T.; Wu, D. C. J. *Nanomate.* **2011**, ID 753705, 8 pages, 2011. doi 10.1155/2011/753705.

25. Zhang, R.; Hummelgård, M.; Lv, G.; Olin, H. *Carbon* **2011,** *49*, 1126.

26. Shi, Q.; Su, Y.; Zhao, W.; Li, C.; Hu, Y.; Jiang, Z.; Zhu, S. *J. Membr. Sci.* **2008,** *319*, 271.

27. Ladd, J.; Zhang, Z.; Chen, S.; Hower, J. C.; Jiang, S. *Biomacromolecules* **2008,** *9*, 1357.

28. Quadir, M. A.; Haag, R. *J. Controlled Release* **2012,** *161*, 484.

29. Shi, M.; Ho, K.; Keating, A.; Shoichet, M. S. *Adv. Funct. Mater.* **2009,** *19*, 1689.