Drug Delivery

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Paclitaxel-Initiated, Controlled Polymerization of Lactide for the Formulation of Polymeric Nanoparticulate Delivery Vehicles**

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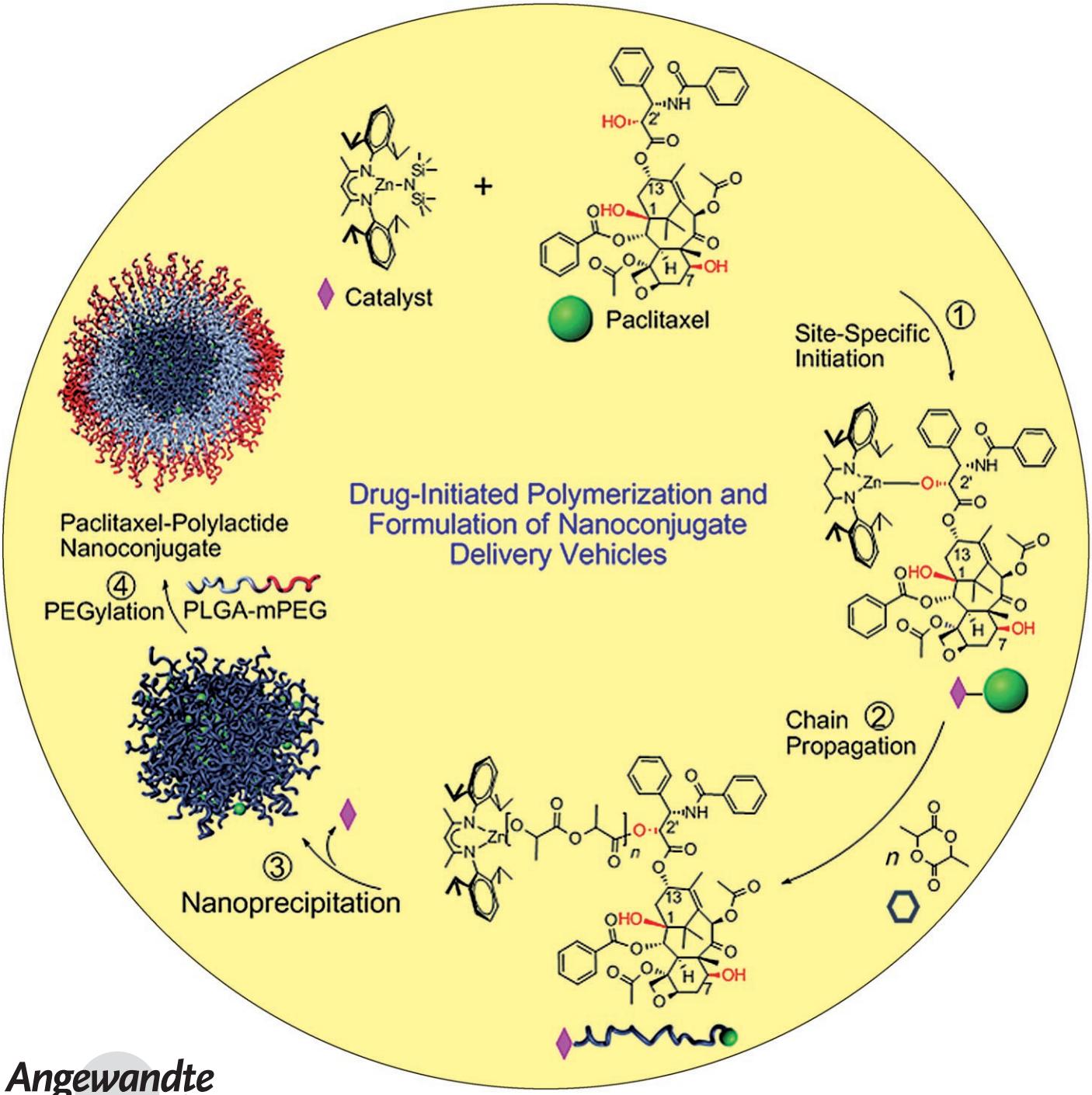

Paclitaxel (Ptxl) is a potent chemotherapeutic agent. However, clinical application of Ptxl is often accompanied by severe, undesirable side effects.[1] To reduce the side effects, various nanoparticulate delivery vehicles have been developed and investigated in the past decade.[2–5] Of the various nanoparticles (NPs) being studied, polymeric nanoencapsulates (NEs), NPs prepared by coprecipitating hydrophobic polymers and drugs, hold particular promise because of their ease of formulation and the potential control of drug release through the degradation of polymers.[6,7] However, current NEs typically have low drug loadings, uncontrolled encapsulation efficiencies, and significant drug burst release effects when used in vivo.[8–11] These formulation challenges significantly limit their potential clinical applications. Here, we report the use of living polymerization to facilitate the controlled preparation of Ptxl–polylactide(PLA)-conjugated NPs with predefined drug loadings, nearly quantitative loading efficiencies, and controlled release kinetics without burst release effects.

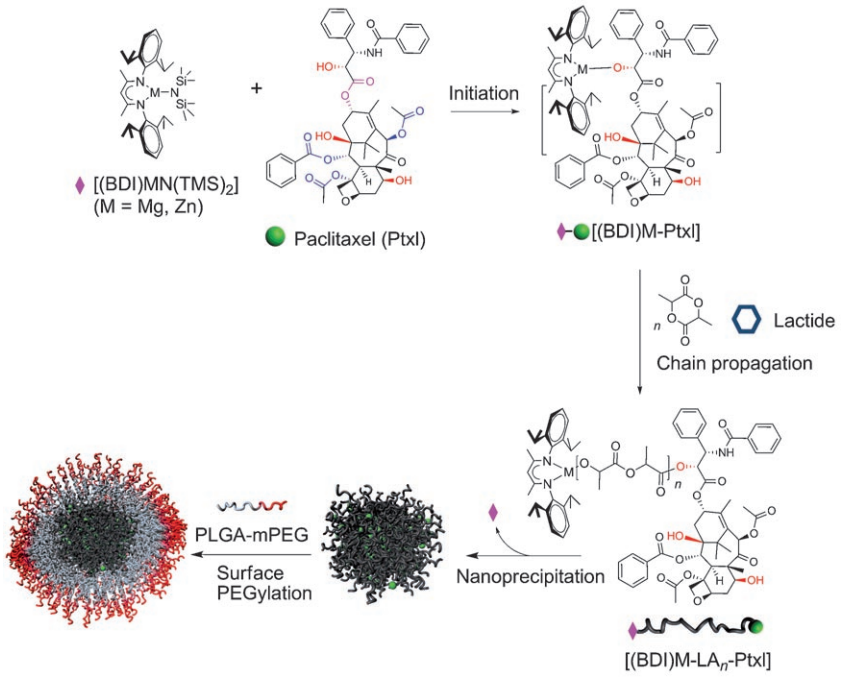

Figure 1. Preparation of poly(ethyleneglycol)ated (PEGylated) Ptxl–PLA NCs by means of Ptxl-initiated LA polymerization in the presence of [(BDI)MN(TMS)$_2$] (M = Mg, Zn), followed by nanoprecipitation and noncovalent surface modification with poly(glycolide-co-lactide)-*b*-methoxylated PEG (PLGA-mPEG) (PLGA-mPEG).

Metal alkoxides (MORs) are well-known initiators for the living polymerizations of cyclic esters, such as DL-lactide (LA) used in this study (Figure 1).[12] They can be prepared in situ by mixing a hydroxy-group-containing compound with an active metal complex, such as a metal–amido complex.[13] If well designed, the MORs formed in situ can initiate controlled polymerization of LA, resulting in quantitative incorporation of the alkoxide (OR) to the PLA terminals with 100% monomer conversion.[12] Since Ptxl has multiple hydroxy groups, we postulated that it may be incorporated into polyesters through the metal–Ptxl-mediated polymerization of LA (Figure 1). Drug loadings can thus be precisely controlled by adjusting the LA/Ptxl ratio. The incorporation efficiency of Ptxl into the resulting PLA should be 100% as the formation of the metal complex is usually quantitative. After polymerization, Ptxl molecules are covalently linked to the terminals of PLA through a hydrolyzable ester linker and are subject to sustained release upon hydrolysis. Followed by nanoprecipitation (Figure 1), polymeric NPs containing covalently linked Ptxl should be readily obtained.

To demonstrate this concept, we utilized [(BDI)MgN(TMS)$_2$] (BDI = 2-((2,6-diisopropylphenyl)amino)-4-((2,6-diisopropylphenyl)imino)-2-pentene, TMS = trimethylsilyl) (Figure 1), an active catalyst developed by Coates and coworkers for the polymerization of LA.[13] After Ptxl was mixed with 1 equiv of [(BDI)MgN(TMS)$_2$], the (BDI)Mg–Ptxl complex formed in situ (structure uncharacterized; tentatively illustrated as a monomeric Mg–Ptxl complex in Figure 1) initiated and completed the polymerization of LA within hours at room temperature; the resulting PLA had nearly quantitative incorporation of Ptxl (entries 1–4, Table 1). The incorporated Ptxl was released in its original form along with degradation species after the Ptxl–PLA was treated with 1M NaOH (see Figure 1 in the Supplementary Information), which demonstrated that Ptxl was linked to PLA through an ester bond. Nanoprecipitation of the Ptxl–PLA conjugates resulted in NPs less than 100 nm in diameter (Table 1). To differentiate these from NEs, these NPs derived from nanoprecipitation of Ptxl–PLA conjugates are called nanoconjugates (NCs); PLA is denoted as LA$_n$ where n is derived from the LA/Ptxl ratio.

NEs prepared from nanoprecipitation are usually polydisperse with multimodal distributions.[7,10] Interestingly, NCs with monomodal particle distributions and low polydisper-

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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

Table 1: Formation of drug–PLA nanoconjugates with high loadings, high incorporation efficiencies, small particle sizes, and low particle distributions.[a]

Entry	NC[b]	M/I	Loading [wt%]	LA conv. [%][c]	Incorp. eff. [%][d]	NC size [nm]	PDI
1	Ptxl–LA_{100}	100	5.6	>99	>99	95.1 ± 2.7	0.04 ± 0.01
2	Ptxl–LA_{50}	50	10.6	>99	>99	80.6 ± 0.2	0.05 ± 0.01
3	Ptxl–LA_{25}	25	19.2	>99	97	55.6 ± 0.5	0.04 ± 0.01
4	Ptxl–LA_{15}	15	28.3	>99	95	85.5 ± 1.4	0.09 ± 0.03
5	Dtxl–LA_{10}	10	35.9	>99	95	77.9 ± 1.5	0.06 ± 0.02
6	CPT–LA_{10}	10	19.5	>99	96	72.5 ± 0.7	0.06 ± 0.02

[a] Abbreviations: M/I = monomer/initiator ratio, NC = nanoconjugates, LA conv. = lactide conversion, Incorp. eff. = incorporation efficiency, PDI = polydispersity derived from particle sizing using dynamic light-scattering, Ptxl = paclitaxel, Dtxl = docetaxel, CPT = camptothecin. [b] NCs are named as drug–$LA_{M/I}$. [c] Determined by analyzing the unreacted lactide using FTIR (band at 1771 cm^{-1}) or using ^{1}H NMR spectroscopy; [d] Based on reversed-phase HPLC analysis of unincorporated drug.

sities (entries 1–4, Table 1), exemplified by the Ptxl–LA_{25} NC (Figure 2a), were consistently obtained through the nanoprecipitation of Ptxl–PLA conjugates. As the multimodal distribution of NEs is due in part to the aggregation of the non-encapsulated free drug,[10] the monomodal distribution observed with NCs is likely related to the unimolecular structures of Ptxl–PLA conjugates.

Both the solvent and the concentration of the polymer have dramatic effects on the size of the NPs prepared by nanoprecipitation. At a fixed concentration of the Ptxl–PLA conjugate, the size of the NCs prepared by precipitating a solution of the Ptxl–PLA conjugate in DMF is typically in a range of 60–100 nm; these particles are 20–30 nm smaller than those prepared with acetone or THF as solvent (data not shown).[10] When the nanoprecipitation was carried out with DMF as the solvent and a DMF/water ratio of 1:20 (v/v), the size of Ptxl–LA_{200} NCs showed a linear correlation with the concentration of Ptxl–LA_{200} conjugate and can be precisely tuned from 60 nm to 100 nm by changing the concentration of Ptxl–LA_{200} (see Figure 2 in the Supporting Information).

Drug burst release is a long-standing formulation challenge of NEs and leads to undesirable side effects and reduced therapeutic efficacy.[11] Conventional NEs typically "burst release" 60–90% of their payloads within a few to tens of hours because the release of drug is controlled solely by diffusion.[14] Since the Ptxl release kinetics of Ptxl–PLA NCs is determined not only by diffusion but also by the hydrolysis of the Ptxl–PLA ester linker, the release of Ptxl from NCs is more controllable and with a significantly reduced burst release effect (Figure 2b). The amount of Ptxl released from Ptxl–LA_{50} (10.6 wt%) and Ptxl–LA_{25} (19.2 wt%) was 7.0% and 8.7% at day 1, and 43% and 70.4% at day 6, respectively. In comparison, 82% of Ptxl was released within 24 h from Ptxl–PLA NE (Figure 2b). The release of Ptxl from Ptxl–LA_{50} NC was slower than that from Ptxl–LA_{25} NC, presumably because of the higher molecular weight of Ptxl–LA_{50} and more compact aggregation in the particle.

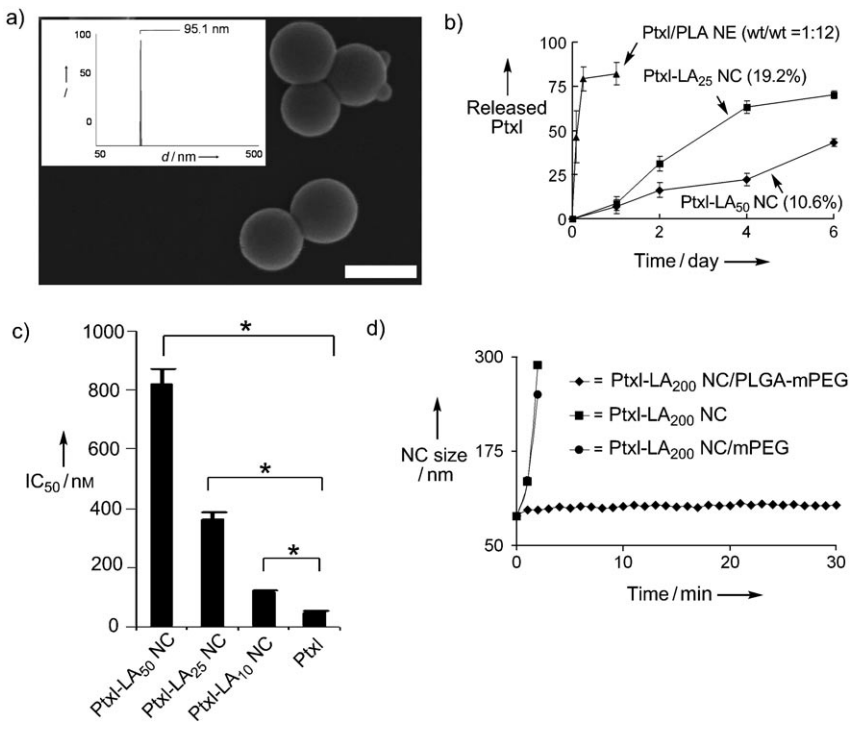

Figure 2. Characterization and properties of Ptxl–PLA NCs. a) Ptxl–LA_{25} (paclitaxel–PLA NC prepared at a LA/Ptxl ratio of 25:1) analyzed by scanning electron microscopy (SEM) and dynamic light scattering (DLS, inset). Scale bar = 150 nm. b) Release kinetics of Ptxl from Ptxl–PLA NCs and Ptxl–PLA NE (prepared by nanoprecipitating a mixture of Ptxl and PLA (Ptxl/PLA (wt/wt) = 1:12) at 37°C in 1×PBS. c) Toxicity evaluation of Ptxl–LA_{50} NC, Ptxl–LA_{25} NC, Ptxl–LA_{10} NC, and Ptxl using MTT assay in PC-3 cells after incubation for 24 h. Significance at 95% confidence interval is marked with an asterisk (*). d) Stability of Ptxl–LA_{200} NC in PBS at 37°C before and after being treated with PLGA–$mPEG_{5k}$ or $mPEG_{5k}$.

The in vitro toxicities of NCs are correlated to the amount of Ptxl released; they show strong correlation with drug loadings (Figure 2c). The IC_{50} values of Ptxl–LA_{15}, Ptxl–LA_{25} and Ptxl–LA_{50} NCs with similar sizes ($\approx$100 nm), which were determined by MTT (MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assays in PC-3 cells, are 111, 370, and 855 nM, respectively. The IC_{50} value of Ptxl–LA_{15} NC is nearly identical to that of free Ptxl (87 nM), while the IC_{50} value of the Ptxl–LA_{50} NC is an order of magnitude higher. As a result, the toxicity of the NCs can be tuned in a wide range simply by controlling NC drug loading.

Surface modification of NPs with poly(ethylene glycol) (PEG) is widely used for prolonged systemic circulation and reduced aggregation of NPs in blood.[15] To reduce the efforts of removing unreacted reagents and by-products, we attempted to use a noncovalent approach to PEGylate the NC surface instead of covalently conjugating PEG to the NCs.[5,7] We used poly(glycolide-co-

lactide)-*b*-methoxylated PEG (PLGA-mPEG), an amphiphilic copolymer that has a 13 kDa PLGA and a 5 kDa PEG segment,[16] to PEGylate the NCs. It is expected that the PLGA block forms strong hydrophobic interactions with the NC to create a stable PEG shell (Figure 1). A similar approach has been used previously for the surface PEGylation of NPs.[17] Sequential addition of 0.4 to 2 equiv (in mass) of PLGA-mPEG to Ptxl–LA_{200} resulted in a linear increase in particle size from 54.5 nm to 100.3 nm (see Figure 3 in the Supporting Information).

Ptxl–LA_n NCs have negative surface zeta potential and thus remain nonaggregated in water as a result of surface charge repulsion. However, aggregation of NCs occurred immediately after they were added to phosphate-buffered saline (PBS), presumably because of the salt-induced screening of the repulsive forces.[18] The PLGA-mPEG modified Ptxl–LA_{200} NCs were significantly more stable in PBS than the untreated NCs or NCs treated with mPEG (Figure 2d), indicating the importance of the hydrophobic PLGA segment to the noncovalent interaction between PLGA-mPEG and NCs.

Ptxl has hydroxy groups at its C2′, C1, and C7 positions (Figure 3a). Any of these three hydroxy groups can potentially initiate LA polymerizations, resulting in Ptxl–PLA conjugates with one to three PLA chains attached to Ptxl. To reduce the heterogeneity of Ptxl–PLA, we investigated whether the initiation can be controlled at a specific hydroxy group of Ptxl to make a Ptxl–PLA conjugate containing a single PLA chain (as illustrated in Figure 1).

The steric environments of the three hydroxy groups of Ptxl differ in terms of steric hindrance in the order of 2′-OH < 7-OH < 1-OH. The tertiary 1-OH group is least accessible and typically inactive.[19] The 7-OH group, however, could potentially compete with the 2′-OH group,[20] the most accessible and active hydroxy group of Ptxl, for coordination with metal catalyst. We postulated that a metal catalyst with a bulky chelating ligand may differentiate between the 2′-OH and 7-OH groups, and thus preferentially or even specifically form a Ptxl–metal complex through the 2′-OH group for site-specific LA polymerization.

Magri et al,[21] reported that tetrabutylammonium borohydride (Bu_4NBH_4) could selectively and quantitatively reduce the 13-ester bond of Ptxl to give baccatin III (BAC) and (1*S*,2*R*)-*N*-1-(1-phenyl-2,3-dihydroxypropyl)benzamide (PDB) (Figure 3a). We attempted to use this reduction reaction to disassemble the Ptxl–LA_5 derived from metal/Ptxl-mediated polymerization and then to analyze whether PLA is attached to these fragments (Figure 3b). It was anticipated that [$Mg(N(TMS)_2)_2$], a catalyst without a chelating ligand, would initiate polymerization nonpreferentially at both the 2′-OH and the 7-OH groups. As expected, both PDB–PLA and BAC–PLA were obtained after the resulting Ptxl–LA_5 was treated with Bu_4NBH_4 (trace 4 in Figure 3c). When [(BDI)MgN(TMS)$_2$] was used, the amount of BAC–PLA derived was significantly reduced (trace 5 in Figure 3c), indicating polymerization of LA was preferentially initiated at the 2′-OH group of Ptxl by Mg catalysts with a proper chelating ligand.

Although [(BDI)MgN(TMS)$_2$] gave significantly improved site-specific control in the metal/Ptxl-initiated polymerization, the resulting Ptxl–PLAs typically have a fairly broad molecular weight distribution (MWD) (e.g., Ptxl–LA_{200} $M_w/M_n = 1.47$). This observation is attributed to fast propagation relative to initiation for the polymerization initiated by Mg catalysts.[13] To reduce the propagation rate and transesterification side reactions, we tested [(BDI)ZnN(TMS)$_2$], a zinc analogue of [(BDI)MgN(TMS)$_2$] that gives more controlled LA polymerization.[13] As expected, Ptxl–PLAs with projected MWs and narrow MWDs were readily prepared by LA polymerization mediated by the (BDI)Zn–Ptxl complex formed in situ. For instance, Ptxl–LA_{200} with M_n = 28100 Da (expected 29700 Da) and a MWD of 1.02

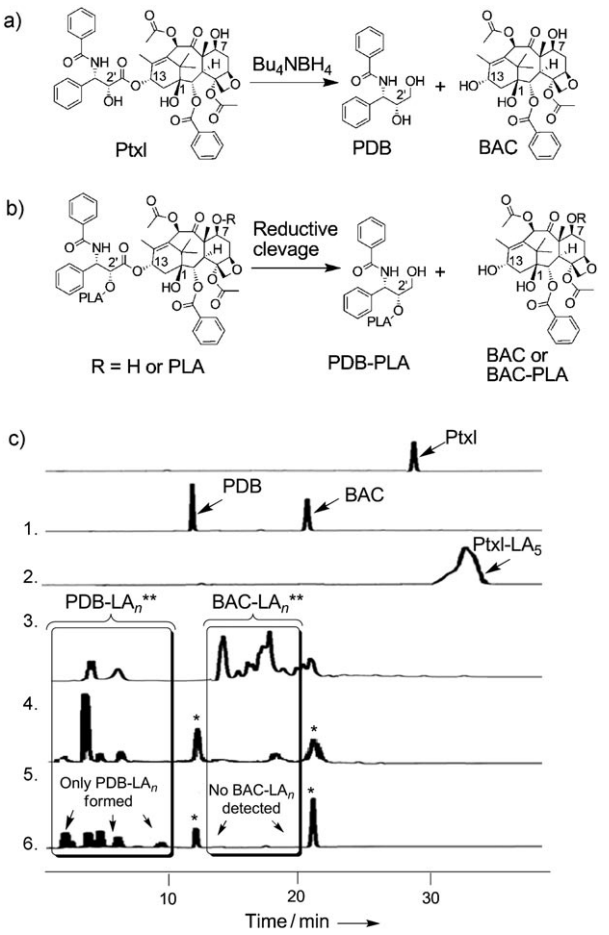

Figure 3. a) Bu_4NBH_4-induced site-specific degradation of Ptxl for the formation of PDB and baccatin (BAC). b) Reductive degradation of 13-ester linkage of Ptxl–PLA; c) HPLC traces of 1. Ptxl, 2. Ptxl treated with Bu_4NBH_4, 3. Ptxl–LA_5 , 4. Ptxl–LA_5 prepared using [$Mg(N(TMS)_2)_2$] followed by treatment with Bu_4NBH_4, 5. same as trace 4 except that [(BDI)MgN(TMS)$_2$] was used, 6. same as trace 4 except that [(BDI)ZnN(TMS)$_2$] was used. The PDB and partial BAC degradation products detected and marked with an asterisk (*) in traces 5 and 6 were from the free Ptxl that was not completely consumed during the polymerization at a very low M/I ratio (M/I = 5). Separation of Ptxl was not attempted. If one compares the BAC and PDB patterns in traces 6 and 2, it is clear that the BAC in trace 6 arose partially from degradation of unreacted Ptxl and partially from the reduction of Ptxl–PLA, where the PLA is attached to the 2′-OH group of Ptxl. The assignments marked with ** were verified by mass spectrosmetry (see Figure 4 in the Supporting Information).

were obtained in the [(BDI)ZnN-Ptxl]-mediated LA polymerization (200 equiv). HPLC analysis of the Ptxl–LA_5 prepared with [(BDI)ZnN$(TMS)_2$] and then treated with Bu_4NBH_4 showed that initiation and polymerization occurred exclusively at the 2′-OH group of Ptxl (trace 6, Figure 3c). Various studies suggests that the polymerization process does not lead to deleterious effect on Ptxl (see Figures 5 and 6 in the Supporting Information).

This drug-initiated polymerization method can be applied to the preparation of NCs of other therapeutic agents containing hydroxy groups. For instance, docetaxel(Dtxl)–LA_{10} and camptothecin(CPT)–LA_{10} NCs with very high drug loading (35.9 wt% and 19.5 wt%, respectively), more than 95% loading efficiencies, and sub-100 nm sizes can be readily prepared using this metal–drug complex initiated LA polymerization followed by nanoprecipitation (entries 5 and 6, Table 1). CPT differs from both Ptxl and Dtxl as it has no intrinsic ester bond. It was quantitatively recovered from the CPT–PLA NC after the NC was treated with NaOH. The CPT separated from the hydrolysis mixture of CPT–PLA in PBS and collected by preparative HPLC showed a ^{1}H NMR spectrum identical to that of the authentic CPT (see Figure 7 in the Supporting Information). This study further demonstrates that the chemical structures of the incorporated drugs remain unchanged under the mild polymerization and nanoprecipitation conditions. The incorporated drugs in NCs can be released in their original forms. Like Ptxl–PLA NCs, hydrolysis of Dtxl–PLA NCs in PBS showed no burst release effects (see Figure 8 in the Supporting Information). The correlation of toxicities with drug loadings for both Dtxl–PLA NC and CPT–PLA NC (see Figure 9 in the Supporting Information) are very similar to that of Ptxl–PLA NCs (Figure 2c).

In conclusion, we have developed a new method for preparing polymeric nanoconjugates using the drug-initiated, controlled, living polymerization of cyclic esters. This unprecedented strategy is alternative to polyester–drug conjugation by means of coupling chemistry,[22–26] and allows preparation of polymer–drug nanoconjugates with very high drug loadings, nearly quantitative loading efficiencies, controlled release profiles without burst release effects, and narrow particle-size distributions. The metal (e.g., Zn, Mg) and the organic chelating ligand are readily removable by solvent extraction. It usually takes only a few hours to prepare salt-stable NCs on a gram or larger scale. The drug release profiles can potentially be further modified by using cyclic esters other than LA. This formulation method can potentially be broadly used for the nanoformulation of numerous hydroxy-group-containing therapeutic agents to achieve excellent control over drug loading and release.

Experimental Section

General procedure for the preparation and formulation of Ptxl–PLA nanoconjugates: [(BDI)MgN$(SiMe_3)_2$] (6.2 mg, 0.01 mmol)[13] and Ptxl (8.5 mg, 0.01 mmol) were mixed in 0.5 mL anhydrous THF. DL-Lactide (144 mg, 1 mmol) in 2 mL anhydrous THF was added dropwise. After the LA was completely consumed (monitored by FTIR or ^{1}H NMR spectroscopy), the polymerization solution was added to ethyl ether (25 mL) to precipitate out the Ptxl-LA_{100} conjugate. Ptxl–LA_{100} in DMF (100 μL, 10 mg mL^{-1}) was precipitated by dropwise addition to vigorously stirred nanopure water (2 mL). PLGA–$mPEG_{5k}$ (MW = 18300 g mol^{-1}, 5 mg mL^{-1} in DMF, 100 μL) was added dropwise to the NCs to give PEGylated Ptxl–LA_{100} NC.

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