

# Anticancer camptothecin-*N*-poly(lactic acid) nanoconjugates with facile hydrolysable linker†

Cite this: *Polym. Chem.*, 2014, 5, 1581Qian Yin,<sup>a</sup> Rong Tong,<sup>‡a</sup> Lichen Yin,<sup>a</sup> Timothy M. Fan<sup>b</sup> and Jianjun Cheng<sup>\*a</sup>Received 6th September 2013  
Accepted 27th September 2013

DOI: 10.1039/c3py01245j

www.rsc.org/polymers

We report a strategy of conjugating CPT to the terminal carboxylate group of polylactide (PLA) with a facile hydrolysable amino ester linker *via* a controlled polymerization method. The obtained CPT-*N*-PLA conjugates were able to self-assemble into 50–100 nanometer-sized conjugates (NCs) with desired *in vitro* physicochemical properties and showed enhanced *in vivo* therapeutic efficacy against Lewis lung carcinoma (LLC) induced in C57BL/6 mice.

## Introduction

20(*S*)-Camptothecin (CPT), a topoisomerase I inhibitor isolated from the Chinese tree *Camptotheca acuminata* in the 1960s, has been demonstrated to have a broad range of remarkable anticancer activities against various tumor models.<sup>1,2</sup> However, the low aqueous solubility of its therapeutically active lactone form has largely hindered its clinical applications. When placed in an aqueous solution at physiological pH, CPT is quickly transformed from its therapeutically active lactone form to the inactive carboxylate form, leading to severe toxicity. Additionally, favorable binding with serum albumin of the carboxylate form of CPT forces the lactone–carboxylate equilibrium toward the formation of the carboxylate.<sup>3–5</sup> Furthermore, CPT tends to be quickly eliminated from the circulation system after being intravenously administered because of its low molecular weight, which significantly diminishes its anticancer activity. To circumvent these drawbacks, there are numerous efforts for synthesizing CPT analogues to achieve improved solubility and enhanced lactone stability.<sup>6–9</sup>

Polymeric nanomedicine, an emerging field that includes the use of drug-containing polymeric nanoparticles (NPs), opens up a new opportunity for overcoming the shortcomings of CPT.<sup>10–14</sup> Utilizing polymeric NPs as drug carriers to deliver CPT has the potential to provide various benefits such as improved water solubility, reduced clearance, reduced drug resistance, and enhanced therapeutic efficacy.<sup>15–17</sup> Poly(lactic acid) (PLA) is one of the extensively used polymeric materials in the formulation of NPs due to its excellent safety profile, tunable degradation kinetics, and ease of synthesis.<sup>18,19</sup> PLA NPs that encapsulate CPT can be readily prepared *via* co-precipitation of the polymer and drug.<sup>20,21</sup> However, such an encapsulation method tends to bring several formulation issues of NPs, such as low encapsulation efficiency, low drug loading, heterogeneous compositions, and “burst” drug release profile, which highly impact their pharmacological and pharmacokinetic properties *in vivo*.<sup>20,22</sup>

To address these challenges, we previously developed CPT-PLA nanoconjugates (NCs) through CPT-initiated ring-opening polymerization (ROP) of lactide (LA) followed by nanoprecipitation of the resulting CPT-PLA conjugates.<sup>23</sup> CPT-PLA NCs possess some properties like nearly 100% loading efficiency, tunable drug loading, and narrowly distributed particle sizes. In these NCs, the release of CPT is attributed to the cleavage of a lactate ester bond between CPT and PLA through hydrolysis at physiological conditions.<sup>23</sup> Because PLA is hydrophobic, the entanglement of polymer chains forms the rigid cores of NCs. Water and ions (*e.g.*, H<sup>+</sup> and OH<sup>−</sup>) are not able to access the ester linkage easily which therefore results in slow release kinetics of CPT: only 50% of CPT is released from conjugates in PBS after two weeks at 37 °C.<sup>23</sup> Such a slow release profile can significantly diminish the side effects of free CPT in circulation, but it brings difficulty in achieving the active drug concentration within a short time in tumors. To improve the efficacy of CPT-PLA NCs, an improved rapid release of CPT from NCs, while still avoiding burst release, is desired. As reported, amine could accelerate the hydrolysis of esters by facilitating the formation of a destabilized intermediate during the

<sup>a</sup>Department of Materials Science and Engineering, University of Illinois at Urbana – Champaign, Urbana, IL 61801, USA. E-mail: jianjunc@illinois.edu

<sup>b</sup>Department of Veterinary Clinical Medicine, University of Illinois at Urbana – Champaign, Urbana, Illinois 61801, USA

† Electronic supplementary information (ESI) available: Experimental procedures, FT-IR spectra and MALDI-TOF MS analysis of synthesized drug–polymer conjugates. See DOI: 10.1039/c3py01245j

‡ Current address: Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA.

hydrolysis process.<sup>24</sup> Thus, we report a simple chemistry that allows for facile conjugation of CPT to the terminal carboxylate group of polylactide (PLA) *via* a hydrolysis-labile amino ester linker. Instead of using poorly controlled coupling chemistry, we developed a ring-opening polymerization (ROP) method to facilitate the incorporation of a hydrophilic amino ester linker between CPT and PLA. The obtained CPT-*N*-PLA conjugates were able to be co-precipitated with methoxy-poly(ethylene glycol)-PLA (mPEG-PLA) to self-assemble into NCs with well-controlled physicochemical properties, such as sub-100 nm size, narrow size distribution, and controlled release kinetics. We also demonstrated that the formulated NCs with rapid drug release kinetic profile could potentially enhance the *in vivo* therapeutic efficacy against Lewis lung carcinoma (LLC) induced in C57BL/6 mice.

## Results and discussion

### Synthesis and characterization of CPT-*N*-LA<sub>*n*</sub> conjugates

It has been suggested that the release of a conjugated anti-cancer drug should occur in a controlled manner within the tumor stroma to maximize its therapeutic efficacy and the drug-oligomer conjugates formed by the hydrolysis of polymer chains during the circulation should be inhibited to minimize the systemic toxicity.<sup>25</sup> Thus, distinctive differences of hydrolysis rates between drug-polymer and polymer backbones are desirable. To achieve this aim, the CPT derivative was first synthesized by condensation of bromoacetic acid with CPT's C20-hydroxyl group to yield CPT-bromide (CPT-Br). Then, CPT-Br was reacted with ethanolamine to obtain *N*-substituted CPT-ester containing a primary hydroxyl group, termed CPT-*N*-OH. As we previously reported, the pendent hydroxyl group of the therapeutic agent allows for one-step drug conjugation to the terminal carboxylate group of PLA *via* a ROP method facilitated by BDI-Zn catalyst.<sup>23,26–28</sup> Through such living polymerization, both the initiation (CPT-*N*-OH incorporation) and the chain propagation can proceed in a well-controlled manner and result in materials with pre-defined drug loadings and narrow molecular weight distributions (MWDs) (Fig. 1).

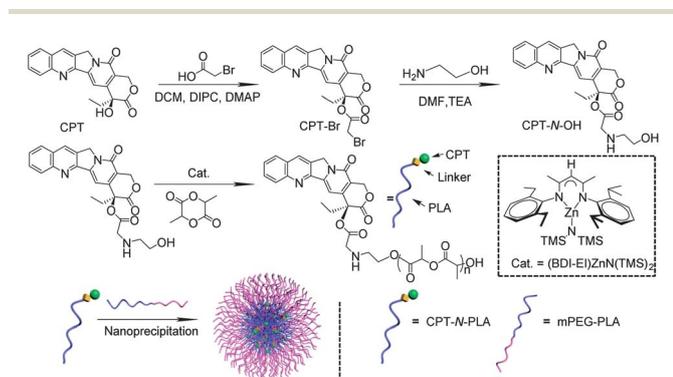


Fig. 1 Preparation of PEGylated CPT-*N*-PLA NCs *via* CPT-*N*-OH initiated LA polymerization in the presence of (BDI-EI)ZnN(TMS)<sub>2</sub>, followed by nanoprecipitation and non-covalent surface modification.

Controlled ROP was performed over a broad range of LA/CPT-*N*-OH ratios from 10 to 200 when the LA polymerizations were mediated by (BDI-EI)ZnN(TMS)<sub>2</sub>/CPT-*N*-OH with quantitative CPT-*N*-OH incorporation efficiencies and narrow MWDs ( $M_w/M_n = 1.09–1.23$ ; Table 1). The obtained MWs of the CPT-*N*-PLA<sub>*n*</sub> conjugates were in excellent agreement with the expected MWs, which followed a linear correlation with the LA/CPT-*N*-OH ratios (Fig. 2B). Monomodal GPC MW distribution curves were observed in all CPT-*N*-PLA<sub>*n*</sub> conjugates prepared with various LA/CPT-*N*-OH ratios (Fig. 2A). The well-controlled polymerization mediated by (BDI-EI)ZnN(TMS)<sub>2</sub>/CPT-*N*-OH presumably proceeded through the insertion-coordination mechanism as reported.<sup>23</sup>

### Preparation and characterization of CPT-*N*-LA<sub>10</sub> NC

Nanoprecipitation, representing a facile, non-extensive and low-energy consuming technique, has been widely used for the preparation of versatile polymeric NPs.<sup>22,29–31</sup> In this study, the CPT-*N*-PLA conjugate was dissolved in a water-miscible organic solvent (DMF) and then dropwise added into fast stirring water, resulting in the formation of CPT-*N*-PLA<sub>10</sub> NCs with CPT embedded in the hydrophobic PLA matrices (Table 2). This method allows for a rapid production of NC with sub-100 nm size and very narrow size distribution in a large quantity (gram scale), which was confirmed with dynamic light scattering (DLS) (Fig. 3A). Furthermore, the sizes of these NCs prepared by nanoprecipitation can be easily controlled by tuning the concentration of polymer.<sup>22</sup> When the DMF-water ratio is fixed at 1/20 (v/v), the size of CPT-*N*-PLA<sub>10</sub> NCs showed a linear correlation with the concentration of CPT-*N*-PLA<sub>10</sub> conjugate and can be precisely tuned from 50 nm to 75 nm (Fig. 3B).

To achieve favorable *in vivo* performance, the NCs are expected to have prolonged circulation time to maximize their therapeutic efficacy.<sup>32–34</sup> However, surface-unmodified NCs are usually found to have non-specific binding with proteins in blood to form large aggregates, subsequently resulting in rapid clearance from the blood stream due to uptake by the reticuloendothelial system (RES).<sup>35</sup> Modification of NC surfaces with PEG, termed “PEGylation”, is the most widely used approach to reduce recognition by RES and prolong systemic circulation.<sup>36</sup> To minimize efforts for complicated chemical synthesis, we applied a facile strategy to coat the surface of NCs with PEG. By mixing mPEG<sub>5k</sub>-PLA<sub>10</sub> (PLA block of 1.4 kDa and mPEG segment of 5 kDa) copolymer with CPT-*N*-PLA<sub>10</sub> conjugate followed by a nanoprecipitation technique, it yields PEGylated NCs *via* the hydrophobic interaction of PLA segments of mPEG<sub>5k</sub>-PLA<sub>10</sub> and CPT-*N*-PLA<sub>10</sub>. As shown in Fig. 3A and D, the nanoparticle size in water increased from 73 to 93 nm after PEGylation, indicating that PEGylation only partially contributed to the increased particle diameter. When dispersed in PBS, cell culture medium (DMEM), and human serum, the particle size was further augmented by 20–40 nm, which was presumably attributed to the salt-induced screening of the repulsive force.<sup>22</sup> No aggregation was noticed according to the DLS histogram, which demonstrated the desired stability of the PEGylated

Table 1 Polymerization of LA mediated by CPT-*N*-OH and (BDI-EI)ZnN(TMS)<sub>2</sub>

Entry <sup>a</sup>	Name	[M]/[cat.]	Conv. (%) <sup>b</sup>	$M_{\text{ncal}} (\times 10^3 \text{ g mol}^{-1})$	$M_n (\times 10^3 \text{ g mol}^{-1})^c$	MWD ( $M_w/M_n$ ) <sup>c</sup>
1	CPT- <i>N</i> -PLA <sub>200</sub>	200/1	>98	29.2	28.9	1.09
2	CPT- <i>N</i> -PLA <sub>100</sub>	100/1	>98	14.9	15.7	1.13
3	CPT- <i>N</i> -PLA <sub>50</sub>	50/1	>98	7.6	7.9	1.17
4	CPT- <i>N</i> -PLA <sub>10</sub>	10/1	>98	1.9	1.4	1.23

<sup>a</sup> All reactions were performed in the glovebox. Abbreviations: Conv. (%) = conversion of monomer %, MWD = molecular weight distribution.

<sup>b</sup> Determined by FT-IR by monitoring the disappearance of the LA peak at 1772 cm<sup>-1</sup>. <sup>c</sup> Determined by gel permeation chromatography (GPC) and MALDI-TOF MS analysis.

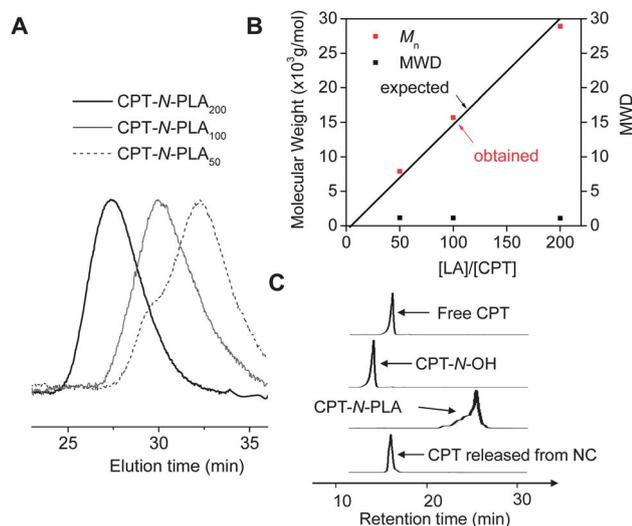


Fig. 2 (A) Overlay of GPC traces of CPT-*N*-PLA<sub>*n*</sub> (*n* = 50, 100 and 200). (B) (BDI-EI)ZnN(TMS)<sub>2</sub>/CPT mediated ring-opening polymerization (ROP) of LA at various ratios of LA/CPT (MWD = molecular weight distribution). (C) HPLC analysis of CPT-*N*-OH initiated polymerization and release of CPT from CPT-*N*-PLA<sub>*n*</sub> NC. (HPLC traces from top to bottom: free CPT, CPT-*N*-OH, CPT-*N*-PLA, CPT released from CPT-*N*-PLA NC).

CPT-*N*-PLA<sub>10</sub> NCs (Fig. 3C). For further clinical applications, it is desirable that NCs are formulated in solid form to retain their original well-controlled properties, feasible for the long-term storage and transport prior to their use in clinic.<sup>37</sup> As shown in Fig. 3D, bovine serum albumin (BSA), a ubiquitous protein in the blood, can be used to stabilize CPT-*N*-PLA<sub>10</sub> NCs and effectively prevent the severe aggregation of NCs during the lyophilization process.<sup>22</sup>

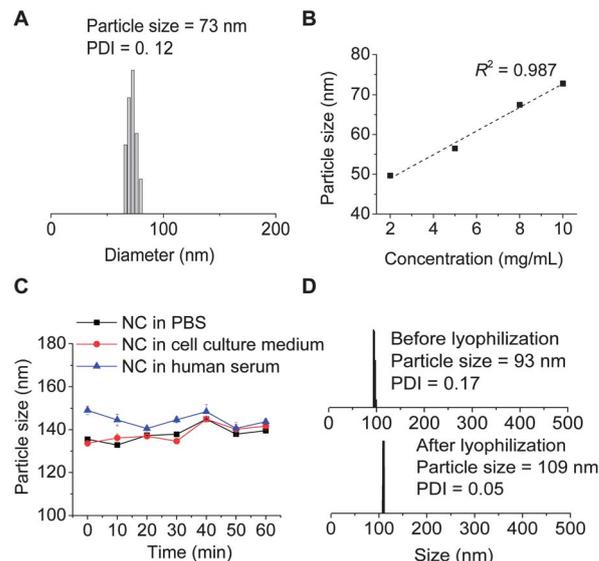


Fig. 3 Formulation and characterization of CPT-*N*-PLA<sub>10</sub> NCs. (A) DLS analysis of CPT-*N*-PLA<sub>10</sub> NC in water (0.5 mg mL<sup>-1</sup>). (B) Precipitation of CPT-*N*-PLA<sub>10</sub> from DMF solution into water at various CPT-*N*-PLA<sub>10</sub> concentrations. (C) Stability of PEGylated CPT-*N*-PLA<sub>10</sub> NCs in PBS, cell culture medium, and human serum buffer (human serum : PBS = 1 : 1, v/v). (D) DLS analysis of NCs reconstitution: monodispersed PEGylated CPT-*N*-PLA<sub>10</sub> NCs mixed with bovine serum albumin (BSA) in water before lyophilization (upper panel), and the reconstituted NCs after lyophilization in the presence of BSA (BSA : NC = 10 : 1, wt/wt) (bottom panel).

### Drug release kinetics and cytotoxicity

We performed the release kinetics study of CPT from PEGylated CPT-*N*-LA<sub>10</sub> NC and PEGylated CPT-LA<sub>10</sub> NC respectively in 50% human serum buffer, which better mimics the release in

Table 2 Characterization of CPT-*N*-PLA NC prepared by LA polymerization mediated by (BDI-EI)ZnN(TMS)<sub>2</sub> catalyst

Entry <sup>a</sup>	Name	M/I ratio	Eff (%) <sup>b</sup>	Loading (%) <sup>c</sup>	Size (nm) <sup>d</sup>	PDI <sup>e</sup>
1	CPT- <i>N</i> -PLA <sub>100</sub>	100	>95	2.3	82	0.06
2	CPT- <i>N</i> -PLA <sub>50</sub>	50	>95	4.6	89	0.09
3	CPT- <i>N</i> -PLA <sub>10</sub>	10	>95	18.5	73	0.12

<sup>a</sup> Abbreviations: NC = nanoconjugates; M/I = monomer/initiator ratio; Eff = incorporation efficiency, the percentage of initiator utilized in the initiation of LA polymerization; PDI = polydispersity derived from particle sizing using DLS. NCs are named as CPT-*N*-PLA<sub>M/I</sub>. <sup>b</sup> The data were based on the reversed-phase HPLC analysis of unincorporated drug. <sup>c</sup> The data were based on the reversed-phase HPLC analysis of unincorporated drug. <sup>d</sup> Characterized by DLS. <sup>e</sup> Characterized by DLS.

physiological conditions. Since the release kinetics of CPT is determined by the hydrolysis of ester linkages between CPT and NCs, the release kinetics of CPT from NCs is more sustainable as compared to the burst release profile often observed in the polymeric encapsulates. As shown in Fig. 4A, CPT-LA<sub>10</sub> NCs with the hydrophobic ester linker between CPT and polymeric NCs exhibited sustained drug release with 18% CPT released over 48 h. In comparison, when the linker was changed to a hydrolysis-labile amino ester linker, the release kinetics of CPT from CPT-N-LA<sub>10</sub> NC could be significantly accelerated with nearly 50% CPT being released within the same period of time. *In vitro* toxicity of NCs is highly correlated with the amount of drug released from NCs; we therefore evaluated the cytotoxicity of PEGylated CPT-LA NCs with two different linkers in MCF-7 cells using MTT assay (Fig. 4B). The IC<sub>50</sub> of PEGylated CPT-LA<sub>10</sub> NC (1435 nM) is nearly three times higher than PEGylated CPT-N-LA<sub>10</sub> NC (454 nM). As a result, the toxicity of PEGylated CPT-NCs against cancer cell proliferation could be improved simply by controlling the linker.

### *In vivo* efficacy of CPT-N-LA NCs

We infer that the NC formulation with rapid drug release kinetic profile will potentially enhance the efficacy. For proof of concept, we then investigated the *in vivo* therapeutic efficacy of NCs against Lewis lung carcinoma (LLC) induced by subcutaneous injection of LLC cells into C57BL/6 mice. The study protocol was reviewed and approved by the Illinois Institutional Animal Care and Use Committee (IACUC) of University of Illinois at Urbana – Champaign (see the ESI† for further details). When the size of tumors reached around 200 mm<sup>3</sup>, the mice were divided into five groups to minimize the differences of body weights and tumor sizes among groups (*N* = 6). Two groups of mice received a single intravenous injection of PEGylated CPT-N-LA<sub>10</sub> NC and PEGylated CPT-LA<sub>10</sub> NC at a dose of 50 mg CPT per kg mice body weight (50 mg kg<sup>-1</sup>), respectively. Irinotecan, a CPT analogue with improved solubility, was administered to a third group as clinically suggested at a single dose of 100 mg kg<sup>-1</sup> intraperitoneally. The other two groups

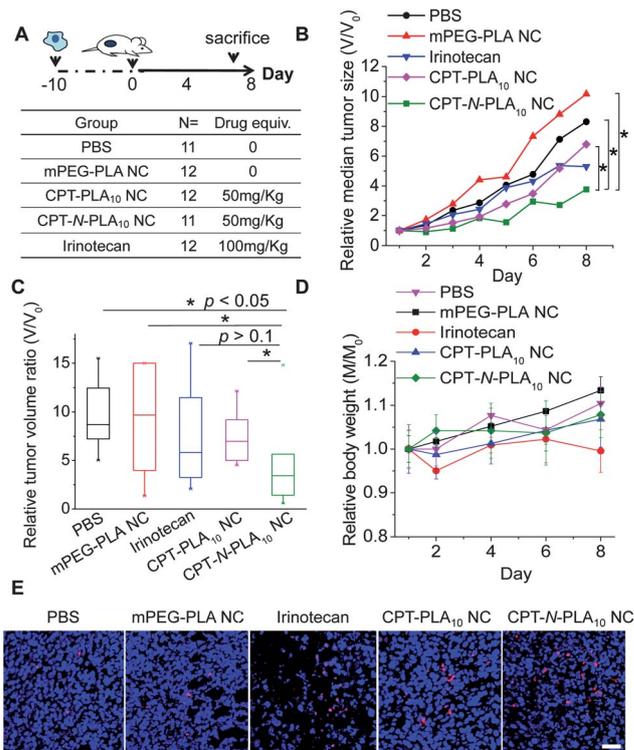


Fig. 5 *In vivo* tumor reduction study. (A) Experimental procedures of the study. (B) Delay and inhibition of LLC (Lewis lung carcinoma) tumor growth in C57BL/6 mice with different treatments (PEGylated CPT-LA<sub>10</sub> NCs, PEGylated CPT-N-LA<sub>10</sub> NCs, irinotecan, mPEG-PLA NCs, and PBS), *N* = 6. Data are presented as relative median tumor size ( $V/V_0$ , compare to the tumor volume at day 0). (C) Box plot of LLC tumor growth in C57BL/6 mice at day 8, after treatment with PEGylated CPT-LA NCs, PEGylated CPT-N-LA NCs, irinotecan, mPEG-PLA NCs, and PBS at day 0. Statistical properties of relative tumor volume ratio ( $V/V_0$ , compare to the tumor volume at day 0) shown in box plot are as follows: box (median with 25/75% percentile), whisker (5/95% percentile), and asterisks (maximum/minimum). Univariate differences between the groups were assessed with Mann-Whitney *U* test.  $P < 0.05$  is considered statistically significant. (D) Relative body weight ( $M/M_0$ ) monitoring over the study (*M*: body weight monitored during the study;  $M_0$ : body weight monitored at day 0). (E) Representative deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end (TUNEL) staining sections of LLC tumors with all treatments (PEGylated CPT-LA<sub>10</sub> NCs, PEGylated CPT-N-LA<sub>10</sub> NCs, irinotecan, mPEG-PLA NCs, and PBS). Scale bar: 40  $\mu$ m.

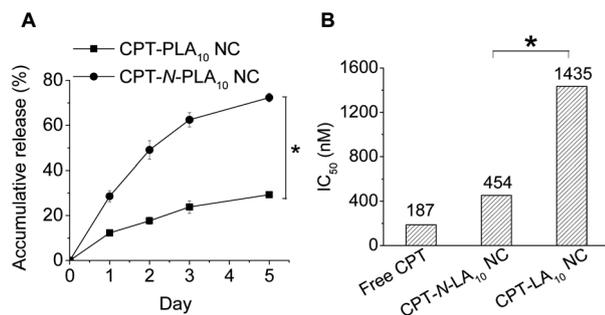


Fig. 4 (A) Release kinetics of CPT from PEGylated CPT-PLA<sub>10</sub> NC and PEGylated CPT-N-PLA<sub>10</sub> NC in human serum buffer (human serum : PBS = 1 : 1, v/v) at 37 °C. (B) Cytotoxicity of free CPT, PEGylated CPT-LA<sub>10</sub> NC and PEGylated CPT-N-PLA<sub>10</sub> NC in MCF-7 cells as determined by MTT assay (37 °C, 72 h). Statistical differences between the groups were assessed with Student's *t*-test.  $*P < 0.05$  is considered statistically significant.

were administered intravenously with PBS or mPEG-PLA NC as negative controls (Fig. 5A). The tumor sizes and body weights of mice in each group were monitored continuously for 8 days post-injection. We found the group of mice treated with PEGylated CPT-N-LA<sub>10</sub> NCs experienced significantly delayed tumor growth and the treatment was more effective than groups treated with CPT-LA<sub>10</sub> NCs, and other control groups ( $P < 0.05$ , Mann-Whitney *U* test, Fig. 5B and C). Our efficacy result is further confirmed by TUNEL staining of tumor sections obtained from the above five groups of mice at day 8. As shown in Fig. 5E, tumors treated with CPT-N-LA<sub>10</sub> NCs had substantially increased apoptotic cell numbers compared with those treated with CPT-LA<sub>10</sub> NCs. The apoptotic index (TUNEL/DAPI, day 8) indicates that CPT-N-LA<sub>10</sub> NCs (12.29%) had a

dramatically improved efficacy compared to the CPT-LA<sub>10</sub> NCs (9.23%) (Fig. 5E). When evaluating the body weight changes of all the mice that received treatments, no significant (<20%) body weight drop was observed (Fig. 5D), which indicated minimal acute toxicities of CPT-loaded polymeric NCs.

## Conclusions

Overall, by taking advantage of the controlled ROP method, we successfully designed and incorporated a hydrolysis-labile amino ester linker to conjugate CPT to PLA *via* a fully controlled manner. The resulting CPT-*N*-PLA conjugates were able to self-assemble into sub-100 nm-sized NCs with desired physicochemical properties, and accelerated release kinetics compared with our previous CPT-PLA NCs. We also demonstrated that such an improvement could contribute to the enhanced *in vivo* efficacy. The growth of Lewis lung carcinomas (LLCs) induced in C57BL/6 mice was significantly delayed compared with CPT-PLA NCs, without acute systemic toxicity.

## Acknowledgements

This work was supported by National Science Foundation (Career Program DMR-0748834 and DMR-1309525) and the National Institute of Health (NIH Director's New Innovator Award 1DP2OD007246-01; 1R21CA152627). Q.Y. was funded at UIUC from NIH National Cancer Institute Alliance for Nanotechnology in Cancer "Midwest Cancer Nanotechnology Training Center" Grant R25 CA154015A.

## Notes and references

- L. F. Liu, S. D. Desai, T. K. Li, Y. Mao, M. Sun and S. P. Sim, *Ann. N. Y. Acad. Sci.*, 2006, **922**, 1–10.
- R. C. Gallo, J. Whangpen and R. H. Adamson, *JNCI, J. Natl. Cancer Inst.*, 1971, **46**, 789–795.
- Q. Y. Li, Y. G. Zu, R. Z. Shi and L. P. Yao, *Curr. Med. Chem.*, 2006, **13**, 2021–2039.
- Z. H. Mi and T. G. Burke, *Biochemistry*, 1994, **33**, 10325–10336.
- Z. H. Mi and T. G. Burke, *Biochemistry*, 1994, **33**, 12540–12545.
- M. C. Bissery, P. Vrignaud, F. Lavelle and G. G. Chabot, *Anti-Cancer Drugs*, 1996, **7**, 437–460.
- B. L. Staker, K. Hjerrild, M. D. Feese, C. A. Behnke, A. B. Burgin and L. Stewart, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 15387–15392.
- F. M. Muggia, I. Dimery and S. G. Arbutck, *Ann. N. Y. Acad. Sci.*, 1996, **803**, 213–223.
- H. Lu, H. X. Lin, Y. Jiang, X. G. Zhou, B. L. Wu and J. M. Chen, *Lett. Drug Des. Discovery*, 2006, **3**, 83–86.
- R. Tong and J. Cheng, *Polym. Rev.*, 2007, **47**, 345–381.
- Q. Yin, R. Tong, Y. X. Xu, K. Baek, L. W. Dobrucki, T. M. Fan and J. J. Cheng, *Biomacromolecules*, 2013, **14**, 920–929.
- R. L. Bhatt, P. de Vries, J. Tulinsky, G. Bellamy, B. Baker, J. W. Singer and P. Klein, *J. Med. Chem.*, 2003, **46**, 190–193.
- M. K. Yu, J. Park and S. Jon, *Theranostics*, 2012, **2**, 3–44.
- Z. G. Me, T. C. Lu, X. S. Chen, Y. H. Zheng and X. B. Jing, *J. Biomed. Mater. Res., Part A*, 2009, **88**, 238–245.
- R. Langer, *Nature*, 1998, **392**, 5–10.
- J. Cheng, K. T. Khin and M. E. Davis, *Mol. Pharmaceutics*, 2004, **1**, 183–193.
- T. Schluep, J. Hwang, J. J. Cheng, J. D. Heidel, D. W. Bartlett, B. Hollister and M. E. Davis, *Clin. Cancer Res.*, 2006, **12**, 1606–1614.
- J. K. Oh, *Soft Matter*, 2011, **7**, 5096–5108.
- X. L. Xu, X. S. Chen, Z. F. Wang and X. B. Jing, *Eur. J. Pharm. Biopharm.*, 2009, **72**, 18–25.
- J. Cheng, B. A. Teply, I. Sherifi, J. Sung, G. Luther, F. X. Gu, E. Levy-Nissenbaum, A. F. Radovic-Moreno, R. Langer and O. C. Farokhzad, *Biomaterials*, 2007, **28**, 869–876.
- D. Bendix, *Polym. Degrad. Stab.*, 1998, **59**, 129–135.
- R. Tong, L. D. Yala, T. M. Fan and J. J. Cheng, *Biomaterials*, 2010, **31**, 3043–3053.
- R. Tong and J. J. Cheng, *Bioconjugate Chem.*, 2010, **21**, 111–121.
- M. L. Bender and B. W. Turnquest, *J. Am. Chem. Soc.*, 1957, **79**, 1656–1662.
- N. Larson and H. Ghandehari, *Chem. Mater.*, 2012, **24**, 840–853.
- R. Tong and J. J. Cheng, *Macromolecules*, 2012, **45**, 2225–2232.
- R. Tong and J. J. Cheng, *Angew. Chem., Int. Ed.*, 2008, **47**, 4830–4834.
- R. Tong and J. J. Cheng, *J. Am. Chem. Soc.*, 2009, **131**, 4744–4754.
- C. Y. Chen, T. H. Kim, W. C. Wu, C. M. Huang, H. Wei, C. W. Mount, Y. Q. Tian, S. H. Jang, S. H. Pun and A. K. Y. Jen, *Biomaterials*, 2013, **34**, 4501–4509.
- Z. Zhao, J. Wang, H. Q. Mao and K. W. Leong, *Adv. Drug Delivery Rev.*, 2003, **55**, 483–499.
- O. C. Farokhzad, J. J. Cheng, B. A. Teply, I. Sherifi, S. Jon, P. W. Kantoff, J. P. Richie and R. Langer, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 6315–6320.
- D. Kim, S. Park, J. H. Lee, Y. Y. Jeong and S. Jon, *J. Am. Chem. Soc.*, 2007, **129**, 7661–7665.
- S. M. Moghimi, A. C. Hunter and J. C. Murray, *Pharmacol. Rev.*, 2001, **53**, 283–318.
- R. S. Burke and S. H. Pun, *Bioconjugate Chem.*, 2008, **19**, 693–704.
- V. Wagner, A. Dullaart, A. K. Bock and A. Zweck, *Nat. Biotechnol.*, 2006, **24**, 1211–1217.
- M. J. Roberts, M. D. Bentley and J. M. Harris, *Adv. Drug Delivery Rev.*, 2002, **54**, 459–476.
- T. Musumeci, L. Vicari, C. A. Ventura, M. Gulisano, R. Pignatello and G. Puglisi, *J. Nanosci. Nanotechnol.*, 2006, **6**, 3118–3125.