ChemComm

Cite this: *Chem. Commun.*, 2012, **48**, 6043–6045

www.rsc.org/chemcomm

COMMUNICATION

Redox-sensitive polymeric nanoparticles for drug delivery†

Hanjoung Cho,[a] Jungeun Bae,[a] Vivek K. Garripelli,[a] Joel M. Anderson,[b] Ho-Wook Jun[b] and Seongbong Jo*[a]

Received 27th February 2012, Accepted 24th April 2012
DOI: 10.1039/c2cc31463k

Bioresponsive polymeric nanoparticles have been extensively pursued for the development of tumor-targeted drug delivery. A novel redox-sensitive biodegradable polymer with "trimethyl-locked" benzoquinone was synthesized for the preparation of paclitaxel-incorporated nanoparticles. The synthesized redox-sensitive nanoparticles released paclitaxel in response to chemically triggered reduction.

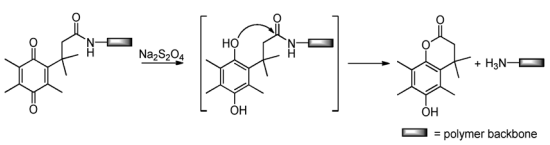
Multifunctional polymeric nanoparticles have been widely adopted for cancer-targeted drug delivery with their potential therapeutic benefit to minimize the dose-limiting systemic toxicity by preferential accumulation in tumors *via* the enhanced permeability and retention (EPR) effect.[1] These types of nanoparticles have frequently been modified to respond to the signals stemming from tumor microenvironments so that they can release incorporated drugs selectively in tumor sites.[2] Thus far, various bioresponsive materials that are sensitive to external stimuli, such as pH,[3] temperature,[4] enzymes,[5] and light,[6] have been intensively considered for targeted drug delivery to cancer.

Since redox changes associated with tumor hypoxia have been identified as a viable biomarker for tumor progression and cancer drug resistance, reductive enzymes overexpressed in tumor microenvironments, such as 12- to 18-fold overexpression of DT-diaphorase (EC 1.6.99.2) in lung cancer,[7] provide an important strategy for selective tumor targeting. Various bioreductive prodrugs have been reported and some of them are currently under clinical trials.[8] However, the prodrug approach to target tumors may be complicated with ubiquitous expression of various reductive enzymes in normal cells.[9]

Trimethyl-locked benzoquinone (TMBQ) is known to be chemically or enzymatically transformed into lactone *via* intramolecular cyclization triggered by two-electron reduction.[10,11] Prodrugs and bio-imaging probes have been based on the bioreductive cleavage of TMBQ by reductive enzymes and suggested for solid tumor-selective drug delivery and imaging based on redox changes occurring in tumor hypoxia.[10] As demonstrated with a bioreductive TMBQ-based aniline mustard prodrug, tested on T47D cells, the active drug resulted in cytotoxicity after the reductive activation by oxidoreductases at a low oxygen concentration.[10a] Even redox-triggered liposomes that electrochemically release chemicals have been designed with the TMBQ chemistry.[11e] Chemical reduction-induced shedding of TMBQ from the liposome surface resulted in the structural change of the supramolecular assemblies, which later destabilized the redox-sensitive liposomes to electrochemically release a hydrophilic fluorescent dye, calcein.[11e] However, the TMBQ-based redox-chemistry has never been applied for a polymeric drug delivery system thus far. The approach to use TMBQ-based redox-sensitive polymeric nanoparticles is advantageous over the TMBQ-based prodrugs or liposomes to target the redox stress in solid tumors. Polymeric nanoparticles can be easily modified with the ligands that selectively interact with the molecules expressed on cancer cells to achieve active targeting. In addition, the nanoparticles may be more flexible than the above-mentioned liposome for the formulation of hydrophobic cancer drugs since they may disrupt exquisitely balanced non-covalent interactions of TMBQ at the liposome surface. Furthermore, the nanoparticles can easily achieve prolonged circulation in blood and preferred accumulation in tumor by poly(ethylene glycol) (PEG) coupling.[12]

In this article, we describe a proof-of-concept novel redox-sensitive polymer containing amino groups protected by TMBQ. The polymer is intended to release lactone and unmask free amino groups under a simulated redox stress with sodium dithionite. Thus, its nanoparticle would be able to release incorporated drugs upon increased polymer hydration and subsequent nanoparticle swelling with the protonation of free amino groups at physiological pH (Scheme 1).[11] We primarily focused to test the feasibility of novel TMBQ-based redox-sensitive polymer nanoparticles as a bioresponsive drug delivery system using a simple *in vitro* drug release study.

A redox-sensitive polymer was designed and synthesized from a monomer containing TMBQ. Initially, benzoquinone carboxylic acid (β,β,2,4,5-pentamethyl-3,6-dioxo-1,4-cyclohexadiene-1-propanoic acid) activated with *N*-hydroxysuccimide



Scheme 1 Chemical reduction of the redox-sensitive polymers based on trimethyl-locked benzoquinone.

[a] *Department of Pharmaceutics, School of Pharmacy, The University of Mississippi, University, MS 38677, USA. E-mail: seongjo@olemiss.edu; Fax: +1 662-915-1177; Tel: +1 662-915-5166*
[b] *Department of Biomedical Engineering, University of Alabama at Birmingham, Birmingham, AL 35294, USA*

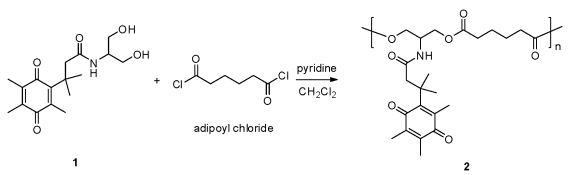
† Electronic supplementary information (ESI) available: Detailed experimental procedures and characterization data. See DOI: 10.1039/c2cc31463k

was synthesized according to the previously reported procedure.[11a] The activated compound was coupled with serinol (2-amino-1,3-propanediol) to yield a redox-sensitive diol monomer (ESI†). Serinol was selected for the polymer synthesis because of its proven biocompatibility as a monomer for biodegradable polyesters.[13] A coupling reaction between TMBQ-succinimidyl ester and serinol consequently yielded a diol with a pendant TMBQ (compound **1**). Synthesized serinol monomer was successfully confirmed by ^{1}H and ^{13}C NMR spectroscopy and elemental analysis. The serinol monomer was polymerized by the reaction described in Scheme 2. The isolated yield of the synthesized redox-sensitive polyester was found to be 85% after purification with precipitation in ether. The number-average polymer molecular weight (M_n) and PDI were determined to be 9800 Da and 1.51, respectively, by gel permeation chromatography (GPC) with polystyrene standards.

The proton NMR of polymer **2** in $CDCl_3$ showed all the characteristic peaks and splitting (ESI†), which indicated successful polymer synthesis. The chemical shift of 4 protons, which are located in adjacent carbonyls in adipoyl groups appeared at δ = 2.33 ppm indicating polyester backbone formation. Additionally, the trimethyl group in benzoquinone ring and dimethyl groups in the serinol monomer were shown at δ = 2.12, 1.95, and 1.41 ppm, respectively. The proton peaks of the monomer were broadened after the polymerization. It is worthwhile to note that this synthetic reaction is also amenable to further PEG modification at the redox-sensitive polymer chain ends by the treatment of adipoyl chloride to secure terminal carboxyl groups that can be exploited for the esterification with methoxy-capped PEG. Even cancer selective cyclic RGD can be modified to the end carboxyl groups.

Redox-sensitive polymeric nanoparticles were prepared from the synthesized polymer by an emulsion method.[14] Tween 80 was used as a surfactant to form emulsion instead of poly(vinyl alcohol) partially because of a low polymer molecular weight of the redox polymer. The final polymeric nanoparticles resulted in a yellowish fluffy powder. Particle size determined by dynamic light scattering (DLS) using a Zetasizer (Malvern Instruments, UK) revealed successful nanoparticle preparation with a Z-averaged hydrodynamic diameter of 180 nm (PDI = 0.138) (Fig. 1) which would allow tumor site accumulation by EPR.[1] A TEM image of the nanoparticles showed that the morphology of the nanoparticles was spherical with the particle size qualitatively comparable to that determined by DLS (Fig. 1), indicating that the nanoparticle preparation *via* an emulsion was reliable.

The effect of redox stress on the nanoparticles was tested with a chemical reductant, sodium dithionite ($Na_2S_2O_4$). It was expected that sodium dithionite would reduce TMBQ to leave free amine groups on the polymer, which would result in physico-chemical changes in nanoparticles that would induce



Scheme 2 Synthesis of redox-sensitive polymer with adipoyl chloride under basic conditions.

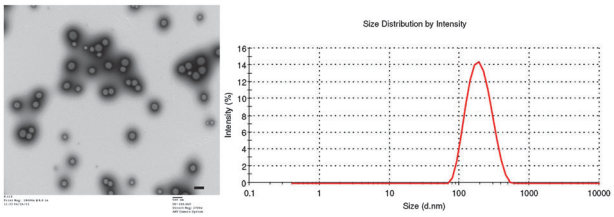


Fig. 1 TEM image and size distribution of polymeric nanoparticles by a dynamic light scattering method (DLS). Scale bar is 500 nm in length.

nanoparticle swelling or dissolution at pH 7.4. Sodium dithionite-induced reduction dramatically increased the hydrodynamic diameter of the nanoparticles from 178 nm in a turbid solution to 29.3 μm in a transparent solution, a size beyond the measurable limit by the Zetasizer as shown in Fig. 2. The size changes might have resulted from the protonation of free amine groups in serinol of which pK_a is around 9.15.

A hydrophobic anticancer drug, paclitaxel, was loaded into the redox-sensitive polymeric nanoparticles of which the particle size was determined to be 267 nm (PDI = 0.21). The slight size increase from 180 nm to 267 nm upon paclitaxel incorporation might be attributed to the hydrophobicity of paclitaxel.[15] As reported with amphiphilic polymer micelles, hydrophobic interaction between paclitaxel and redox-sensitive polymer could expand the particles.[15] However, the morphology of drug-loaded nanoparticles observed by TEM was similar to that of blank nanoparticles and spherical (ESI†). The paclitaxel loading efficiency in the nanoparticles was determined to be 77.9% when a drug to polymer ratio of 1 : 10 was used.

Redox-triggered drug release from the nanoparticles was tested by an *in vitro* release study using a medium containing sodium dithionite. At a 200 molar excess of sodium dithionite to the molar amount of benzoquinone, the release of paclitaxel and lactone was dramatically increased over 36 h as shown in Fig. 3. The amount of sodium dithionite used in the experiment might exceed the redox stress occurring in tumors. The primary purpose of using an excess amount of sodium dithionite was to test the feasibility of the nanoparticles as a novel redox-sensitive drug delivery system. Further investigation with DT-diaphorase and NADPH mimicking tumor microenvironments would better evaluate the TMBQ-based polymer nanoparticles.

The inclusion of sodium salicylate at a concentration of 80 mM maintained sink conditions during the release study. It has been known that sodium salicylate is able to increase paclitaxel

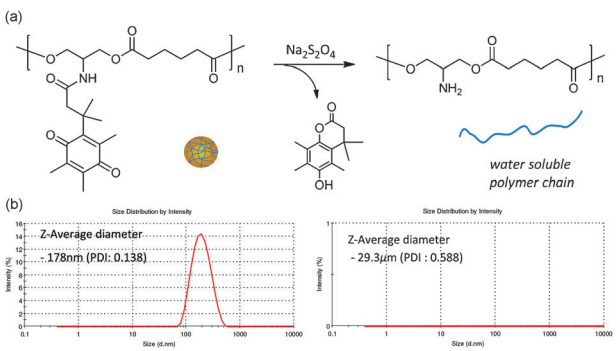


Fig. 2 Change in nanoparticle size in the presence of sodium dithionite determined by DLS.

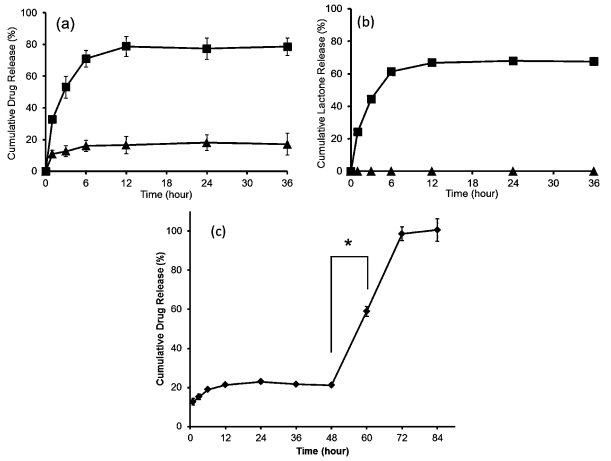


Fig. 3 *In vitro* release profiles of (a) paclitaxel and (b) lactone in the presence of sodium dithionite (■) and without sodium dithionite (▲) in PBS. Triggered paclitaxel release was tested with an alternating addition of sodium dithionite (c). Significant increase (*) in cumulative paclitaxel release ($P < 0.05$) was noted during the release study. Data represent the mean ± SD ($n = 3$).

solubility in aqueous solution without affecting physical properties of paclitaxel.[16] As shown in Fig. 3, about 52% of encapsulated paclitaxel was released within 3 h in the presence of sodium dithionite while 13% of paclitaxel was released over 12 h in the absence of the reducing agent. An achieved cumulative paclitaxel release over 36 h was 79% in the presence of the reducing agent. However, only 17% of incorporated paclitaxel was released from the redox-sensitive nanoparticles without the chemical reductant. Lactone release from the paclitaxel-loaded nanoparticles was also consistent with the paclitaxel release. Cumulative lactone release lasted for 12 h to reach 66.8% while no lactone was released without reduction (Fig. 3b). Therefore, lactone release upon nanoparticle reduction indicates that property change occurred in the redox-sensitive nanoparticles was indeed mediated by the two-electron reduction of TMBQ moiety. Furthermore, the consequent size change occurred to nanoparticles might be translated into increased paclitaxel release.

We also challenged the nanoparticles with the reducing agent after incubating them in PBS for 48 h. An addition of sodium dithionite immediately increased drug release, which peaked within 24 h (Fig. 3c). The percentage release of paclitaxel within initial 48 h was only 20%. However, paclitaxel release increased to a cumulative release of 90% within 24 h after the addition of sodium dithionite. Taken together, the results indicated that paclitaxel could be released from the TMBQ-based redox-sensitive polymer nanoparticles in response to redox changes. Although a gap needs to be filled with better simulation of the redox stress using tumor-related reductive enzymes that are able to reduce TMBQ, the TMBQ-based nanoparticles would be useful for targeting solid tumors.

Novel redox-sensitive polymeric nanoparticles were prepared from a synthesized monomer containing TMBQ as a redox sensitive group. A hydrophobic cancer drug, paclitaxel, was incorporated into the polymeric nanoparticles and released by sodium dithionite-mediated reduction in a triggered manner. Since the polymeric nanoparticles are able to release incorporated drugs in response to a simulated redox stress, these nanoparticles would be useful for targeted drug delivery to solid tumors.

We acknowledge that funding for this work came from the Department of Defense W81XWH-10-1-0414 and National Science Foundation EPS-0903787 (S.J.). We also acknowledge the HRSA grant off which the Malvern Zetasizer was purchased. Individual fellowship support was provided by the Ruth L. Kirschstein National Research Service Award Individual Fellowship (F31DE021286) from the National Institute of Dental & Craniofacial Research (J.M.A.)

Notes and references

1 H. Maeda, J. Wu, T. Sawa, Y. Matsumura and K. Hori, *J. Controlled Release*, 2000, **65**, 271–284.
2 (*a*) S. Sengupta, D. Eavarone, I. Capila, G. Zhao, N. Watson, T. Kiziltepe and R. Sasisekharan, *Nature*, 2005, **436**, 568–572; (*b*) W. Wang and C. Alexander, *Angew. Chem., Int. Ed.*, 2008, **47**, 7804–7806.
3 (*a*) A. P. Griset, J. Walpole, R. Liu, A. Gaffey, Y. L. Colson and M. W. Grinstaff, *J. Am. Chem. Soc.*, 2009, **131**, 2469–2471; (*b*) N. Murthy, M. Xu, S. Schuck, J. Kunisawa, N. Shastri and J. M. Frechet, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 4995–5000; (*c*) 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.
4 (*a*) J. E. Chung, M. Yokoyama, M. Yamato, T. Aoyagi, Y. Sakurai and T. Okano, *J. Controlled Release*, 1999, **62**, 115–127; (*b*) S. Q. Liu, Y. W. Tong and Y. Y. Yang, *Biomaterials*, 2005, **26**, 5064–5074; (*c*) K. Na, K. H. Lee, D. H. Lee and Y. H. Bae, *Eur. J. Pharm. Sci.*, 2006, **27**, 115–122.
5 F. M. Veronese, O. Schiavon, G. Pasut, R. Mendichi, L. Andersson, A. Tsirk, J. Ford, G. Wu, S. Kneller, J. Davies and R. Duncan, *Bioconjugate Chem.*, 2005, **16**, 775–784.
6 (*a*) N. Fomina, C. McFearin, M. Sermsakdi, O. Edigin and A. Almutairi, *J. Am. Chem. Soc.*, 2010, **132**, 9540–9542; (*b*) A. P. Goodwin, J. L. Mynar, Y. Ma, G. R. Fleming and J. M. Frechet, *J. Am. Chem. Soc.*, 2005, **127**, 9952–9953.
7 S. Y. Sharp, L. R. Kelland, M. R. Valenti, L. A. Brunton, S. Hobbs and P. Workman, *Mol. Pharmacol.*, 2000, **58**, 1146–1155.
8 (*a*) C. P. Guise, M. R. Abbattista, R. S. Singleton, S. D. Holford, J. Connolly, G. U. Dachs, S. B. Fox, R. Pollock, J. Harvey, P. Guilford, F. Donate, W. R. Wilson and A. V. Patterson, *Cancer Res.*, 2010, **70**, 1573–1584; (*b*) M. R. Albertella, P. M. Loadman, P. H. Jones, R. M. Phillips, R. Rampling, N. Burnet, C. Alcock, A. Anthoney, E. Vjaters, C. R. Dunk, P. A. Harris, A. Wong, A. S. Lalani and C. J. Twelves, *Clin. Cancer Res.*, 2008, **14**, 1096–1104.
9 F. P. Guengerich and W. W. Johnson, *Biochemistry*, 1997, **36**, 14741–14750.
10 (*a*) M. Volpato, N. Abou-Zeid, R. W. Tanner, L. T. Glassbrook, J. Taylor, I. Stratford, P. M. Loadman, M. Jaffar and R. M. Phillips, *Mol. Cancer. Ther.*, 2007, **6**, 3122–3130; (*b*) S. T. Huang, Y. X. Peng and K. L. Wang, *Biosens. Bioelectron.*, 2008, **23**, 1793–1798; (*c*) S. T. Huang and Y. L. Lin, *Org. Lett.*, 2006, **8**, 265–268.
11 (*a*) A. Zheng, D. Shan and B. Wang, *J. Org. Chem.*, 1999, **64**, 156–161; (*b*) W. Ong and R. L. McCarley, *Chem. Commun.*, 2005, 4699–4701; (*c*) C. Yan, W. Matsuda, D. R. Pepperberg, S. C. Zimmerman and D. E. Leckband, *J. Colloid Interface Sci.*, 2006, **296**, 165–177; (*d*) W. Ong and R. L. McCarley, *Macromolecules*, 2006, **39**, 7295–7301; (*e*) W. Ong, Y. Yang, A. C. Cruciano and R. L. McCarley, *J. Am. Chem. Soc.*, 2008, **130**, 14739–14744.
12 V. P. Torchilin, *Pharm. Res.*, 2007, **24**, 1–16.
13 J. Rickerby, R. Prabhakar, A. Patel, J. Knowles and S. Brocchini, *J. Controlled Release*, 2005, **101**, 21–34.
14 J. Jung, I. H. Lee, E. Lee, J. Park and S. Jon, *Biomacromolecules*, 2007, **8**, 3401–3407.
15 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.
16 (*a*) Y. W. Cho, L. Lee, S. C. Lee, K. M. Huh and K. Park, *J. Controlled Release*, 2004, **97**, 259–257; (*b*) K. M. Huh, H. S. Min, S. C. Lee, H. J. Lee, S. Kim and K. Park, *J. Controlled Release*, 2008, **126**, 122–129.