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COMMUNICATION

A facile strategy for polymers to achieve glucose-responsive behavior at neutral pH†

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A novel type of amphiphilic block polymer with phenylboronate ester as a leaving group in the hydrophobic block was designed and synthesized as a nanocarrier to successfully realize glucose-responsiveness at neutral pH.

Recently, considerable impetus has been given to the research on saccharide-responsive materials due to their practical significance in the development of sensors and drug releasing carriers for glucose-related human disorders such as diabetes, a life-threatening disease with an increasing tendency around the world.[1,2] Boron-containing polymers, in particular, polymeric phenylboronic acids, have attracted growing scientific attention as potential insulin-delivering candidates because of being prone to form water-soluble phenylborate derivatives between phenylboronic acid and sugar molecules.[3–8] To realize their application as drug delivery vehicles under physiological conditions, one of the most challenging problems in the development of organoboron polymers is how to achieve the saccharide-responsiveness at neutral pH. Although the incorporation of the electron-rich nitrogen atom into the polymers has been evidenced to be an effective method to lower the pK_a value of phenylboronic acid residues *via* intra- or intermolecular interaction between B and N atoms, thus realizing sugar-responsiveness at neutral pH,[9,10] it is still an extremely important and arduous job to explore more organoboron macromolecules to satisfy various requirements of the sensing and delivery applications.

It is well-known that the rigid *cis*-diols found in many saccharides generally exhibit higher affinities with organoboronic acids through reversible boronate formation than acyclic diols like ethylene glycol,[11] which suggests that the boronic acid–acyclic diol complexes are easily dissociated in the presence of appropriate saccharide molecules.[4e,12–14] This competition mechanism between sugar molecule and acyclic diol provides an excellent opportunity for developing a novel type of sugar-responsive materials. Inspired by the competition concept, we envision that amphiphilic block polymers with hydrophobic block bearing pinacolboronate ester units generated *via* the phenylboronic acid–acyclic diol complexation may act as effective sugar-responsive materials. Due to saccharide molecules having predominant association force with organoboron, the presented pinacol phenylboronate moieties on the polymer are prone to combine with sugar molecules and detach from the polymer architecture as a phenylboronate–saccharide complex. The disengagement of phenylboronate groups will trigger the polarity transfer of the polymer from amphiphile to double hydrophile, thus leading to disruption of the initial nanoaggregates formed *via* self-assembly of the amphiphilic polymer, and

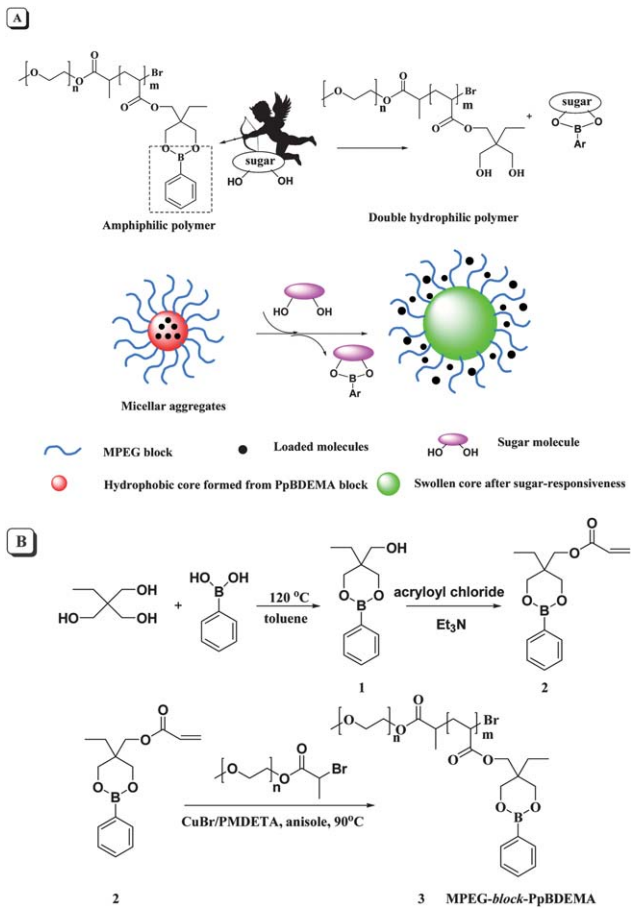

Fig. 1 (A) Schematic diagram for sugar-responsive behavior. (B) Synthesis of monomer and block copolymer.

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releasing the loaded molecules (Fig. 1A). Alternatively, boronate ester architecture exhibits a relatively lower pK_a than boronic acid itself,[11] thus also providing one potential to explore smart sugar-responsive polymers available to physiological need. Herein, we employed this polarity switch mechanism induced by the competition between sugar and acyclic diol to develop one type of block copolymer with pinacolboronate moieties and evaluate their stimuli-responsive ability in the presence of D-glucose at neutral pH. Fig. 1B schematically shows our synthesis approach of monomer and corresponding amphiphilic block copolymer.

The acrylate monomer **2** containing pinacol phenylboronate was prepared from phenylboronic acid and 1,1,1-tris(hydroxymethyl) propane as starting materials in a two-step simple transformation. Subsequently, the amphiphilic block polymer **3** was fabricated *via* ATRP of monomer **2** using monomethoxy poly(ethylene glycol)–Br (MPEG–Br, M_n = 2100 g mol^{-1}) as macroinitiator. Polymerization was conducted at 90 °C in anisole at the monomer concentration of 1.10 g mL^{-1}, and the experimental molecular weight of the polymer was linearly increased with polydispersity index controlled in the range of 1.20–1.30 over the course of the polymerization (Fig. 2A). The resulting block polymer MPEG-*block*-PpBDEMA *via* 18 h polymerization had hydrophilic and hydrophobic repeating unit ratio of 44 to 64 (calculated by ^{1}H NMR in Fig. S9†, M_w/M_n = 1.22). This successful preparation is further confirmed by a GPC trace which showed a sharp signal free from any residual MPEG–Br (Fig. 2B). Compared to the previously reported synthetic method of organoboron macromolecules,[6,9] the current synthesis was of ease and well operation.

The critical micelle concentration (CMC) measured by the fluorescence technique[15] was 49 mg L^{-1} for the polymer **3** (Fig. S11 in the ESI†). Particles can be fabricated from the polymer using the reported technique.[16] A transmission electron microscopy (TEM) graph revealed that these particles were close to spherical micelles having core–shell structure with a hydrophobic core composed of PpBDEMA and the overall size about 30 nm (Fig. 3). The size distribution was corroborated by dynamic light scattering (DLS) measurements in water, which indicated that the particles had an average diameter of 90 nm.

In this study, the dissociation of pinacolboronate ester from the polymer would unmask the hydroxyl groups of the polymers and change the polymer polarity, which would trigger the release of the molecules encapsulated in the hydrophobic core of nanocarriers. To test the release mechanism induced by the competition effect of the sugar molecules on pinacolboronate ester, particles formed from MPEG-*block*-PpBDEMA were suspended in aqueous solutions at pH 7.4 and 7.8 with or without 8 mg mL^{-1} glucose. Their glucose-responsive behavior at 37 °C was investigated by fluorescence

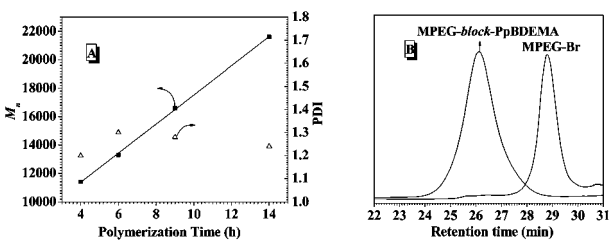

Fig. 2 (A) M_n and polydispersity index *versus* polymerization time for ATRP of monomer **2**; (B) GPC traces of the block polymer **3** compared to MPEG–Br (M_n = 2100 g mol^{-1}).

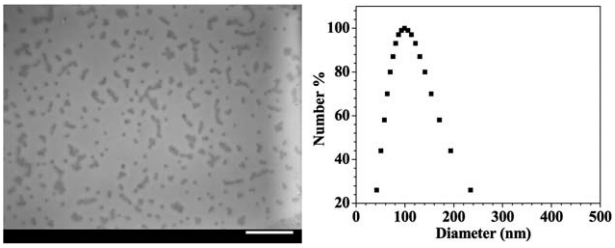

Fig. 3 TEM graph of particles formed *via* self-assembly of the block polymer **3** (left, scale bar stands for 900 nm); hydrodynamic diameter distribution of particles in water measured by DLS (right).

techniques using nile red as probe molecule and cargo over time. The releasing percentage was calculated by a comparison of the fluorescence intensity at determined time and initial time. In Fig. 4A, upon the addition of glucose, the nanoparticles from MPEG-*block*-PpBDEMA promptly generated response, and nearly 40 percent of the encapsulated nile red escaped from the nanoparticles in 3 min at pH 7.8. Although the environmental pH decrease would weaken the affinities of boronate and sugar molecules, rapid release of nile red also occurred at pH 7.4 as well, and 20 percent of the encapsulated molecules was liberated during the experimental time. The blank experiment without glucose was conducted, and no obvious nile red release trend was detected in 6 min incubation regardless of pH values.

In the human body, the blood glucose concentration is tightly controlled in a normal range of 4.4–6.6 mM. The blood glucose concentration undulation would induce insulin response to different degrees. To confirm the controllable release ability of these nanoparticles with the change of environmental stimuli, we investigated the effect of glucose concentration on the stimuli-responsiveness of nanoparticles in aqueous solution at pH 7.4 (Fig. 4B). At the given

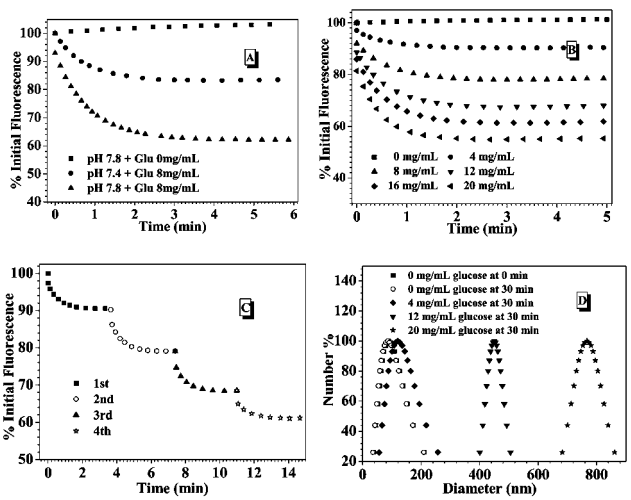

Fig. 4 (A) Nile red release from the particles with and without 8 mg mL^{-1} glucose at various pH environments as measured by fluorescence intensity decrease at 620 nm; (B) time dependence of nile red release at various glucose concentrations at pH 7.4 and 37 °C; (C) nile red release from the particles under the stimuli of glucose in batched addition, each batch of glucose at 4 mg mL^{-1}; (D) particles size changing measured by DLS during incubating at various concentrations of glucose for 30 min at pH 7.4 and 37 °C.

glucose concentration of 4 mg mL^{-1} (22.0 mM), 10 percent of the encapsulated nile red was released in 2 min. As more glucose was added to the particle aqueous solution, the competition driving force increase for glucose–boronate complexation is of benefit to more phenylboronate detachment from the hydrophobic core of nanoparticles. Therefore, higher release rates were observed at glucose concentrations of 12 mg mL^{-1} (66.0 mM) and 16 mg mL^{-1} (88.0 mM) in the similar period of incubation. Moreover, nile red release percentage was almost linearly increased depending on the initially given glucose concentration (Fig. S11†). Meanwhile, it is interestingly found that the release of nile red triggered by glucose at any given concentration reached a plateau within 5 min.

Besides, glucose-responsive behavior of the nanoparticles was further investigated by consecutive addition of glucose at pH 7.4 and 37 °C. When the added glucose of each batch was maintained at 4.0 mg mL^{-1} in the measured solution, interestingly, it is detected that 10 percent of the encapsulated nile red was released during 4 min observation under the stimuli of the first 4 mg mL^{-1} glucose, then following one release plateau (Fig. 4C). Upon the addition of more 4.0 mg mL^{-1} glucose, additional 10% nile red escaped from the nanoparticles in the second 4 min incubation. The similar release behavior occurred when the third and fourth batch of glucose at the given concentration were added into the above particles solution. The nanoparticle solution turned cloudy after the fourth glucose-responsive release. The overall release percentage of nile red reached 40% at the cumulative glucose concentration of 16.0 mg mL^{-1}, which is similar to the results upon the addition of the identical glucose concentration in one portion (Fig. 4B). This effect of the consecutive release under the given glucose concentration indicates that the studied nanocarriers have potential to be one type of on–off glucose-responsive switch which can controllably release the encapsulated molecules depending on the changing glucose concentration.

The size change of the nanoparticles in response to glucose was followed by DLS measurement. It is noteworthy that the addition of glucose molecule led to the rapid and remarkable expanding of the nanoparticles at pH 7.4 and 37 °C (Fig. 4D). The nanoparticle size increased from the initial 90 nm to 140 nm after 30 min incubation at the given glucose concentration of 4 mg mL^{-1}. More obviously, the micellar bulk was increased to 450 nm and 760 nm when the glucose concentration was determined at 12 mg mL^{-1} and 20 mg mL^{-1}, respectively. In contrast, the micellar size remained stable in the absence of glucose during 30 min observation. Under the stimuli of glucose, the micellar bulk expansion instead of disruption was likely to result from the removal of pinacolboronate ester from the nanoparticles' hydrophobic core uncovered the hydroxyl groups which are highly hydrophilic but not water-soluble,[17] thus resulting in the maintenance of micellar structures with bigger size, as shown in Fig. 1A.

In addition, the glucose-triggered insulin release from the polymeric micelles was also preliminarily conducted in this study. After fluorescein-labeled insulin (FITC-insulin, 3.0 mg) and the polymer MPEG-*block*-PpBDEMA (5.0 mg) dissolved in 0.3 mL THF was added to 5.0 mL deionized water with stirring at room temperature overnight, the insulin-encapsulated micellar solution transferred into dialysis membranes (molecular weight cut off 12 000) was dialyzed against distilled water until the fluorescence signal of the dialysis bag inside at the emission wavelength of 519 nm was stable. The insulin release behavior was tested with or without glucose at the concentration of 4 mg mL^{-1} at pH 7.4 and 37 °C, respectively. The micelles

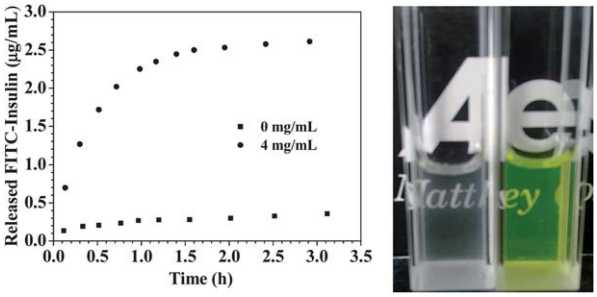

Fig. 5 FITC-insulin release from the nanoparticles at the glucose concentration of 4 mg mL^{-1} at pH 7.4 and 37 °C (left). The images of nanoparticle solution before and after insulin release (right, yellow one was prior to release and the colorless one was after releasing).

loading FITC-insulin exhibited quite low leaching in the absence of glucose stimuli, suggesting a good capping efficiency (Fig. 5, left). In contrast, obvious insulin release triggered by 4 mg mL^{-1} glucose was observed within 2 h, and showed a similar diffusion-controlled kinetic profile. The color of the micellar solution turned from light yellow to colorless with FITC-insulin escaping from the polymeric compartment, as shown in the right image of Fig. 5. Further exploration of insulin release under glucose-stimuli is underway in our laboratory.

In conclusion, utilizing the affinity disparity of saccharide molecules and acyclic diol binding with organoboron and the characteristics of boronate ester having relatively low pK_a, the polymeric nanocarriers containing pinacolboronate ester architecture can indeed release its payload in the presence of glucose at neutral pH. This study provides one well-operation method to realize glucose-responsive release under physiological conditions. Further work will investigate the detailed smart regulation of sugar-responsive behavior and the biocompatibility of the polymers and dissociated products during the responsiveness.

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Notes and references

1 T. D. James and S. Shinkai, *Top. Curr. Chem.*, 2002, **218**, 159.
2 F. Jakle, *J. Inorg. Organomet. Polym. Mater.*, 2005, **15**, 293.
3 (*a*) J. N. Cambre, D. Roy, S. R. Gondi and S. B. Sumerlin, *J. Am. Chem. Soc.*, 2007, **129**, 10348; (*b*) D. Roy, J. N. Cambre and B. S. Sumerlin, *Chem. Commun.*, 2008, 2477; (*c*) D. Roy, J. N. Cambre and S. B. Sumerlin, *Chem. Commun.*, 2009, 2106; (*d*) P. De, S. R. Gondi, D. Roy and B. S. Sumerlin, *Macromolecules*, 2009, **42**, 5614.
4 (*a*) Y. J. Zhang, Y. Guan and S. Q. Zhou, *Biomacromolecules*, 2006, **7**, 3196; (*b*) Y. J. Zhang, Y. Guan and S. Q. Zhou, *Biomacromolecules*, 2007, **8**, 3842; (*c*) T. Hoare and R. Pelton, *Biomacromolecules*, 2008, **9**, 733; (*d*) X. Jin, X. Zhang, Z. Wu, D. Teng, X. Zhang, Y. Wang, Z. Wang and C. Li, *Biomacromolecules*, 2009, **10**, 1337; (*e*) Z. B. Ding, Y. Guan, Y. J. Zhang and X. X. Zhu, *Soft Matter*, 2009, **5**, 2302.
5 (*a*) V. Lapeyre, C. Ancla, B. Catargi and V. Ravaine, *J. Colloid Interface Sci.*, 2008, **327**, 316; (*b*) T. Chen, D. P. Chang, T. Liu, R. Desikan, R. Datar, T. Thundat, R. Berger and S. Zauscher, *J. Mater. Chem.*, 2010, **20**, 3391.

6 (*a*) Y. Qin, G. Cheng, A. Sundararaman and F. Jäkle, *J. Am. Chem. Soc.*, 2002, **124**, 12672; (*b*) Y. Qin, G. Cheng, O. Achara, K. Parab and F. Jäkle, *Macromolecules*, 2004, **37**, 7123; (*c*) Y. Qin, V. Sukul, D. Pagakos, C. Cui and F. Jäkle, *Macromolecules*, 2005, **38**, 8987.
7 (*a*) V. Ravaine, C. Ancla and B. Catargi, *J. Controlled Release*, 2008, **132**, 2; (*b*) D. Wang, T. Liu, J. Yin and S. Y. Liu, *Macromolecules*, 2011, **44**, 2282.
8 (*a*) B. Wang, R. Ma, G. Liu, Y. Li, X. Liu, Y. An and L. Shi, *Langmuir*, 2009, **25**, 12522; (*b*) B. Wang, R. Ma, G. Liu, X. Liu, Y. Gao, J. Shen, Y. An and L. Shi, *Macromol. Rapid Commun.*, 2010, **31**, 1628.
9 (*a*) I. Hisamitsu, K. Kataoka, T. Okano and Y. Sakurai, *Pharm. Res.*, 1997, **14**, 289; (*b*) A. Matsumoto, S. Ikeda, A. Harada and K. Kataoka, *Biomacromolecules*, 2003, **4**, 1410; (*c*) A. Matsumoto, R. Yoshida and K. Kataoka, *Biomacromolecules*, 2004, **5**, 1038; (*d*) A. Matsumoto, K. Yamamoto, R. Yoshida, K. Kataoka, T. Aoyagi and Y. Miyahara, *Chem. Commun.*, 2010, **46**, 2203.
10 (*a*) K. T. Kim, J. J. L. M. Cornelissen, R. J. M. Nolte and J. C. M. van Hest, *J. Am. Chem. Soc.*, 2009, **131**, 13908; (*b*) K. T. Kim, J. J. L. M. Cornelissen, R. J. M. Nolte and J. C. M. van Hest, *Adv. Mater.*, 2009, **21**, 2787.
11 J. P. Lorand and J. O. Edwards, *J. Org. Chem.*, 1959, **24**, 769.
12 G. Springsteen and B. H. Wang, *Chem. Commun.*, 2001, 1608.
13 C. Cannizzo, S. Amigoni-Gerbier and C. Larpent, *Polymer*, 2005, **46**, 1269.
14 Y. Zhao, B. G. Trewyn, I. I. Slowing and V. S. Y. Lin, *J. Am. Chem. Soc.*, 2009, **131**, 8398.
15 I. Astafieva, X. F. Zhong and A. Eisenberg, *Macromolecules*, 1993, **26**, 7339.
16 (*a*) L. Zhang and A. Eisenberg, *Macromol. Symp.*, 1997, **113**, 221; (*b*) A. Choucair and A. Eisenberg, *Eur. Phys. J. E*, 2003, **10**, 37; (*c*) D. E. Discher and A. Eisenberg, *Science*, 2002, **279**, 967.
17 E. J. Vandenberg and D. Tian, *Macromolecules*, 1999, **32**, 3613.