Nanotechnology

DOI: 10.1002/anie.201201179

Dual-Responsive Boronate Crosslinked Micelles for Targeted Drug Delivery**

*Weixuan Chen, Yunfeng Cheng, and Binghe Wang**

boron · drug delivery · FRET · micelles · nanotechnology

Driven by the need to improve specificity and bioavailability of therapeutic agents, reduce drug cytotoxicity, and overcome drug resistance, continuous efforts have been dedicated over the last few decades to the development of stimuli-responsive drug-delivery nanoplatforms for controlled drug release in response to specific cellular signals.[1] Among them, polymeric micelles have drawn a great deal of attention because of their many desirable features for targeted drug delivery.[2] Micelles can accommodate a wide variety of compounds with a high drug loading capacity. They have the potential to accumulate preferentially at the target site because of the enhanced permeation and retention effect (EPR),[3] and to release the payload in response to biological stimuli such as pH,[4] temperature,[5] redox reactions,[6] and enzymes.[7] Despite all the progress, this field is still faced with tremendous challenges and somewhat unresolved problems such as premature release and drug loss during storage because of de-micellization. The strong shear stress in blood circulation also causes problems for micelle stability.[8] Therefore, the interest is high in developing crosslinked micelles for improved stability and circulation time. However, crosslinked micelles may present difficulties in drug release because of the increased stability. One potential solution is the use of reversible crosslinked micelles, which break their crosslink in response to the appropriate stimuli and thus release the payload. Remarkable progress has been made in developing stimuli-responsive crosslinked micelles (SCMs), including pH-cleavable,[9] disulfide-bond-containing,[6b] and hydrolysable ester-bond-containing SCMs.[2a] Even more promising are SCMs which are responsive to multiple stimuli as reported recently for the precise spatiotemporal drug release in the complex in vivo microenvironment.[10]

On the basis of previously reported telodendrimer systems for efficient anticancer drug delivery, Lam and coworkers developed another class of smart SCM-based nanocarriers, which are termed dual-responsive boronate crosslinked micelles (BCM), for targeted drug delivery.[11] The central hypothesis is that through using the well-known reversible boronic acid/catechol complexation to crosslink the core–shell polymer of SCMs, the resulting micelles should retain the stability of crosslinked systems and minimize premature payload release under physiological conditions. The encapsulated drug can be released selectively at the targeted sites when triggered by low pH values or exogenous competing diols (Figure 1). This study clearly demonstrated the potential of using boronic acid–diol interactions for the preparation of SCMs, and will certainly stimulate additional research on using boronic acid–diol for targeted drug delivery.

Specifically, a class of cross-linkable telodendrimer pair, PEG^{5k}-NBA_n/BA_n-CA_8 and PEG^{5k}-$Catechol_n$-CA_8 were carefully designed and synthesized (PEG = polyethylene glycol; CA = cholic acids; NBA = nitroboronic acid; BA = boronic acid; $n = 2$ or 4). PEG^{5k}-CA_8 was also synthesized to generate non-crosslinked micelles (NCM) as a control. The formation of BCMs were achieved by using a solvent evaporation method and verified by the Alizarin Red S. (ARS) method for boronic acid binding studies developed by the Wang group.[12]

Next, the physical properties of a series of BCMs, formed by using equal molar ratios of the boronic-acid- and catechol-containing telodendrimers, were evaluated. The NCMs had similar sizes to those of the BCMs (around 20 nm with a narrow distribution), and significantly reduced critical micelle concentrations (CMC = 50 $\mu g\,mL^{-1}$) compared to that of the control NCM (4–10 $\mu g\,mL^{-1}$).[11] The stabilities of BCMs against plasma proteins and severe micelle-disrupting conditions were further investigated, and BCM4, constructed from two types of modified telodendrimers, 3,4-dihydroxybenzoic-acid-containing PEG^{5k}-$catechol_4$-CA_8 (**1**) and 3-carboxy-5-nitrophenylboronic-acid-containing PEG^{5k}-NBA_4-CA_8 (**2**; Figure 2), stood out as the best candidates for additional drug delivery evaluation. BCM4 showed no significant particle size alteration when exposed to 50% (v/v) human plasma for 24 hours. It is remarkable that even after being exposed to a 2.5 $mg\,mL^{-1}$ SDS solution for 2 days, BCM4 showed little particle size alteration. However, as expected, this stability was strongly interrupted by lowering the pH value from 7.4 to 5.0 or by addition of 100 mM mannitol (a safe FDA approved drug for diuresis with high blood level of > 50 mM achieved clinically at recommended dose). Encouraged by the preliminary results, the drug release

[*] W. Chen, Dr. Y. Cheng, Prof. Dr. B. Wang
Department of Chemistry, Center for Biotechnology and Drug Design, and Center for Diagnostics and Therapeutics Georgia State University, Atlanta, GA 30302-4098 (USA)
E-mail: wang@gsu.edu
Homepage: http://chemistry.gsu.edu/Groups/Bing_Wang/default.html

[**] Work performed in the lab of the author in related areas is supported by the National Institutes of Health (GM086925 and GM084933) and the GSU Molecular Basis of Disease program.

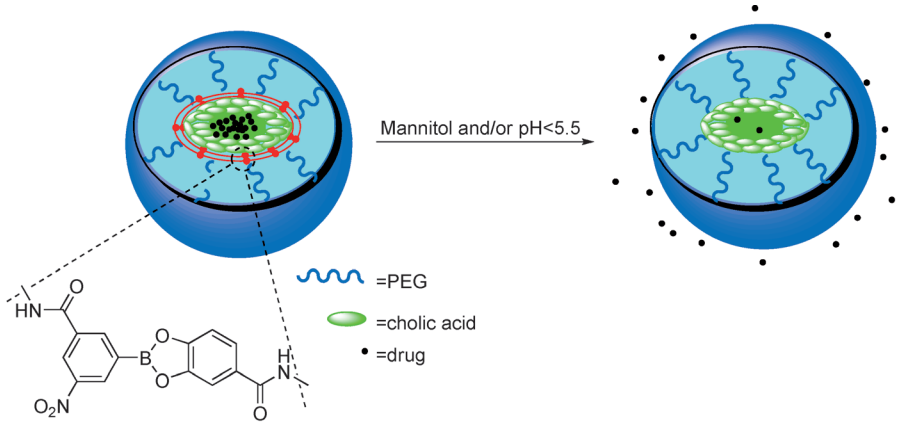

Figure 1. Structure of the boronate crosslinked micelles and drug release in response to mannitol or acidic pH.

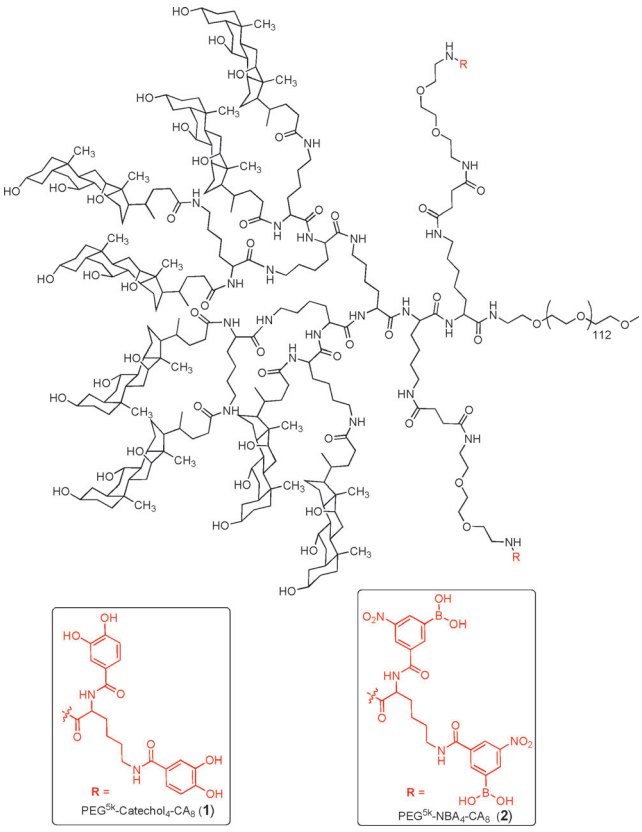

Figure 2. Telodendrimer pair [PEG^{5k}-(nitroboronic acid/catechol)$_4$-CA_8].

profiles were explored by using paclitaxel (PTX) as a model drug. PTX release from BCM4 was significantly slower than that from the control (NCM), thus demonstrating stability. In contrast, drug release from BCM4 was tremendously accelerated by addition of 100 mM mannitol or by adjusting the pH value to 5.0. Similar results were obtained in the in vitro MTT assay against SKOV-3 ovarian cancer cells. It was found that PTX-BCM4 was nearly twofold less cytotoxic than Taxol (free drug of PTX) and PTX-NCM at equal dose levels, and the cytotoxicity was enhanced at pH 5.0 in the presence of mannitol (100 mM).

Furthermore, in vivo stability tests were done by using a Förster resonance energy transfer (FRET) system, which was constructed by using DiO, a green dye, as the donor and rhodamine B, a red dye, as the acceptor. DiO was trapped inside, which mimics the drug, and rhodamine B was covalently linked to the telodendrimers to track the wall component of the nanocarriers. When the micelles are intact, excitation of DiO leads to fluorescence corresponding to rhodamine B emission. Thus rhodamine fluorescence intensity is an indication of micelle stability. The FRET-BCM4 and FRET-NCM micelles were each intravenously injected into nude mice. Blood was then collected at different time points and the FRET ratio ($I_{\text{rhodamine B}}/(I_{\text{rhodamine B}} + I_{\text{DiO}})$) was used as a measure of the micelle integrity. The result clearly showed that the FRET ratio of FRET-BCM4 decreased much more slowly than that of FRET-NCM, thus indicating that FRET-BCM4 was more stable than FRET-NCM.

Moreover, blood elimination kinetic studies of the micelles were conducted using rhodamine B-NCM and rhodamine B-BCM4. The intensity of rhodamine B fluorescence of the rhodamine B-BCM4 micelle was six times higher than that of rhodamine-NCM 10 hours post injection. The prolonged (24 h) fluorescence intensity for BCM4 is also indicative of its long circulation in the blood.

Finally, imaging results of organs harvested from the mice models showed in general, the preferential uptake of DiO and PTX co-loaded onto BCM4 in the SKOV-3 ovarian tumor, however, there is a visible payload release in the liver as well.

In summary, the strategy described by Lam and co-workers has clearly shown the potential of boronic acid–diol interactions as a way to build dual-responsive (pH and diols) and crosslinked micelles with improved stability and quick payload release in response to pH and diol addition. Before translating this promising platform into clinical use, more needs to be done. For instance, the antitumor efficacy needs to be demonstrated in animal models. Regardless, the work described by Lam andco-workers opens up many new possibilities in the design of stimuli-responsive drug-delivery nanoplatforms. For example, boronic acid–catechol complexation is known to be sensitive to oxidizing agents such as the H_2O_2, thus suggesting that BCMs can be used as nanocarriers for oxidation-triggered drug release in oxidative microenvir-

onments. However, the sensitivity of the boronate system to oxidative conditions is a double-edged sword, and may have undesirable effects on the stability and release site or rate of the described micelles in vivo.[13] In contrast, a combination of other redox sensitive groups, such as disulfide, on teloden-drimers may lead to improved specificity in targeted drug release since solid tumor tends to have low pH values and a reducing microenvironment.

Received: February 13, 2012
Published online: April 18, 2012

[1] a) S. Ganta, H. Devalapally, A. Shahiwala, M. Amiji, *J. Controlled Release* **2008**, *126*, 187 – 204; b) E. S. Lee, Z. Gao, Y. H. Bae, *J. Controlled Release* **2008**, *132*, 164 – 170; c) V. Torchilin, *Adv. Drug Delivery Rev.* **2006**, *58*, 1532 – 1555.
[2] a) C. J. Rijcken, C. J. Snel, R. M. Schiffelers, C. F. van Nostrum, W. E. Hennink, *Biomaterials* **2007**, *28*, 5581 – 5593; b) Y. Bae, K. Kataoka, *Adv. Drug Delivery Rev.* **2009**, *61*, 768 – 784.
[3] a) H. Maeda, L. W. Seymour, Y. Miyamoto, *Bioconjugate Chem.* **1992**, *3*, 351 – 362; b) R. K. Jain, *J. Controlled Release* **2001**, *74*, 7 – 25.
[4] a) E. S. Lee, K. Na, Y. H. Bae, *Nano Lett.* **2005**, *5*, 325 – 329; b) Y. Bae, N. Nishiyama, K. Kataoka, *Bioconjugate Chem.* **2007**, *18*, 1131 – 1139.
[5] a) Y. Li, S. Pan, W. Zhang, Z. Du, *Nanotechnology* **2009**, *20*, 065104; b) S.-W. Choi, Y. Zhang, Y. Xia, *Angew. Chem.* **2010**, *122*, 8076 – 8080; *Angew. Chem. Int. Ed.* **2010**, *49*, 7904 – 7908.
[6] a) R. A. Petros, P. A. Ropp, J. M. DeSimone, *J. Am. Chem. Soc.* **2008**, *130*, 5008 – 5009; b) M. Talelli, M. Iman, A. K. Varkouhi, C. J. F. Rijcken, R. M. Schiffelers, T. Etrych, K. Ulbrich, C. F. van Nostrum, T. Lammers, G. Storm, W. E. Hennink, *Biomaterials* **2010**, *31*, 7797 – 7804.
[7] B. Law, C.-H. Tung, *Bioconjugate Chem.* **2009**, *20*, 1683 – 1695.
[8] J. Dai, S. Lin, D. Cheng, S. Zou, X. Shuai, *Angew. Chem.* **2011**, *123*, 9576 – 9580; *Angew. Chem. Int. Ed.* **2011**, *50*, 9404 – 9408.
[9] K. Zhou, Y. Wang, X. Huang, K. Luby-Phelps, B. D. Sumer, J. Gao, *Angew. Chem.* **2011**, *123*, 6233 – 6238; *Angew. Chem. Int. Ed.* **2011**, *50*, 6109 – 6114.
[10] a) N. Ma, Y. Li, H. Xu, Z. Wang, X. Zhang, *J. Am. Chem. Soc.* **2010**, *132*, 442 – 443; b) E. S. Olson, T. Jiang, T. A. Aguilera, Q. T. Nguyen, L. G. Ellies, M. Scadeng, R. Y. Tsien, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 4311 – 4316; c) C. Wei, J. Guo, C. Wang, *Macromol. Rapid Commun.* **2011**, *32*, 451 – 455.
[11] Y. Li, W. Xiao, K. Xiao, L. Berti, J. Luo, H. P. Tseng, G. Fung, K. S. Lam, *Angew. Chem.* **2012**, *124*, 2918 – 2923; *Angew. Chem. Int. Ed.* **2012**, *51*, 2864 – 2869.
[12] G. Springsteen, B. Wang, *Chem. Commun.* **2001**, 1608 – 1609.
[13] S. G. Rhee, *Science* **2006**, *312*, 1882 – 1883.

