33rd Annual International Conference of the IEEE EMBS
Boston, Massachusetts USA, August 30 - September 3, 2011

Drug-Polyester Conjugated Nanoparticles for Cancer Drug Delivery

Rong Tong, Li Tang, Qian Yin and Jianjun Cheng

***Abstract*—We report here the synthesis of camptothecin-polylactide conjugate via camptothecin-initiated lactide polymerization and the formation of nanoconjugates for drug delivery applications.**

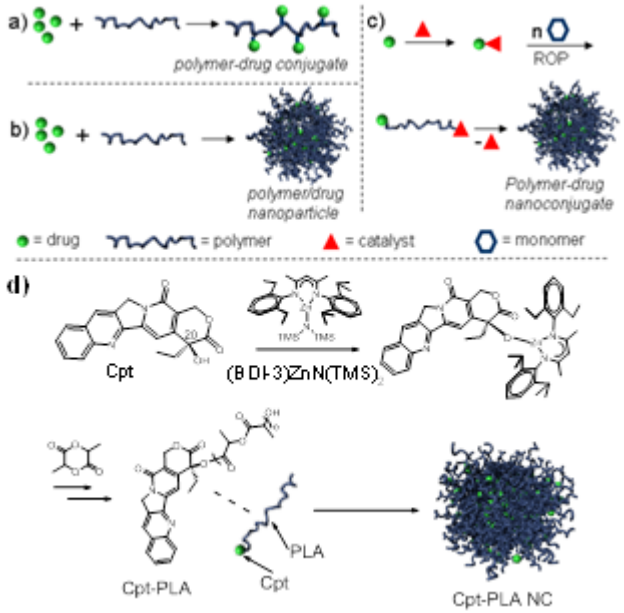
I. Introduction

POLYMERIC nanomedicine, an emerging field that involves the development of polymeric nanostructures for cancer treatment, is expected to alter the landscape of oncology [1]. In current formulation of anticancer polymeric nanostructures, drug molecules are either covalently linked to hydrophilic polymers through conventional coupling chemistry (Scheme 1a) [2] or non-covalently encapsulated into hydrophobic polymeric nanoparticles (NPs) (Scheme 1b) [3]. Several systems derived from these two strategies have been approved for clinical use of cancer therapy [4]. In this communication we report a new nanoparticle formulation method through a camptothecin (Cpt)-initiated highly efficient lactide (LA) ring-opening polymerization (ROP) followed by nanoprecipitation of the resulting Cpt-polylactide (Cpt-PLA) conjugate (Scheme 1d) [5, 6].

ROPs of LA are usually mediated by a metal-alkoxide (RO-M) [7] or by a metal-carboxylate in the presence of a hydroxyl-containing initiator (R'OH) with RO (or R'O) being attached to the terminus of the resulting PLA through an ester linker. This process has been extensively used for incorporation of hydroxyl-containing substrate such as vitamin [8], macromolecule [9], drug [6] and even nanoparticle to PLA. [10] To incorporate Cpt, a complex therapeutic agent containing less active hydroxyl group (Scheme 1d) and lactone, control of catalyst activity is essential to ensure the success of this proposed formulation strategy (Scheme 1c).

II. Results and Discussion

Tin(II) ethylhexanoate [9], a well-known LA polymerization catalyst, was first chosen to study. However, this catalyst gave poorly controlled Cpt incorporation and LA polymerization (data not shown). Luckily, when (BDI)MN(TMS)$_2$ (M = Mg and Zn), a series of catalysts developed by Coates and coworkers [7], were mixed with Cpt to initiate LA polymerizations, excellent control over chemoselective incorporation of Cpt to PLA was observed. When 100 equiv. LA was added to the mixture of (BDI-1)MgN(TMS)$_2$ and Cpt, the polymerization completed within 12 h with 100% Cpt incorporation efficiency. After Cpt-LA$_{100}$, the Cpt-PLA conjugate prepared at a LA/Doxo ratio of 100, was treated with 0.1M NaOH, Cpt in its original form was observed. The present study demonstrated for the first time that the C20-OH of Cpt, a tertiary hydroxyl group, could also be activated by (BDI-1)MgN(TMS)$_2$ to initiate LA polymerization. (Scheme 1d).



Scheme 1. Illustration of (a) polymer-drug conjugate, (b) polymer/drug nanoparticle, and (c) polymer-drug nanoconjugate via drug-initiated ROP followed by nanoprecipitation. (d) Preparation of Cpt-PLA conjugate through Cpt/(BDI-3)ZnN(TMS)$_2$ initiated LA ROP.

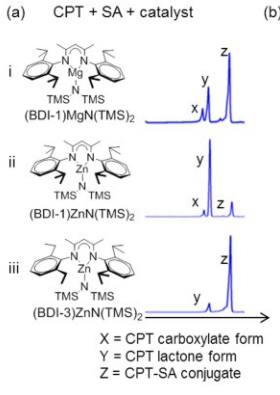
In order to achieve better controlled polymerization, we next tested (BDI-1)ZnN(TMS)$_2$, a Zn analogue of (BDI-1)MgN(TMS)$_2$. As reported by Coates and coworkers, Zn catalysts in general outperform Mg catalysts for LA polymerization [7]. This (BDI-1)ZnN(TMS)$_2$/Cpt complex mediated LA polymerization at a M/I ratio of 100 and gave Cpt-PLA conjugate with a very narrow MWD (M_w/M_n = 1.07). However, the Cpt incorporation efficiency was only 61% based on the HPLC analysis, indicating the poor efficiency of forming Zn-Cpt complex during the initiation step. The actual M/I ratio in this reaction was 163 (calculated based on the LA/Cpt ratio of 100:0.61). It was therefore not surprising that the obtained M_n (2.83×10^4 g/mol) was substantially higher than the M_n calculated based on the LA/Cpt ratio of 100:1

This work was by the National Science Foundation (Career Program DMR-0748834).

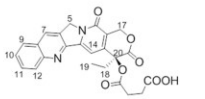
R. Tong is with the Massachusetts Institute of Technology, Cambridge, MA 02138 USA (Phone: 217-417-8722, e-mail: rtong@mit.edu). Li Tang, Qian Yin and Jianjun Cheng are with the University of Illinois at Urbana-Champaign (217-244-3924; e-mail: jianjunc@illinois.edu).

(expected $M_n = 1.47 \times 10^4$ g/mol). The poor efficiency for the coordination of Cpt with (BDI-1)ZnN(TMS)$_2$ was due in part to the relatively low activity of Zn as compared to Mg as well as the steric bulk of BDI-1 ligand surrounding the coordination site. We next studied whether enhanced incorporation efficiency of Cpt could be achieved by using a Zn catalyst with a BDI ligand with reduced steric bulk and/or altered electronic property.

We synthesized a series of zinc catalysts containing BDI ligands with variable 2, 6-aryl substituents and then used these catalysts in Cpt-initiated LA polymerizations. BDI-3 is an analogue of BDI-1 whose *i*Pr groups were replaced by ethyl groups only at both R^1 position. As expected, the steric bulk of the BDI at its R^1 and R^2 positions had profound effect on the capability of the Zn catalysts to form coordination complexes with Cpt during the initiation step. It was observed 100% incorporation efficiencies in (BDI-3)ZnN(TMS)$_2$/Cpt mediated polymerizations, with narrow MWDs ($M_w/M_n < 1.2$). The corresponding M_n's (1.73×10^4 g/mol) were much closer to the expected M_n than that of the PLA-Cpt obtained from the (BDI-1)ZnN(TMS)$_2$/Cpt mediated LA polymerization. Excellently controlled polymerizations were observed over a broad range of LA/Cpt ratios from 75 to 400 when the LA polymerizations were mediated by (BDI-3)ZnN(TMS)$_2$/Cpt . Quantitative Cpt incorporation efficiencies and very narrow MWDs (M_w/M_n = 1.02-1.18) were observed in all experiments performed. The obtained MWs of the Cpt-PLA conjugates were in excellent agreement with the expected MWs, which followed a linear correlation with the LA/Cpt ratios. Monomodal GPC MW distribution curves were observed in all Cpt-PLA conjugates prepared with various LA/Cpt ratios. The well-controlled polymerization mediated by (BDI-3)ZnN(TMS)$_2$ likely proceeded through the conventional insertion-coordination mechanism.



(b)



	CPT-SA (δ, ppm)	CPT (δ, ppm)
7-H	8.63	8.64
12-H	8.21	8.21
9H	8.07	8.07
11-H	7.87	7.87
10-H	7.71	7.72
14-H	7.43	7.46
17-H	5.57, 5.44	5.58, 5.41
5-H	5.35	5.36
-C*H*$_2$-COOH	2.81	
-OC(O)C*H*$_2$-	2.55	
18-H	2.23	1.97
19-H	0.99	1.01

Fig. 1. (a) HPLC analysis of the reaction of SA and Cpt in the presence of (i) (BDI-1)MgN(TMS)$_2$, (ii) (BDI-1)ZnN(TMS)$_2$, (iii) (BDI-3)ZnN(TMS)$_2$; (b) The chemical shift values of Cpt and Cpt-SA derived from the corresponding ^{1}H-NMR spectra

In the polymerization, Cpt does not involve the subsequent chain propagation. Apparently, the most critical step determining whether the lactone ring of Cpt is opened or remains closed is the initiation step. As LA is subject to rapid polymerization and the resulting Cpt-PLA conjugate is difficult to be precisely characterized, we used succinic anhydride (SA) as the model monomer to study (BDI)ZnN(TMS)$_2$/Cpt-mediated initiation. Such a reaction led to the formation of Cpt-succinic acid (Cpt-SA), a small molecule instead of a polymer, whose structure can be easily determined by routine characterization methods. (BDI-1)MgN(TMS)$_2$-mediated conjugation of Cpt and SA resulted in Cpt-SA conjugates with 59.5% yield when the reaction was carried out at 40^{o}C for 4 h. Cpt in carboxylate form was also detected (peak x, Figure 1a-i), indicating the Cpt lactone ring was opened in the presence of (BDI-1)MgN(TMS)$_2$ and that it may function not only as a ROP catalyst but also as a strong base. When such a reaction was mediated by (BDI-1)ZnN(TMS)$_2$, a weaker base and a less reactive ROP catalyst compared to its Mg analogue, Cpt-SA in 18.8% yield was obtained. Formation of small amount of Cpt in its carboxylate form was also detected (peak x, Figure 1a-ii). Cpt-SA in 89.1% yield was obtained in the presence of (BDI-3)ZnN(TMS)$_2$. The substantially increased yield of Cpt-SA in this experiment indicated that (BDI-3)ZnN(TMS)$_2$ activated the tertiary C20-OH group of Cpt very effectively to allow for facile nucleophilic ring opening of SA. Very interestingly, Cpt-SA was the only product formed; no carboxylate form of Cpt was detected (Figure 1a-iii). The high yield of Cpt-SA obtained in this experiment indicated that (BDI-3)ZnN(TMS)$_2$ was capable of efficiently activating the C20-OH of Cpt to facilitate the ring-opening reaction with SA. The Cpt/SA ring-opening conjugation mediated by BDI-Zn catalyst appeared to be a versatile method for converting the C20-hydroxyl group of Cpt to a carboxylic acid end group with a degradable ester linker, which can be further used for conjugation to various drug delivery vehicles through the conventional carboxylate-amine coupling chemistry.

Table 1. Preparation and Characterization of CPT-PLA NCs.[a]

NC	[LA]/[CPT]	LD (%)	CV (%)	IE (%)	NC sizes ± SD (nm)	PD ± SD
CPT-LA$_{100}$	100	2.36	>99	>99	80.6 ± 0.8	0.083 ± 0.016
CPT-LA$_{50}$	50	4.61	>99	>99	70.7 ± 0.7	0.096 ± 0.007
CPT-LA$_{25}$	25	8.82	>99	>99	76.6 ± 0.9	0.092 ± 0.006
CPT-LA$_{10}$	10	19.48	>99	>99	72.5 ± 0.7	0.056 ± 0.015

[a] Abbreviations: NC = nanoconjugates; LD = Cpt loading in wt%; CV = conversion of LA; IE = incorporation efficiency; PD = polydispersity of NCs; STD = standard deviation.

Cpt-PLA conjugated nanoparticles, or called nanoconjugates (NCs), were readily prepared through the nanoprecipitation of PLA-Cpt conjugates (Scheme 1d). Sub-100 nm NCs with narrow, monomodal particle distribution were obtained in all cases (Table 1). This narrow size distribution is in contrast to the multimodal particle distributions frequently observed in NPs prepared by co-precipitation of polymers and drugs [12, 13]. As the multimodal particle distributions were attributed in part to the self-aggregation of non-encapsulated drugs [12], nanoprecipitation of unimolecular structured polymer-drug conjugates has clear advantage for eliminating particle heterogeneity.

Because both monomer conversions and drug incorporation were quantitative (Table 1), Cpt loadings in Cpt-PLA NCs could thus be precisely pre-determined by adjusting LA/Cpt feeding ratios. At a low M/I ratio of 10, drug loading could be as high as 19.4% (Cpt-LA_{10}, Table 1). To our knowledge, this is by far the highest loading ever reported in Cpt-containing polymeric NPs. Even at this high drug loading, sustained release of Cpt from Cpt-LA_{10} NC was observed through the hydrolysis of the ester linker connecting the Cpt and the PLA. No burst release of Cpt was observed in Cpt-LA_{10}, which was in sharp contrast to the burst release of PLA/Cpt NP prepared by co-precipitation. The release rates correlate to drug loadings; therefore toxicities of NCs can be tuned by controlling drug loadings.

III. Conclusion

Preparation of Cpt-PLA conjugates with controlled loading and release profiles have been previously reported using conventional coupling chemistry [14]. Our method allows for facile incorporation of Cpt to PLA and forms PLA-Cpt conjugates with low polydispersities, pre-determined drug loadings (as high as 20%) and 100% loading efficiencies. By controlling the metal and the chelating ligands of the catalysts, the initiation of LA polymerization can be specifically controlled at the C20-OH of Cpt with negligible lactone ring opening in Cpt. Cpt conjugated to PLA should maintain its lactone form (the therapeutically active form). This method has been extended to the formation of PLA NCs with several other hydroxyl-containing therapeutic agents, which demonstrates its potentially widespread utility for the formulation of polymer nanoparticles for drug delivery applications.

IV. Experimental

(BDI-3)ZnN$(TMS)_2$ (5.5 mg, 8.9 μmol) was dissolved in anhydrous THF (300 μL). The solution was added to a vial containing Cpt (3.0 mg, 8.6 mmol) and the mixture was stirred for 15 min until the Cpt was completely dissolved in THF. LA (124 mg, 0.86 mmol) was dissolved in a vial containing THF (940 μL) and the resulting solution was added to the mixture of Cpt/(BDI-3)ZnN$(TMS)_2$ ([LA]0 = 0.69 M). FT-IR was used to follow the conversion of the LA in the polymerization solution by monitoring the intensity of the lactone band at 1772 cm-1. After the LA was completely consumed, the polymerization was quenched with ice-cold methanol (10 mL). The precipitate (Cpt-PLA) was collected by centrifugation and then dried under vacuum. The resulting Cpt-PLA conjugate was denoted as Cpt-LAn where n is the monomer/initiator (LA/Cpt) molar ratio. For particle preparation, A DMF solution containing the Cpt-LA10 conjugate (100 μL, 5 mg/mL) was added dropwise to nanopure water (4 mL). The resulting Cpt-LA10 NCs were collected by ultrafiltration (15 min, 3000 × g, Ultracel membrane with 10,000 NMWL, Millipore, Billerica, MA) and were characterized by DLS and SEM for particle sizes and by HPLC for drug loading and release kinetics

References

[1] R. Tong and J. Cheng, "Anticancer polymeric nanomedicines," *Polymer Reviews,* vol. 47, pp. 345-381, 2007.

[2] R. Duncan, "Polymer conjugates as anticancer nanomedicines," *Nature Reviews Cancer,* vol. 6, pp. 688-701, Sep 2006.

[3] K. S. Soppimath, T. M. Aminabhavi, A. R. Kulkarni, and W. E. Rudzinski, "Biodegradable polymeric nanoparticles as drug delivery devices," *Journal of Controlled Release,* vol. 70, pp. 1-20, Jan 29 2001.

[4] V. Wagner, A. Dullaart, A. K. Bock, and A. Zweck, "The emerging nanomedicine landscape," *Nature Biotechnology,* vol. 24, pp. 1211-1217, Oct 2006.

[5] Tong, R.; Cheng, J. J., "Controlled Synthesis of Camptothecin-Polylactide Conjugates and Nanoconjugates." *Bioconjugate Chemistry*, vol. 21, pp 111-121 , Jan 2010

[6] R. Tong and J. Cheng, "Paclitaxel-initiated, controlled polymerization of lactide for the formulation of polymeric nanoparticulate delivery vehicles," *Angewandte Chemie-International Edition,* vol. 47, pp. 4830-4834, 2008.

[7] B. M. Chamberlain, M. Cheng, D. R. Moore, T. M. Ovitt, E. B. Lobkovsky, and G. W. Coates, "Polymerization of lactide with zinc and magnesium beta-diiminate complexes: Stereocontrol and mechanism," *Journal of the American Chemical Society,* vol. 123, pp. 3229-3238, APR 11 2001.

[8] Z. P. Zhang and S. S. Feng, "Nanoparticles of poly(lactide)/vitamin E TPGS copolymer for cancer chemotherapy: Synthesis, formulation, characterization and in vitro drug release," *Biomaterials,* vol. 27, pp. 262-270, Jan 2006.

[9] Y. Hu, X. Q. Jiang, Y. Ding, L. Y. Zhang, C. Z. Yang, J. F. Zhang, J. N. Chen, and Y. H. Yang, "Preparation and drug release behaviors of nimodipine-loaded poly(caprolactone)-poly(ethylene oxide)-polylactide amphiphilic copolymer nanoparticles," *Biomaterials,* vol. 24, pp. 2395-2404, Jun 2003.

[10] F. H. Chen, Q. Gao, G. Y. Hong, and J. Z. Ni, "Synthesis of magnetite core-shell nanoparticles by surface-initiated ring-opening polymerization of L-lactide," *Journal of Magnetism and Magnetic Materials,* vol. 320, pp. 1921-1927, Jul 2008.

[11] M. H. Chisholm, J. Gallucci, and K. Phomphrai, "Coordination chemistry and reactivity of monomeric alkoxides and amides of magnesium and zinc supported by the diiminato ligand CH(CMeNC6H3-2,6-Pr-i(2))(2). A comparative study," *Inorganic Chemistry,* vol. 41, pp. 2785-2794, May 2002.

[12] 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, "Formulation of functionalized PLGA-PEG nanoparticles for in vivo targeted drug delivery," *Biomaterials,* vol. 28, pp. 869-876, Feb 2007.

[13] O. C. Farokhzad, J. Cheng, B. A. Teply, I. Sherifi, S. Jon, P. W. Kantoff, J. P. Richie, and R. Langer, "Targeted nanoparticle-aptamer bioconjugates for cancer chemotherapy in vivo," *Proceedings of the National Academy of Sciences of the United States of America,* vol. 103, pp. 6315-6320, Apr 18 2006.

[14] H. S. Yoo, K. H. Lee, J. E. Oh, and T. G. Park, "In vitro and in vivo anti-tumor activities of nanoparticles based on doxorubicin-PLGA conjugates," *Journal of Controlled Release,* vol. 68, pp. 419-431, Sep 2000.