Controlled Formulation of Doxorubicin-Polylactide Nanoconjugates for Cancer Drug Delivery

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Abstract—We report here the synthesis of doxorubicin-polylactide conjugate via doxorubicin-initiated lactide polymerization.

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 doxorubicin (Doxo)-initiated, chemo- and regionselective lactide (LA) ring-opening polymerization (ROP) followed by nanoprecipitation of the resulting Doxo-polylactide (Doxo-PLA) conjugate (Scheme 1c) [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 Doxo, a complex therapeutic agent containing multiple functional groups (Scheme 1d), control of chemoselectivity 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 Doxo 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 Doxo to initiate LA polymerizations, excellent control over chemoselective incorporation of Doxo to PLA was observed. When 100 equiv. LA was added to the mixture of $(BDI)MgN(TMS)_2$ and Doxo, the polymerization completed within 12 h with 100% Doxo incorporation efficiency. After $Doxo-LA_{100}$, the Doxo-PLA conjugate prepared at a LA/Doxo ratio of 100, was treated with 0.1M NaOH, nearly quantitative recovery of Doxo in its original form was observed. This study demonstrated that Doxo molecules are linked to PLA exclusively through ester linkers that are subject to base-induced rapid hydrolysis (Scheme 1d).
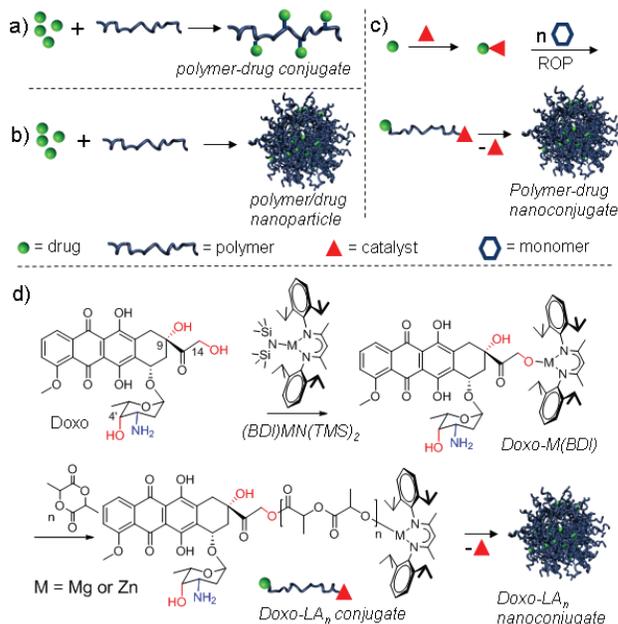
Scheme 1. Illustration of (a) polymer-drug conjugate, (b) polymer/drug nanoparticle, and (c) polymer-drug nanoconjugate via drug-initiated ROP followed by nanoprecipitation. (d) Preparation of Doxo-PLA conjugate through $Doxo/(BDI)MN(TMS)_2$ (M = Mg or Zn)-initiated LA ROP.

Formation of amide bonds is usually favored in conventional carboxylate coupling when both amine and hydroxyl groups are present. Interestingly, the unprotected amine group of Doxo did not interfere with this Mg-Doxo initiated polymerization and remained intact in the resulting conjugates. To verify the observed chemoselectivity, we used 1-pyrenemethanol (Pyr-OH) and 1-pyrenemethylamine

(Pyr-NH$_2$) as the corresponding model hydroxyl and amine initiator. An equal molar mixture of Pyr-NH$_2$ and Pyr-OH with 1 equiv. (BDI)MgN(TMS)$_2$ initiated a LA polymerization exclusively through Pyr-OH, whereas Pyr-NH$_2$ remained intact in the polymerization solution.

Doxo/(BDI)MgN(TMS)$_2$-mediated LA polymerizations gave Doxo-PLAs with well-controlled molecular weights (MWs). The obtained M_n of Doxo-LA$_{200}$ (3.12 × 10^4 g/mol) was in good agreement with the expected M_n (2.93 × 10^4 g/mol). Nonetheless, a broad MW distribution (MWD) (M_w/M_n = 1.50) was observed, which is attributed presumably to the slow initiation relative to chain propagation as well as to potential trans-esterification reactions [6, 7]. It is known that Zn-alkoxide undergoes slightly slower but better controlled living polymerization compared with their Mg analogue [7, 11]. When (BDI)ZnN(TMS)$_2$ was used in a similar polymerization, the resulting Doxo-LA$_{200}$ had the expected M_n (3.01 × 10^4 g/mol) with significantly reduced MWD (M_w/M_n = 1.15). The MWs of Doxo-PLA conjugates could be readily controlled by adjusting the LA/Doxo feeding ratio. Covalent attachment of Doxo to the resulting Doxo-PLA was confirmed by end group analysis of the Doxo-PLA using MALDI-TOF MS.
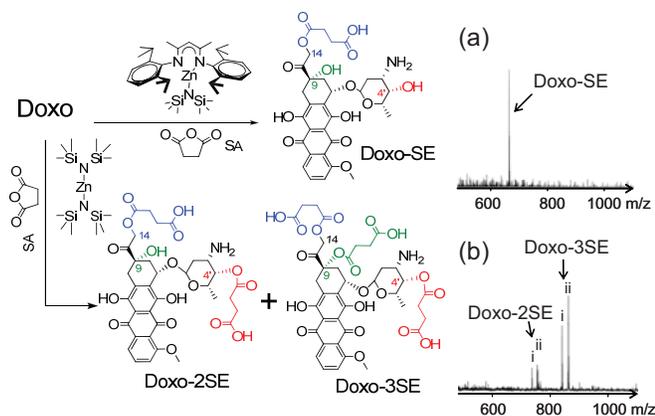
Fig. 1. MS analysis of the mixture of (a) Doxo, 1 eq. (BDI)ZnN(TMS)$_2$ and 3 eq. SA, and (b) Doxo, 1 eq. Zn(N(TMS)$_2$)$_2$ and 3 eq. SA. Peak assignment: (a) Doxo-SE: (M+H) 644.3; (b) Doxo-2SE: (i = (M+H) 742.3, ii = (M+NH$_4$) 759.7; Doxo-3SE: (i = (M+H) 842.3, ii = (M+Na) 863.3)

Because of the steric bulk of BDI, Doxo likely forms alkoxide complex with the BDI-metal catalyst more readily through its less sterically hindered 14-OH group than through the more sterically hindered 4'-OH or 9-OH groups to facilitate regioselective initiation and polymerization. To prove this regioselectivity, we mixed Doxo/(BDI)ZnN(TMS)$_2$ with succinic anhydride (SA) to mimic the initiation step of Doxo/(BDI)ZnN(TMS)$_2$-mediated LA polymerization, and characterized the reaction mixture by MS (Figure 1a) and NMR. As expected, Doxo-14-succinic ester (Doxo-SE) was the predominate product (Figure 1a). When (BDI)ZnN(TMS)$_2$ was replaced by Zn(N(TMS)$_2$)$_2$, the regionselectivity disappeared and Doxo-4',14-bissuccinic ester (Doxo-2SE) and Doxo-4',9,14-trisuccinic ester (Doxo-3SE) became the predominant

products (Figure 1b). These and additional studies demonstrated that both ligand and metal catalyst have dramatic effect on regioselectivity during initiation and on polymer MWs.

Table 1. A Mixture of Doxo and (BDI)ZnN(TMS)$_2$ Initiated LA Polymerization and NC Formation[a]

M/I	LD(%)	CV(%)	IE(%)	NC[b]	NC size ± SD (nm)	PD ± SD
100	3.6	>99	>99	Doxo-LA$_{100}$	90.6 ± 0.6	0.08 ± 0.01
50	7.1	>99	>99	Doxo-LA$_{50}$	76.0 ± 1.1	0.09 ± 0.01
25	13.1	>99	96	Doxo-LA$_{25}$	76.1 ± 8.1	0.16 ± 0.02
10[c]	27.4	>99	94	Doxo-LA$_{10}$	125.2 ± 2.3	0.11 ± 0.01

[a]Abbreviations: M/I = LA/Doxo ratio; LD = Doxo loading in wt%; CV = conversion of LA; IE = incorporation efficiency of Doxo; SD = standard deviation; PD = polydispersity of NC. [b]NCs are denoted by Doxo-LA$_{M/I ratio}$. [c]Prepared with (BDI)MgN(TMS)$_2$

Doxo-PLA conjugated nanoparticles, or called nanoconjugates (NCs), were readily prepared through the nanoprecipitation of PLA-Doxo 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), Doxo loadings in Doxo-PLA NCs could thus be precisely pre-determined by adjusting LA/Doxo feeding ratios. At a low M/I ratio of 10, drug loading could be as high as 27.4% (Doxo-LA$_{10}$, Table 1). To our knowledge, this is by far the highest loading ever reported in Doxo-containing polymeric NPs. Even at this high drug loading, sustained release of Doxo from Doxo-LA$_{10}$ NC was observed through the hydrolysis of the ester linker connecting the Doxo and the PLA. No burst release of Doxo was observed in Doxo-LA$_{10}$, which was in sharp contrast to the burst release of PLA/Doxo 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 Doxo-PLA conjugates with controlled loading and release profiles have been previously reported using conventional coupling chemistry [14]. Here we report a different conjugation method by taking advantage of Doxo-initiated, chemo- and regioselective ROPs for facile formation of PLA-Doxo conjugates with pre-determined drug loadings and quantitative loading efficiencies. The metal catalysts do not have deleterious effects on Doxo. 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

In a glove box, Doxo (5.5 mg, 0.01mmol) was dissolved in anhydrous THF (1 mL). (BDI)MgN(TMS)$_2$ (18.1 mg, 0.03 mmol) was added to the Doxo solution. The mixture was stirred for 15-20 min at room temperature. LA (144 mg, 1.0 mmol) in DMF (1 mL) was added dropwise to the vigorously stirred mixture of Doxo and (BDI)MgN(TMS)$_2$. The polymerization was monitored by following the lactone band at 1772 cm^{-1} using FTIR or by checking the methine (-CH-) peak of LA around 5.2-5.0 ppm using ^{1}H-NMR. After the polymerization was complete (usually within 90 min), an aliquot of the polymerization solution was injected to HPLC to quantify the unreacted Doxo in order to determine the incorporation efficiency of Doxo to the Doxo-PLA conjugate. One drop of water was added to the polymerization solution to hydrolyze the Mg-Doxo alkoxide and to terminate the polymerization. The resulting Doxo-LA$_{100}$ was precipitated with ethyl ether (10 mL), washed with ether and methanol/acetic acid (v/v = 100/1, 10 mL) to remove BDI ligand, and dried under vacuum. Complete removal of BDI from Doxo-LA$_{100}$ conjugate was verified by NMR. A Doxo-LA$_{100}$ conjugate in DMF (100 µL, 10 mg/mL) was dropwise added to nanopure water (2 mL). The resulting Doxo-LA$_{100}$ NC was collected by ultrafiltration (15 min, 3000 × g, Ultracel membrane with 10,000 NMWL, Millipore, Billerica, MA) and used for characterization of size, drug loading, release kinetics, etc.

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] R. Tong and J. Cheng, "Ring-Opening Polymerization Mediated-Controlled Formulation of Polylactide-Doxorubicin Nanoconjugates," *Journal of the American Chemical Society,* vol. 131, pp. 4744-4754, 2009.

[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.