Polymer—Cisplatin Conjugate Nanoparticles for Acid-Responsive Drug Delivery

Santosh Aryal, Che-Ming Jack Hu, and Liangfang Zhang*

Department of Nanoengineering and Moores Cancer Center, University of California San Diego, La Jolla, California 92093

ABSTRACT We report the synthesis of novel acid-responsive therapeutic nanoparticles (NPs) with sub-100 nm size consisting of polymer—cisplatin conjugates. The uniqueness of these drug delivery polymeric NPs lies in the covalent conjugation of each cisplatin drug to the hydrophobic segment of two biocompatible diblock copolymer chains through a hydrazone bond, resulting in highly differential drug release profile at different environmental acidity. We demonstrate that the synthesized polymer—cisplatin conjugates can readily precipitate to form sub-100 nm NPs in aqueous solution due to their very low critical micelle concentration (CMC). The resulting NPs show well-controlled cisplatin loading yield, excellent acid-responsive drug release kinetics, and enhanced *in vitro* cytotoxicity against ovarian cancer cells as compared to free cisplatin. As an environmentally sensitive drug delivery vehicle, these NPs can potentially minimize the drug loss during NP circulation in the blood, where the pH value is neutral, and trigger rapid intracellular drug release after the NPs are endocytosed by the target cells. This characteristic drug release profile holds the promise to suppress cancer cell chemoresistance by rapidly releasing a high dose of chemotherapy drugs inside the tumor cells, thereby improving the therapeutic efficacy of the drug payload.

KEYWORDS: polymeric nanoparticle · cisplatin · drug delivery · controlled release · stimuli-responsive

Cisplatin, *cis*-diamminedichloroplatinum(II), has been widely used in the clinic to treat a variety of cancers such as ovarian, breast, bladder, head and neck, and small cell lung cancer because of its potent activity to cross-link DNA upon entering the cells.[1] It preferentially binds to the N7 atoms of guanine bases in DNA double-helix strands, thereby preventing the strands from uncoiling and separating. This prohibits the division of the cells and ultimately results in cellular apoptosis.[2−4] However, cisplatin is vulnerable to attack by plasma proteins, particularly those containing thiol groups, such as human serum albumin.[5] It has been reported that 1 day after cisplatin administration, 65−98% of the platinum in the blood was protein-bound.[6,7] This undesirable protein binding deactivated the drugs, leading to less therapeutic efficacy, and accounted for some severe side effects of cisplatin therapy.[8−10]

To improve the therapeutic index of cisplatin while minimizing its adverse side effects, cisplatin analogue Pt(IV) prodrugs have been synthesized.[11−14] These Pt(IV) prodrugs usually have less potency and toxicity than Pt(II) before they are reduced to their corresponding Pt(II) derivatives by cysteine or other stimuli. In these Pt(IV) prodrugs, two additional coordination sites become available which offer great synthetic flexibility for further chemical modification. For instance, axial oxidation of cisplatin yields a dihydroxy product, *cis,cis,trans*-$Pt(NH_3)_2Cl_2(OH)_2$, which can be further esterified with carboxyl or acid anhydride groups to provide different functionality. Ang *et al.* have synthesized ethacraplatin *via* the acylation of *cis,cis,trans*-$Pt(NH_3)_2Cl_2(OH)_2$ with an excess of ethacrynic acid chloride and demonstrated the capacity of ethacraplatin to overcome glutathione-*s*-transferase mediated drug resistance.[11] More recently, Mukhopadhyay *et al.* have modified cisplatin with acid anhydride, followed by conjugation with targeting peptides for selective delivery of Pt(IV) to angiogenic tumor vasculature.[15] These cisplatin analogue Pt(IV) prodrugs mainly have been applied directly as small molecule drugs for cancer treatment.

A further approach to improve the therapeutic efficacy of these cisplatin analogue Pt(IV) prodrugs is to load them into a drug nanocarrier,[16,17] for example, a polymeric nanoparticle (NP), and then preferentially deliver the drug-loaded nanocarriers to the cells or tissues of interest instead of administering free drugs. Many advantages of using polymeric NPs to deliver drugs have been recognized in the past decades.[18,19] It prolongs the half-life of drugs in the

*Address correspondence to zhang@ucsd.edu.

Received for review October 12, 2009 and accepted December 17, 2009.

Published online December 29, 2009.
10.1021/nn9014032

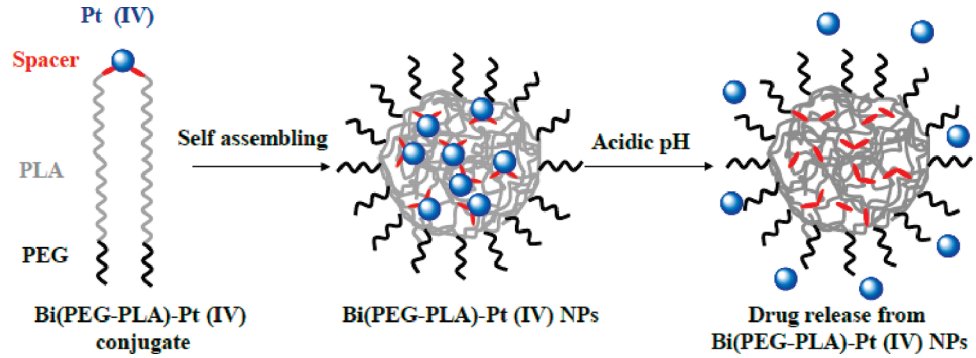


Figure 1. Schematic illustrations of the structure of polymer−cisplatin prodrug conjugate, the formation of Bi(PEG-PLA)-Pt(IV) NPs through self-assembling, and acid-responsive drug release from the NPs.

systemic circulation, releases drugs at a sustained rate or in an environmentally responsive manner, delivers drugs in a targeted manner to minimize systemic side effects, and delivers two or more drugs simultaneously

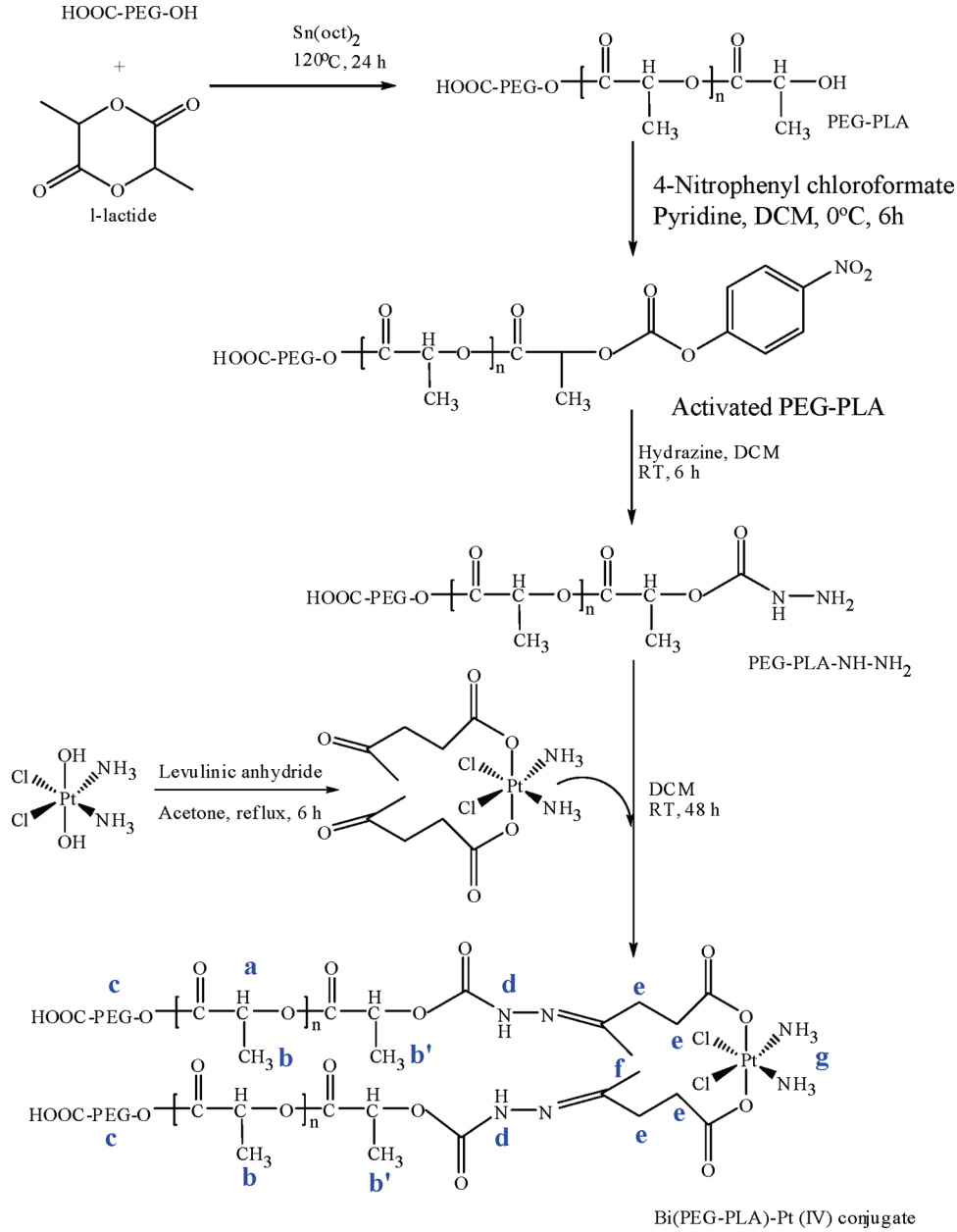


Figure 2. Schematic description of the synthesis of Bi(PEG-PLA)-Pt(IV) polymer−prodrug conjugate.

for combination therapy to generate a synergistic effect and suppress drug resistance.[19] However, loading cisplatin into polymeric NPs is challenging because of the poor solubility of cisplatin in organic solvents and its partial solubility in water.[12,20] A pioneer study by Dhar *et al.* has physically encapsulated cisplatin analogue Pt(IV) prodrugs into polymeric NPs by attaching two linear hexyl chains to the Pt(IV) prodrugs and thus converting the prodrug to a more hydrophobic derivative. They have successfully demonstrated the enhanced therapeutic efficacy of the Pt(IV) prodrug-encapsulated NPs against prostate cancer.[12]

Here we hypothesized that, by covalently conjugating Pt(IV) prodrugs to the building blocks of polymeric NPs through a stimuli-sensitive bond (*e.g.*, an acid-responsive bond), one is able to not only load cisplatin analogue Pt(IV) prodrugs into polymeric NPs but also control cisplatin release kinetics from the NPs in an environmentally sensitive manner. This would allow for minimizing the amount of drugs leaching out of the NPs during their circulation in the blood (pH = 7.4) and enable rapid intracellular drug release when the NPs are endocytosed by the target cells (pH = 5−6[21]). To this end, we synthesized a hydrazine terminated poly(ethylene glycol)-*b*-poly(L-lactide) (PEG-PLA-NH-NH_2) copolymer and a levulinic acid modified cisplatin analogue Pt(IV) prodrug. Subsequently, these two compounds were covalently conjugated to each other with a stoichiometric ratio of 2:1 through an acid-responsive hydrazone bond, resulting in a polymer−cisplatin prodrug conjugate, Bi(PEG-PLA)-Pt(IV). This conjugate was then precipitated to form sub-100 nm polymeric NPs, as illustrated in Figure 1. We demonstrated that these polymer−cisplatin prodrug conjugate NPs had well-controlled drug loading yield, excellent acid-responsive drug release characteristics, and potent cytotoxicity against ovarian cancer.

RESULTS AND DISCUSSION

In the study, we first synthesized a Bi(PEG-PLA)-Pt(IV) polymer−cisplatin prodrug conjugate following the protocol illustrated in Figure 2. Briefly, carboxyl-functionalized poly(ethylene glycol) (COOH-PEG-OH, M_n = 3500 g mol^{-1}) was used as macroinitiator for the ring-opening polymerization of L-lactide in the presence of stannous octoate as a catalyst. The resultant poly(ethylene glycol)-*b*-poly(L-lactide) (PEG-PLA) was characterized by gel permeation chromatography (GPC) with a molecular weight of 10 000 g mol^{-1} and a polydispersity index of 1.12. Then the PEG-PLA was activated by 4-nitrophenyl chloroformate and derivatized to its hydrazine derivative (PEG-PLA-NH-NH_2). Subsequently, this hydrazine terminated polymer was reacted with presynthesized *cis,trans,cis*-$PtCl_2(OCOCH_2CH_2COCH_3)_2(NH_3)_2$ cisplatin analogue prodrug, in which levulinic acid was used as a spacer, resulting in a Bi(PEG-PLA)-Pt(IV).

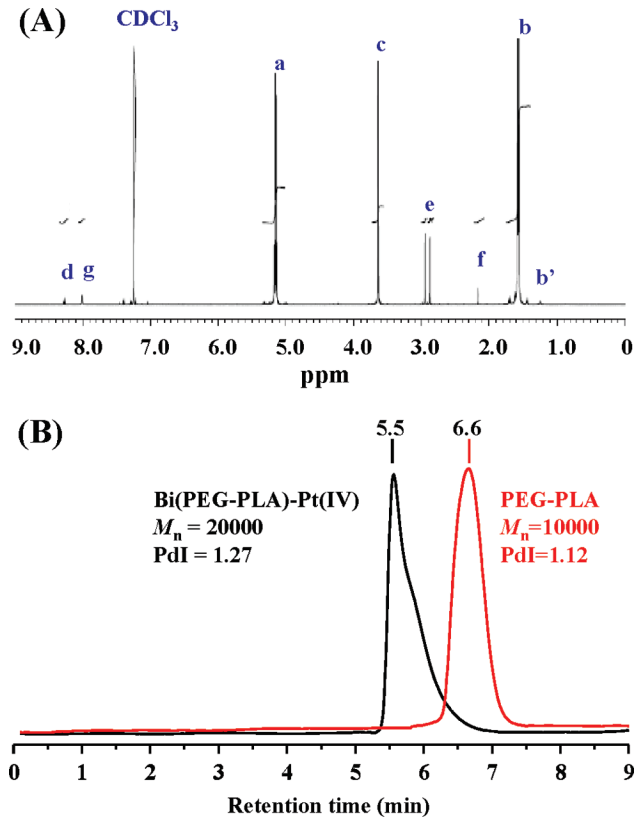
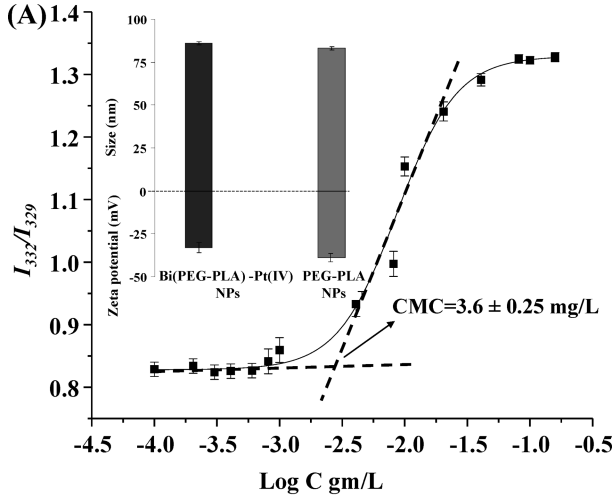


Figure 3. Characterization of Bi(PEG-PLA)-Pt(IV) polymer−prodrug conjugate. (A) ^{1}H NMR spectrum of the synthesized Bi(PEG-PLA)-Pt(IV) conjugate. (B) GPC chromatogram of PEG-PLA polymer and Bi(PEG-PLA)-Pt(IV) conjugate.

The entire reaction processes were monitored by ^{1}H NMR spectroscopy and GPC. The ^{1}H NMR spectra of all intermediate products are provided in the Supporting Information (Figures S1−S4). As indexed in Figure 3A, the ^{1}H NMR spectrum of the final polymer−cisplatin prodrug conjugate included all characteristic resonance peaks of the PEG-PLA polymer, the levulinic acid spacer, and the Pt(IV) prodrug, for example, the characteristic peaks of −NH-*N* = at δ 8.28 ppm, $-CH_2$ of levulinic acid at δ 2.1 and 2.95 ppm, and $-NH_3$ of cisplatin at δ 8.0 ppm. A considerable ^{1}H NMR resonance shift was observed for the $-CH_2$ of levulinic acid and the $-NH_3$ of cisplatin in the final product as compared to those in $PtCl_2(OCOCH_2CH_2COCH_3)_2(NH_3)_2$ (Figure S4). This is likely due to the presence of two polyester chains that sandwich the Pt(IV) metal from the axial position in the Bi(PEG-PLA)-Pt(IV) conjugate. Such conformation creates local magnetic field inhomogeneity accounting for the resonance shift. In addition, the quadrupolar effect of the ^{14}N nucleus may also contribute to the resonance shift because it makes protons resonating in a broad spectrum.[14] The formation of Bi(PEG-PLA)-Pt(IV) conjugate was further confirmed by the GPC measurements. As shown in Figure 3B, the characteristic peak of PEG-PLA at 6.6 min disappeared in the chromatogram of the polymer−cisplatin prodrug conjugate. Instead, a dominant peak appeared at the retention time of 5.5



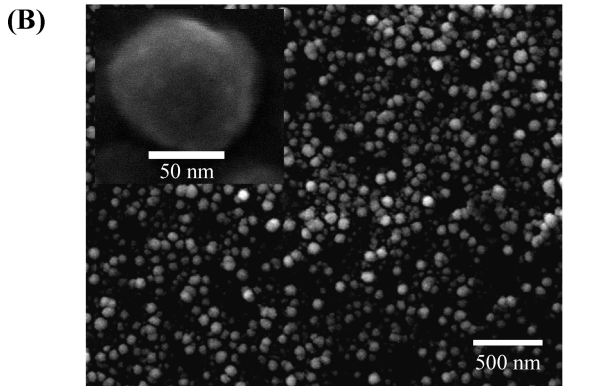


Figure 4. (A) Determination of the critical micelle concentration (CMC) of Bi(PEG-PLA)-Pt(IV) conjugate using pyrene probe. Inset: NP size, surface ζ-potential of Bi(PEG-PLA)-Pt(IV) NPs and PEG-PLA NPs measured by DLS. (B) Representative SEM image of Bi(PEG-PLA)-Pt(IV) conjugate NPs. Inset: High-resolution SEM image of a single Bi(PEG-PLA)-Pt(IV) NP.

min, corresponding to the molecular weight of about 20 000 g mol^{-1}. This clearly indicates the formation of Bi(PEG-PLA)-Pt(IV) conjugate, which contains two PEG-PLA polymer chains.

Next, we measured the critical micelle concentration (CMC) of the synthesized Bi(PEG-PLA)-Pt(IV) conjugate to evaluate its feasibility of forming NPs. CMC was determined using pyrene as a hydrophobic probe that has been widely used for this purpose because of its characteristic fluorescence spectra sensitive to environmental polarity.[22] The fluorescence emission of pyrene was fixed at 390 nm, while its excitation spectra were monitored at various concentrations of Bi(PEG-PLA)-Pt(IV) conjugate. The intensity ratio at 332 and 329 nm was plotted against polymer−cisplatin prodrug concentration in a semilog graph. As shown in Figure 4A, the CMC of the Bi(PEG-PLA)-Pt(IV) conjugate was 3.6 ± 0.25 mg/L. This very low CMC value indicates that the conjugate is prone to form NPs *via* precipitation method. Indeed, dynamic light scattering (DLS) measurement showed that the self-assembled Bi(PEG-PLA)-Pt(IV) conjugate NPs had an unimodal size distribution with an average diameter of 86 ± 2.0 nm (Figure 4A, inset), which was consistent with the findings from SEM images (Figure 4B). The surface ζ-potential of the NPs was about −33 ± 1.2 mV (Figure 4A, inset). We further found that the size and surface ζ-potential of the polymer−prodrug conjugate NPs were similar to those of the corresponding PEG-PLA polymeric NPs, 83 ± 2.0 nm and −36 ± 2.0 mV, respectively. This suggests that conjugation of cisplatin prodrug to the PEG-PLA polymer chain has negligible effect on formation of the polymeric NPs.

After having prepared Bi(PEG-PLA)-Pt(IV) NPs, we then quantified cisplatin loading yield of the NPs and cisplatin release kinetics from the NPs at different pH values using inductively coupled plasma optical emission spectrometry (ICP-OES). Here the drug loading yield is defined as the weight ratio of the cisplatin payload to the NPs including both polymer excipients and cisplatin. The drug release kinetics represents how fast the drugs leak out of the NPs, plotted as the weight ratio of the accumulative released cisplatin to the total cisplatin payload against time. As shown in Figure 5A, when more Pt(IV) prodrugs were used to react with PEG-PLA during the polymer−prodrug conjugation process, a higher cisplatin drug loading yield was achieved. For example, the initial Pt(IV)/PEG-PLA reaction molar ratio of 2:1, 4:1, and 6:1 resulted in a final cisplatin drug loading yield of 0.35 ± 0.01, 0.89 ± 0.02, and 1.05 ± 0.03 wt % (mean ± SD, $n = 3$), respectively. However, when the Pt(IV)/PEG-PLA molar ratio was higher than 6:1 (*e.g.*, 8:1), no considerable drug loading yield increase occurred. This is likely due to the saturation of polymer chains, in which all PEG-PLA polymers have been conjugated with Pt(IV) prodrugs. Figure 5B showed the cisplatin release kinetics from the Bi(PEG-PLA)-Pt(IV) NPs at three distinct pH values, pH = 5.0, 6.0, and 7.4. The cisplatin release rate from the NPs at pH = 5.0 and 6.0 was significantly faster than at pH = 7.4. When the cisplatin loading yield was 1.05 wt % (Figure 5B), it took the Bi(PEG-PLA)-Pt(IV) NPs around 4 and 6 h to release 50% of total cisplatin payload at pH = 5.0 and 6.0, respectively, *versus* 22 h at pH = 7.4. The contrast of the cisplatin release rate was even more sharper within the first a few hours. For example, during the first 2 h period, 17 and 15% of the cisplatin payload was released at pH = 5.0 and 6.0, respectively, while only 2% was released at pH = 7.4. These results suggest that cisplatin release kinetics from the Bi(PEG-PLA)-Pt(IV) NPs is pH-dependent. This is mainly because the cisplatin analogue Pt(IV) prodrugs were covalently conjugated to the polymer chains through hydrazone bond, which is an acid-labile bond. At pH = 5−6, hydrazone bond can be easily cleaved within a few minutes to free the drugs, which will diffuse out of the NPs. In contrast, this bond is relatively stable at pH = 7.4.[23] The observed sustained cisplatin release at pH = 7.4 may be due to the degradation of the PLA polymers, to which the cisplatin analogue prodrugs were covalently

linked. As a biodegradable polymer, PLA ester can be hydrolyzed to small segments or monomers at both neutral pH and acidic pH.[24] Here we incubated the PEG-PLA NPs in pH = 5.0, 6.0, and 7.4 PBS solutions at 37 °C. At each time point, an aliquot of the PEG-PLA NPs was collected to measure the polymer molecular weight (M_w) using GPC. As shown in Figure 5B inset, after 50 h incubation, the polymer M_w decreased by a factor of 20, 18, and 8% at pH = 5.0, 6.0, and 7.4, respectively. These data reasonably explain the sustained drug release kinetics at neutral pH, as shown in Figure 5B, but also raises a concern that the observed rapid drug release at pH = 5.0 and 6.0 might be because of fast PLA degradation at acidic pH rather than the cleavage of hydrazone bond. However, negligible difference of M_w loss was observed within the first 24 h of incubation at these three pH values. This confirms that the drug burst at pH = 5.0 and 6.0 during the first a few hours is due to the cleavage of the hydrazone bond but not polymer degradation.

Lastly, we examined the *in vitro* cellular cytotoxicity of the synthesized acid-responsive Bi(PEG-PLA)-Pt(IV) NPs. To this end, we chose A2780 human ovarian carcinoma cell line as a model cancer cell because of the well-known toxicity of cisplatin against ovarian cancer. Following a well-established cellular cytotoxicity measurement protocol,[25,26] the A2780 cells were incubated with Bi(PEG-PLA)-Pt(IV) NPs for 4 h. After the incubation, the excess NPs were removed and the cells were washed three times with fresh buffer followed by the addition of fresh culture media. Subsequently, the cells were incubated for 72 h before being assessed by MTT assay. Cell culture media and PEG-PLA NPs (without cisplatin analogue prodrugs) were used as negative controls. Free cisplatin drug at different concentrations (10, 50, and 100 μM) served as positive controls. As shown in Figure 6, the cell viability of the Bi(PEG-PLA)-Pt(IV) NPs decreased to about 65% after 4 h incubation. In contrast, PEG-PLA NPs had negligible cytotoxicity against ovarian cancer cells, similar as the cell media. The cell viability of free cisplatin at 10, 50, and 100 was 99, 62, and 35%, respectively. On the basis of the cisplatin loading yield of 1.05 wt % measured in Figure 5A, we calculated that the amount of cisplatin loaded in the Bi(PEG-PLA)-Pt(IV) NPs for this cytotoxicity study was equivalent to 7 μM free cisplatin. Surprisingly, the NPs with an equivalent 7 μM free cisplatin had cellular cytotixicity against ovarian cancer cells as high as 50 μM free cisplatin. To ensure that the measured cytotoxicity was due to the internalized NPs but not the free drugs in the media released from the NPs, the culture media were filtered through a membrane with a molecular weight cutoff of 10 kDa after the 4 h incubation with Bi(PEG-PLA)-Pt(IV) NPs. The filtrate was collected to quantify Pt content using ICP-OES. Negligible amount of free Pt drug (0.05 μM) was observed in the media, which was consistent with the slow drug release profile of the NPs at pH = 7.4. This approximate 7-fold cytotoxicity increase of Bi(PEG-PLA)-Pt(IV) NPs might be attributed to the burst drug release in the acidic intracellular environment. Upon internalization, the acid-responsive NPs caused a surge in intracellular drug concentration that possibly overwhelmed some chemoresistance mechanisms of tumor cells, such as the P-glycoprotein (P-gp) membrane proteins mediated drug efflux mechanism.[27] Our results are consistent with an earlier study by Xu *et al.*, who have examined the activity of cisplatin encapsulated in different NP systems and have observed enhanced cytotoxicity of those NPs with fast cisplatin release profile.[28]

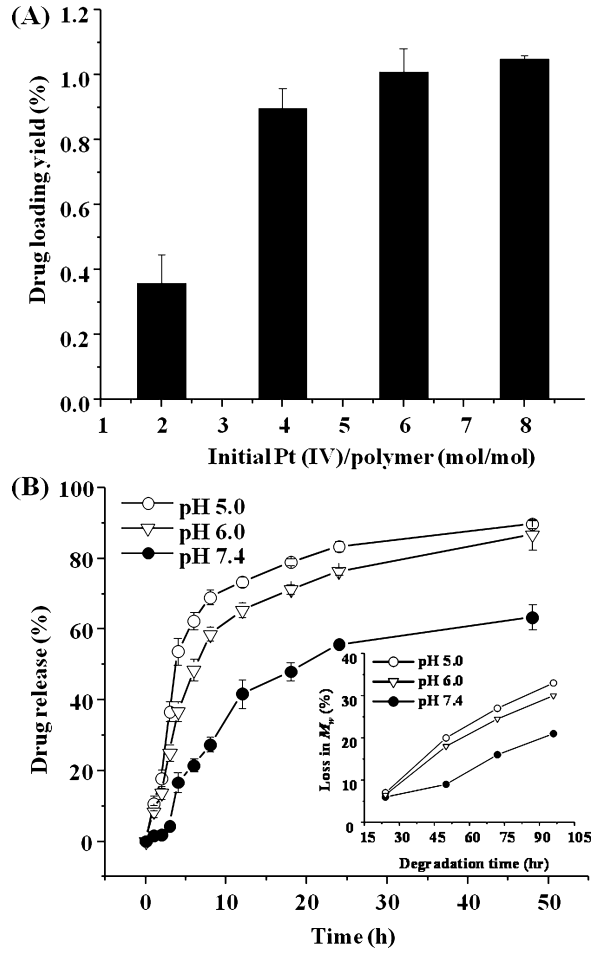


Figure 5. (A) Cisplatin loading yield of Bi(PEG-PLA)-Pt(IV) NPs at various initial Pt(IV)/PEG-PLA reaction molar ratios. (B) Cisplatin release profile from Bi(PEG-PLA)-Pt(IV) NPs at pH = 5.0 (open circles), pH = 6.0 (open triangles), and pH = 7.4 (solid circles) PBS buffer at 37 °C. Inset: Hydrolytic degradation rate of PEG-PLA NPs at pH = 5.0, 6.0, and 7.4.

CONCLUSIONS

In conclusion, a novel acid-responsive Bi(PEG-PLA)-Pt(IV) polymer−cisplatin prodrug conjugate NP was synthesized as a new cisplatin delivery vehicle. The uniqueness of this drug delivery NP system is that the cisplatin analogue prodrug was covalently linked to the hydrophobic segment of two PEG-PLA copolymer chains through pH-sensitive hydrazone bond. We dem-

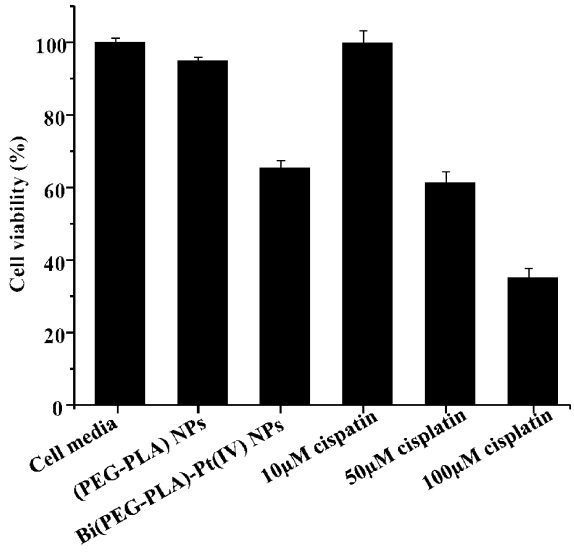


Figure 6. MTT assay to measure the cytotoxicity of Bi(PEG-PLA)-Pt(IV) NPs against A2780 human ovarian carcinoma cell line in comparison with cell culture media, PEG-PLA NPs and free cisplatin (10, 50, and 100 μM). The amount of cisplatin loaded in the Bi(PEG-PLA)-Pt(IV) NPs was equivalent to 7 μM free cisplatin drug. All samples were incubated with cells for 4 h, and the cells were subsequently washed and incubated for a total of 72 h before assessing cell viability in each group ($n = 6$).

onstrated that sub-100 nm Bi(PEG-PLA)-Pt(IV) NPs were readily formed due to the very low CMC of the Bi(PEG-PLA)-Pt(IV) conjugate. These NPs had well-controlled cisplatin loading yield and showed excellent acid-responsive drug release kinetics, leading to enhanced *in vitro* cytotoxicity against tumor cells as compared to free cisplatin. As an environmentally sensitive drug delivery vehicle, these NPs can potentially minimize the drug loss during their circulation in the blood, where the pH value is neutral, and trigger rapid intracellular drug release when the NPs are endocytosed by the target cells. This characteristic drug release kinetics may suppress cancer cell chemoresistance and improve the therapeutic efficacy of the drug payload. We also speculate that, by attaching targeting ligands onto the surface of these NPs, one can improve the binding specificity of the NPs, thereby further enhancing the therapeutic index of these polymer−prodrug conjugate NPs. Moreover, although cisplatin was selected as a specific model drug in this study, the concept and technique of developing acid-responsive drug delivery polymeric NPs by covalently conjugating drug molecules to polymer chains through double or multiple stimuli-responsive linkers can be generalized to deliver many other types of therapeutic or diagnostic agents, including both hydrophilic and hydrophobic agents.

EXPERIMENTAL SECTION

Materials. L-Lactide (*cis*-3,6-dimethyl-1,4-dioxan-2,5-dione), stannous octoate, K_2PtCl_4, $AgNO_3$, and all other chemicals for wet chemistry synthesis of cisplatin were purchased from Sigma-Aldrich. Poly(ethylene glycol) ($M_n = 3500$ g mol^{-1}) was obtained from JenKem Technology, Shanghai, China. Cell culture reagents and media were purchased from Media Tech. 3-(4,5-Dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) cell proliferation assay kit was obtained from Promega Corporation, USA. A2780 human ovarian carcinoma cells were received from Dr. Stephen Howell in the Moores Cancer Center at UCSD.

Synthesis of PEG-PLA and Activated PEG-PLA Copolymers. The PEG-PLA diblock copolymer was synthesized by ring-opening polymerization (ROP) and activated using 4-nitrophenyl chloroformate. Briefly, L-lactide (1.6 g, 11.1 mmol, purified by recrystallization in ethyl acetate) and COOH-PEG_{3500}-OH (0.2 g, 0.057 mmol) were heated to 120 °C under nitrogen atmosphere for complete melting. Then stannous octoate in anhydrous toluene was added into the melting mixture with a monomer/catalyst molar ratio of 500:1 to initiate ROP. Polymerization was carried under reflux condition at 120 °C under nitrogen atmosphere for 24 h. After the completion of the reaction, the crude product was cooled to room temperature, stirred with cold water to hydrolyze unreacted L-lactide monomers, and extracted with chloroform. The synthesized PEG-PLA diblock polymers were then purified by precipitation in cold diethyl ether and dried under vacuum. This washing process was repeated three times. To activate the obtained PEG-PLA for further conjugation, 3 g of the diblock copolymers was dissolved in 50 mL of methylene chloride (MC) and activated by 141.0 mg of 4-nitrophenyl chloroformate while adding 99.0 mg of pyridine (stoichiometric molar ratio, PPEG-PLA/4-nitrophenyl chloroformate/pyridine = 1:3:5) in a dropwise manner at 0 °C. Then the reaction was carried out for 12 h at room temperature in nitrogen. The activated PEG-PLA diblock copolymers were recovered by precipitation in ice-cold diethyl ether and dried under reduced pressure. The final activated polymers and all polymer intermediates were characterized by Varian Mercury 400 nuclear magnetic resonance (NMR) spectroscopy. ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm) spectra of PEG-PLA: 1.2 (t, $-CH_3$ PLA end group, $J = 7.0$ Hz), 1.55 (m, $-CH_3$, PLA repeating unit), 3.47 (q, $-CH_2$, PEG end group, $J = 7.0$ Hz), 3.63 (m, $-CH_2$, PEG repeating unit) 5.15 (m, −CH, PLA repeating unit). ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm) spectra of the activated PEG-PLA: 1.2 (t, $-CH_3$ PLA end group, $J = 7.0$ Hz), 1.55 (m, $-CH_3$, PLA repeating unit), 3.47 (q, $-CH_2$, PEG end group, $J = 7.0$ Hz), 3.63 (m, $-CH_2$, PEG repeating unit) 5.15 (m, −CH, PLA repeating unit), 7.93 (t, aromatic −CH, $J = 6.9$ Hz), 8.78 (d, aromatic −CH, $J = 5.0$ Hz). The molecular weight of the synthesized polymer was determined using gel permeation chromatography (GPC) (Viscotek, USA). For the GPC measurements, tetrahydrofuran (THF) was used as a mobile phase with a flow rate of 1 mL/min. Weight average molecular weights as well as polydispersity indices were calculated from a calibration curve using a series of polystyrene standards.

Synthesis of *cis*,*trans*,*cis*-$PtCl_2(OCOCH_2CH_2COCH_3)_2(NH_3)_2$ Prodrug. Cisplatin and $PtCl_2(OH)_2(NH_3)_2$ were first synthesized following a previously published protocol.[29] The obtained *cis*,*trans*,*cis*-$PtCl_2(OH)_2(NH_3)_2$ was then used to prepare *cis*,*trans*,*cis*-$PtCl_2(OCOCH_2CH_2COCH_3)_2(NH_3)_2$. An excess of levulinic anhydride was added to an acetone solution containing 100 mg (0.3 mmol) of $PtCl_2(OH)_2(NH_3)_2$ in reflux condition. After 12 h of reaction, cold water was added to hydrolyze excess levulinic anhydride. The reaction mixture was left at 2 °C for 16 h. The acetone was removed from the reaction mixture under reduced pressure leaving a white residue. The residue was purified by washing with water, ethanol, and ether, respectively, resulting in a final product yield of 39.0%. ^{1}H NMR (DMSO-d_6, 500 MHz, δ ppm): 2.13 (s, 3H, $-CH_3$), 2.72 (s, 2H, $-CH_2$), 6.2−6.8 (br, 3H, NH_3).

Synthesis of Bi(PEG-PLA)-Pt(IV) Conjugate. The activated PEG-PLA (100 mg) was dissolved in 20 mL of dimethylformamide (DMF) and reacted with hydrazine (stoichiometric ratio of activated PEG-PLA/hydrazine = 1:10) in nitrogen at room temperature for 12 h. By precipitating in ice-chilled diethyl ether, the resulting hydrazine functionalized PEG-PLA diblock copolymer (PEG-PLA-NH-NH_2) was retrieved. The PEG-PLA-NH-NH_2 was then mixed

with $PtCl_2(OCOCH_2CH_2COCH_3)_2(NH_3)_2$ with a stoichiometric ratio of 1:8 in 30 mL of MC/DMF (1:1 volume ratio) in the presence of 3 Å molecular sieves. The reaction was allowed to proceed for 48 h at 50 °C under reflux in nitrogen. The crude product Bi(PEG-PLA)-Pt(IV) conjugate was purified by repeated precipitation in diethyl ether and was subsequently extracted with water and chloroform. The purified Bi(PEG-PLA)-Pt(IV) conjugate was kept at −20 °C for further use. ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 1.2 (t, $-CH_3$ PLA end group, $J = 7.0$ Hz), 1.55 (m, $-CH_3$, PLA repeating unit), 2.1 (s, $-CH_3$, spacer), 2.9 (d, $-CH_2$ spacer, $J = 2.8$ Hz), 3.47 (q, $-CH_2$, PEG end group, $J = 7.0$ Hz), 3.63 (m, $-CH_2$, PEG repeating unit) 5.15 (m, −CH, PLA repeating unit), 8.0 (br, 3H, $-NH_3$), 8.2 (br, 1H, −NH). The conjugation yield was determined by inductively coupled plasma optical emission spectroscopy (ICP-OES). Intensity of spectral line at 265.945 nm was measured for all samples and standards. The platinum level in the samples was determined by comparing the measured intensity with a standard curve of platinum.

Preparation of Bi(PEG-PLA)-Pt(IV) Conjugate NPs. The NPs were prepared *via* a nanoprecipitaton method following a previously published protocol.[30] Ten milligrams of Bi(PEG-PLA)-Pt(IV) conjugate was dissolved in 3 mL of acetonitrile and added to a vial containing 10 mL of water under constant stirring. After the completion of the nanoprecipitation, the organic solvent was evaporated in the hood for 2 h and under reduced pressure for additional 3 h to ensure the complete removal of acetonitrile. Then the NP solutions were washed three times using an Amicon Ultra-4 centrifugal filter (Millipore, Billerica, MA) with a molecular weight cutoff of 10 kDa. The NP size and surface ζ-potential were obtained from three repeat measurements using a dynamic light scattering (Malvern Zetasizer, ZEN 3600) with backscattering angle of 173°. The morphology and particle size were further characterized using scanning electron microscopy (SEM). Samples for SEM were prepared by dropping 5 μL of NPs solutions onto a polished silicon wafer. After drying the droplet at room temperature overnight, the sample was coated with chromium and then imaged by SEM.

Drug Loading Yield and Drug Release Studies. To measure the drug loading yield and release profile of cisplatin from the Bi(PEG-PLA)-Pt(IV) NPs, 100 μL of the prepared NP solutions was loaded into each Slide-A-Lyzer MINI dialysis microtube with a molecular weight cutoff of 3.5 kDa (Pierce, Rockford, IL). The NPs were then dialyzed against pH = 5.0, 6.0, and 7.4 PBS buffer at 37 °C. PBS buffers were changed every 12 h during the whole dialysis process. At each predetermined time point, NP solutions from three mini dialysis units were collected separately for drug quantification. The concentration of cisplatin was quantified using ICP-OES as described above.

Polymer Degradation Study. Hydrolytic degradation of PEG-PLA NPs was studied by incubating the NPs in pH = 5.0, 6.0, and 7.4 PBS buffer at 37 °C. At each time point, an aliquot of NPs was collected, extracted with chloroform, and re-precipitated in cold diethyl ether. The change of polymer molecular weight was determined by using GPC.

Cell Viability Assay. Cytotoxicity of Bi(PEG-PLA)-Pt(IV) NPs was assessed against A2780 human ovarian carcinoma cell line using the MTT assay. First, A2780 human ovarian carcinoma cells were seeded (2×10^4) in 96-well plates and incubated for 24 h. Next, the medium was replaced with 150 μL of fresh medium and incubated with 50 μL of Bi(PEG-PLA)-Pt(IV) NPs for 4 h. Then the excess NPs were removed, and cells were washed three times with fresh buffer followed by the addition of fresh medium. The plates were then incubated for 72 h and measured by MTT reagent following a protocol provided by the manufacturer. Fresh cell media and PEG-PLA NPs were used as negative controls. Free cisplatin at various concentrations was used as positive controls.

Acknowledgment. We thank Drs. Andrew Kummel, Stephen Howell, and Sadik Esener for valuable discussion, and Dr. William Trogler for the use of GPC. This work is supported by the National Institutes of Health Grant U54CA119335 and the University of California San Diego.

Supporting Information Available: ^{1}H NMR spectra of PEG-PLA, activated PEG-PLA, PEG-PLA-NH-NH_2, $PtCl_2(OCOCH_2CH_2,COCH_3)_2(NH_3)_2$, and Pt calibration curve of ICP-OES. This material is available free of charge *via* the Internet at http://pubs.acs.org.

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