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Preparation of Polymeric Prodrug Paclitaxel-Poly(lactic acid)-*b*-Polyisobutylene and Its Application in Coatings of a Drug Eluting Stent

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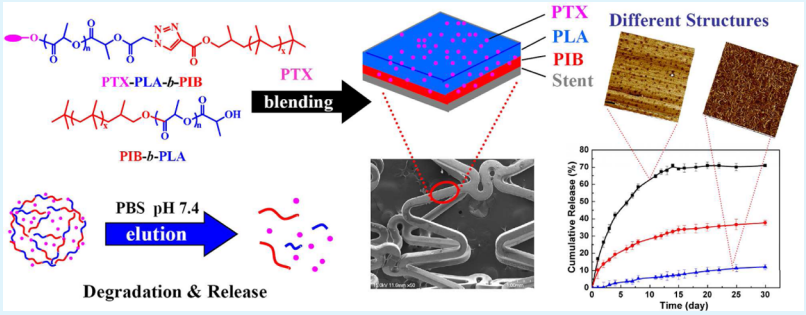

ABSTRACT: To develop a novel biodegradable and quite adhesive coating material for fabricating a paclitaxel (PTX)-containing eluting stent, herein, we report two kinds of drug eluting stent (DES) materials. One of them is a prodrug, PTX end-capped poly(lactic acid)-*b*-polyisobutylene (PTX-PLA-*b*-PIB) diblock copolymer, which possesses favorable biodegradability and biocompatibility. The other is a mixture of PIB-*b*-PLA diblock copolymer and PTX. PIB-*b*-PLA was synthesized via the ring-opening polymerization (ROP) using hydroxyl-terminated polyisobutylene (PIB-OH) as the initiator, while the PTX-PLA-*b*-PIB prodrug was prepared through a combination of ROP and Cu(I)-catalyzed azide−alkyne cycloaddition "click" reaction. The chemical structures and compositions as well as the molecular weights and molecular weight distributions of these copolymers have been fully characterized by ^{1}H nuclear magnetic resonance, Fourier transform infrared, and gel permeation chromatography measurements. The thermal degradation behavior and glass transition temperature (T_g) of the copolymers were studied by thermogravimetric analysis and differential scanning calorimetry, respectively. The solutions of PTX-PLA-*b*-PIB and the PIB-*b*-PLA/PTX mixture were separately coated onto the bare metal stents to form the PTX-containing DES. Subsequently, the surface structures and morphologies of the bare stent and DES were studied by atomic force microscopy and scanning electron microscopy, respectively. The *in vitro* release of PTX from these stents was conducted in a buffer medium (PBS 7.4) at 37 °C. The results showed that the coating formed by a blend of PTX-PLA-*b*-PIB, PIB-*b*-PLA, and PTX yielded a release that was better sustained than those of the individual PTX-PLA-*b*-PIB prodrug or PIB-*b*-PLA/PTX mixture. MTT assays demonstrated that the stent coated with PTX-PLA-*b*-PIB displayed a cytotoxicity lower than that of the PIB-*b*-PLA/PTX mixed layer, and the biocompatibility of coatings can be effectively improved by the prodrug.

KEYWORDS: *drug eluting stent, prodrug, biodegradability, drug delivery, paclitaxel*

INTRODUCTION

Coronary artery disease has been one of the most dangerous diseases in the world. Since the 1990s, the implantation of bare metal stents (BMS) revolutionized the field of interventional cardiology. However, the results of long-term investigation have been shattered by the dual problems of in-stent restenosis (ISR) and stent thrombosis associated with BMS.[1−5] To address these issues, polymer-based carriers loaded with drugs are utilized to coat the BMS, which is called a drug eluting stent (DES).[6−9] For a successful commercial DES product, the stent coating materials chosen to deliver drugs should be able to meet several key requirements, including nonreactivity with the incorporated drug, good stability, nontoxicity to cells, biocompatibility with vascular tissue, and the capacity to control the elution of the drug from the stent, as well as good physical properties that accommodate stent deformation during application without cracking or peeling.[10,11]

Since Pinchuk's group prepared triblock copolymer PS-*b*-PIB-*b*-PS (SIBS) via cationic polymerization,[12,13] Boston Scientific has developed several kinds of stents based on this

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soft elastomeric polymer, such as Taxus, Promus, and ION Stent.[14−16] SIBS has many advantages for stent application, such as favorable adhesion to metal, good elongation to accommodate stent expansion and deformation, processing compatibility with paclitaxel, chemical stability, fatigue resistance, and control of drug release.[17] Subsequently, Faust's group prepared various ABA-type copolymers based on polyisobutylene (PIB), such as PMMA-*b*-PIB-*b*-PMMA, PSOH-*b*-PIB-*b*-PSOH, PVF-*b*-PIB-*b*-PVF, etc.[18−21] They proved that PIB can be used as the polymer coating for DES because of its good physical properties and biocompatibility. However, the amount of PTX released from the blends of PIB-based copolymer coatings is relatively low, which somewhat limits their applications.

In recent years, biodegradable and bioabsorbable polymers that are used as DES coatings have made tremendous progress.[2−4,22−26] Poly(lactic acid) (PLA) is widely used because of its excellent biocompatibility and biodegradability.[27] A number of commercial DES based on PLA, like Axxess, JACTAX HD, and BioMatrix, were developed.[28−35] Researchers have demonstrated the satisfactory efficacy and safety profiles for these biodegradable polymer-based DES in treating patients, with low rates of stent thrombosis. Ge Junbo's group utilized poly(LA-*co*-MBC) in reaction with heparin and prepared a fully biodegradable polymer for DES.[36,37] The results showed that the polymer coated on the stent was almost degraded after 16 weeks and the As_2O_3 drug could be completely released.

A study of stent thrombosis after DES implantation during long-term follow-up showed that late-onset stent thrombosis occurred in some patients,[38,39] and this phenomenon was probably ascribed to the inflammation that resulted from the direct contact between the drug and the tissues. Meanwhile, the drug released in the lesion site can hardly maintain a long-term and sustained release, which would cause an ISR risk. In recent years, a polymeric drug delivery system has been widely used because of its advantages in prolonging the circulation time of the drug *in vivo* and decreasing drug exposure to normal tissues,[40−42] which can reduce the risk of late-onset stent thrombosis.

In this study, a PTX-immobilized biocompatible prodrug PTX-PLA-*b*-PIB was synthesized and used as the coating material to fabricate PTX-eluting stents for late-stage restenosis. In comparison, a PIB-*b*-PLA diblock copolymer was synthesized and used to physically load PTX. The *in vitro* release results showed that the DES coated by a blend of PTX-PLA-*b*-PIB, PIB-*b*-PLA, and PTX could release a relatively large amount of PTX at first and maintain a sustained release in the long term, while the prodrug used in the stent coating can effectively improve the biocompatibility of DES.

EXPERIMENTAL SECTION

Materials. Exo-olefin-terminated highly reactive polyisobutylene (HRPIB) was provided by the China PetroChina Jilin Chemical Group ($\bar{M}_n \approx 3600$ g mol^{-1}; PDI = 1.80). Stannous octoate [$Sn(Oct)_2$, 95%, Sigma-Aldrich] was distilled under reduced pressure before being used. L-Lactide (LA, 99%, TCI) was distilled from toluene before being used. Bromoacetic acid (C.P., Sinopharm Chemical Reagent), *N*,*N*′-diisopropylcarbodiimide (DIC, 99%, Sigma-Aldrich), propiolic acid (97%, TCI), 4-(dimethylamino)pyridine (DMAP, 99%, Shanghai Medpep), sodium azide (NaN_3, 98%, Sinopharm Chemical Reagent), *N*,*N*,*N*′,*N*″,*N*″-pentamethyl diethylenetriamine (PMDETA, 99%, J&K Chemical), paclitaxel (PTX, 99%, Beijing Zhongshuo Pharmaceutical Technology Development), and *N*,*N*-dimethylformamide (DMF, A.R., Sinopharm Chemical Reagent) were used as received. Cuprous bromide (CuBr, 95%, Sinopharm Chemical Reagent) was purified by being washed three times with glacial acetic acid and acetone, followed by drying under vacuum at room temperature overnight. Dichloromethane (CH_2Cl_2, A.R., Sinopharm Chemical Reagent) and toluene (A.R., Sinopharm Chemical Reagent) were separately dried by being refluxed over CaH_2 and distilled before being used. All the other chemicals were analytic reagents and were used as received unless otherwise mentioned. All the cell culture-related reagents were purchased from Invitrogen/Life Technologies.

Preparation of Hydroxyl-Terminated Polyisobutylene (PIB-OH). PIB-OH was produced by hydroboration/oxidation of commercial HRPIB using a previously published method.[43,44] The reaction was conducted in a three-neck 500 mL round-bottom flask with a magnetic stirring bar, equipped with a constant-pressure dripping funnel and a nitrogen inlet/outlet, which was immersed in an ice bath. Anhydrous tetrahydrofuran (THF) (150 mL) was added to the flask with sodium borohydride (3.79 g, 0.10 mol) under a dry argon atmosphere. The contents were stirred and equilibrated at 0 °C. Then, a certain amount of boron trifluoride diethyl etherate [$BF_3(C_2H_5)_2O$] (19 mL, 0.14 mol) was slowly added to the flask, and the reaction was allowed to proceed at 0 °C for 3 h to form BH_3 *in situ*. Subsequently, a solution of HRPIB (21 g, 6.0 mmol) dissolved in 60 mL of anhydrous THF was added dropwise to the solution at 0 °C while it was being stirred for 24 h. Afterward, an aqueous NaOH solution was added to this solution to adjust the pH value to ∼12, and then a required amount of hydrogen peroxide (30%, 50 mL) was added dropwise to the reactor. The reaction continued to proceed while the mixture was being stirred at 0 °C for 8 h. The resulting mixture was extracted with 50 mL of anhydrous diethyl ether. The organic layer was separated, washed with Milli-Q water to remove any remaining salts until the layer was neutral, and then dried over anhydrous $MgSO_4$. Finally, the solution was filtered and concentrated with a rotary evaporator, and the product was dried under vacuum at 40 °C for at least 2 days (17.2 g, 81.9% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.99 [s, 9H, $(CH_3)_3C$-], 1.11 [s, 360H, -$C(CH_3)_2$-], 1.41 [s, 120H, -$CH_2C(CH_3)_2$-], 1.58 [d, 2H, -$C(CH_3)_2CH_2$-], 1.73 [m, 1H, -$(CH_3)CHCH_2$-], 3.32, 3.48 [m, 2H, -$(CH_3)CHCH_2OH$].

Synthesis of PIB-*b*-PLA Diblock Copolymer. PIB-*b*-PLA was prepared by ring-opening polymerization (ROP) of the L-Lactide (LA) monomer using PIB-OH as the initiator and $Sn(Oct)_2$ as the catalyst. In brief, a 100 mL dry flask containing 40 mL of anhydrous toluene was charged with PIB-OH (1.8 g, 0.50 mmol), LA (2.55 g, 30 mmol), and $Sn(Oct)_2$ (0.10 g, 0.25 mmol). The flask was degassed through three exhausting−refilling nitrogen cycles, and the reaction was then conducted at 90 °C for 12 h while the mixture was being continuously stirred. Afterward, the mixture was concentrated and precipitated twice in cold hexane, and the viscous product was then dried under vacuum at 25 °C to a constant weight (3.86 g, 88.7% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.99 [m, 9H, $(CH_3)C$-], 1.11 [m, 360H, -$C(CH_3)_2$-], 1.41 [t, 120H, -$CH_2C(CH_3)_2$-], 1.58 [t, 2H, -$CH(CH_3)O$-], 5.18 [s, 1H, -$CH(CH_3)O$-].

Synthesis of PTX-PLA-N_3. An azide group end-capped prodrug was synthesized in three steps. In the first step, PTX-PLA-OH was prepared by a ROP reaction of LA monomer using the 2′-hydroxyl group in PTX as the initiating site and $Sn(Oct)_2$ as the catalyst. In brief, a 50 mL dry flask containing 15 mL of anhydrous toluene was charged with PTX (0.17 g, 0.20 mmol), LA (1.7 g, 12 mmol), and $Sn(Oct)_2$ (0.04 g, 0.10 mmol). The flask was degassed via three exhausting−refilling nitrogen cycles, and the reaction was conducted at 90 °C for 12 h while the mixture was being continuously stirred. Afterward, the mixture was concentrated and precipitated twice in cold methanol, and the viscous product was then dried under vacuum at 25 °C to a constant weight (1.48 g, 78% yield).

In the second step, PTX-PLA-Br was synthesized by the esterification between PTX-PLA-OH and bromoacetic acid. In a 50 mL dry flask, PTX-PLA-OH (0.60 g, 0.10 mmol), bromoacetic acid (0.084 g, 0.60 mmol), and DMAP (0.012 g, 0.10 mmol) were dissolved in 25 mL of anhydrous CH_2Cl_2 under a dry nitrogen atmosphere. The mixture was degassed via three exhausting−refilling

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nitrogen cycles. Then, DIC (0.04 g, 0.30 mmol) was added dropwise to the flask over 30 min. The reaction was conducted at 25 °C for 24 h. The mixture was filtered, and the solution was diluted with 50 mL of CH_2Cl_2 and washed twice with 10 mL of a PBS solution (pH 10.0). The combined organic phase was dried over anhydrous $MgSO_4$ for 2 h, and the filtrate was then concentrated and precipitated twice into 100 mL of cold methanol. The precipitates were collected and dried under vacuum at 30 °C to a constant weight to give PTX-PLA-Br (0.55 g, 80% yield).

The third step was the preparation of PTX-PLA-N_3. In a 50 mL dry flask, PTX-PLA-Br (1.74 g, 0.26 mmol) and NaN_3 (0.085 g, 1.3 mmol) were dissolved in 10 mL of DMF, and the reaction was conducted at 40 °C for 36 h. The mixture was passed through a short column of basic alumina to remove the insoluble salt. The solution was concentrated under vacuum, diluted with 50 mL of CH_2Cl_2, and washed twice with a PBS solution (pH 10.0). The combined organic phase was dried over anhydrous $MgSO_4$ for 2 h, and the filtrate was then concentrated and precipitated twice into 150 mL of cold methanol. The precipitates were collected and dried under vacuum at 30 °C to a constant weight to give PTX-PLA-N_3 (1.15 g, 68.5% yield).

Synthesis of PIB-Alkyne. The functional PIB-Alkyne was prepared by esterification of hydroxyl groups of PIB-OH with propiolic acid. In brief, in a 50 mL dry flask, PIB-OH (1.80 g, 0.50 mmol), DMAP (0.061 g, 0.50 mmol), and propiolic acid (0.35 g, 5.0 mmol) were dissolved in 20 mL of anhydrous CH_2Cl_2 under a dry nitrogen atmosphere. The mixture was degassed via three exhausting−refilling nitrogen cycles, and DIC (0.040 g, 0.30 mmol) was then added dropwise to the flask over 30 min. The reaction was conducted at 25 °C for 24 h. The mixture was filtered and the solution diluted with 50 mL of CH_2Cl_2 and washed twice with 20 mL of a PBS solution (pH 10.0). The combined organic phase was dried over anhydrous $MgSO_4$ for 2 h, and the filtrate was then concentrated and precipitated twice into 100 mL of cold methanol. The precipitates were collected and dried under vacuum at 30 °C to a constant weight to give PIB-Alkyne (1.42 g, 67.2% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.99 [s, 9H, $(CH_3)_3C$-], 1.11 [s, 360H, -$C(CH_3)_2$-], 1.41 [s, 120H, -$CH_2C(CH_3)_2$-], 1.61 [d, 2H, -$C(CH_3)_2CH_2$-], 2.87 [m, 1H, -$(CH_3)CHCH_2$-], 3.42 (s, 1H, -C≡C*H*), 3.91, 4.04 [m, 2H, -$(CH_3)CHCH_2O$-].

Synthesis of Polymeric Prodrug PTX-PLA-*b*-PIB. PTX-PLA-*b*-PIB was synthesized by Cu(I)-catalyzed azide−alkyne cycloaddition (CuAAC) "click" reaction between PTX-PLA-N_3 and PIB-Alkyne. A typical procedure was as follows. In a 50 mL dry flask, PTX-PLA-N_3 (0.44 g, 0.065 mmol), PMDETA (22.5 mg, 0.13 mmol), and PIB-Alkyne (0.257 g, 0.07 mmol) were dissolved in 10 mL of anhydrous CH_2Cl_2. The reactants were degassed by three exhausting−refilling nitrogen cycles. Subsequently, CuBr (0.0186 g, 0.13 mmol) was added to the flask under the protection of nitrogen, and the solution was degassed once again and stirred at 25 °C under a nitrogen atmosphere for 24 h. The reaction was stopped by exposing the system to air. The mixture was diluted with 100 mL of CH_2Cl_2 and passed through a short column of basic alumina. The solution was then concentrated and precipitated twice into 100 mL of cold hexanes. The precipitates were collected and dried under vacuum to give the polymeric prodrug PTX-PLA-*b*-PIB (0.71 g, 61% yield).

Characterizations. ^{1}H NMR spectra were recorded with an INOVA-400 (Varian) NMR spectrometer at room temperature with $CDCl_3$ as the solvent and TMS as the internal reference. Fourier transform infrared (FT-IR) spectra were recorded on a Nicolet 6700 spectrometer using the KBr disk method. The number-averaged molecular weights and molecular weight distributions (PDIs) of polymers were measured on a Waters 1515 GPC instrument with a Waters 1515 isocratic high-performance liquid chromatography (HPLC) pump, a Waters 2414 refractive index detector, a Waters 717 plus autosampler, and a set of MZ-Gel SD plus columns (300 mm × 8.0 mm; 500, 103, and 104 Å). THF was used at a flow rate of 1 mL min^{-1}, and the narrowly distributed polystyrene standards were used for calibration.

Contact Angle Measurement. The thin films of PTX-PLA-*b*-PIB and the PIB-*b*-PLA/PTX mixture were prepared by casting the polymer solutions in CH_2Cl_2 (0.5 wt %) onto the silicon pellets. The films on the slides were dried by being placed in a desiccator for 12 h, followed by vacuum drying for 3 days. The static contact angle of the films was measured using a JC2000D6 contact angle goniometer (Shanghai Zhongchen Powereach). A droplet of Milli-Q water (5 μL) was placed on the surface of the tested film at room temperature; the contact angle was measured after a static time of 30 s, and a tangent method was applied to obtain the contact angle value in degrees.

Thermogravimetric Analysis (TGA). TGA was performed using a TG/DTA 6300 (SII Nano Technology) instrument, and the data were recorded over a temperature range of 100−650 °C under a nitrogen atmosphere at a heating rate of 10 °C min^{-1}.

Differential Scanning Calorimetry (DSC). The PTX-PLA-*b*-PIB and PIB-*b*-PLA/PTX films blended with 5−15% PTX at 5% increments were prepared for DSC analysis. The glass transition temperatures (T_g) of the copolymer blends were determined by a Q200 (TA) instrument using a heating rate of 10 °C min^{-1}.

Atomic Force Microscopy (AFM). The polymer morphologies on the coating surface of stents were determined using AFM. A Dimension 3100 atomic force microscope (Digital Instruments/Veeco Metrology) controlled with NanoScope IV and NanoScope Extender electronics was used. Silicon sheets coated with PIB-*b*-PLA and PTX-PLA-*b*-PIB copolymers with and without PTX were analyzed by AFM. Samples were examined in the dry state using tapping mode.

Scanning Electron Microscopy (SEM). Bare metal stents were washed with ethanol and 2-propanol and then dried under vacuum at 25 °C for 1 day. The polymer-coated stents were prepared via a dip-coating method with 1 mg of copolymer dissolved in CH_2Cl_2 at 0.1 wt % and then dried under vacuum at 25 °C for 3 days. The morphologies of the coated stents were examined using a JSM-6460 scanning electron microscope (JEOL). The coated stents did not require an additional coating of a conductive thin layer (for example, gold or carbon) prior to examination. An accelerating voltage of 1 kV was used for collecting the secondary electron images.

***In Vitro* Drug Release.** The profile of *in vitro* release of PTX from the stents was evaluated by immersing each stent in a 50 mL tube equilibrated with 30 mL of PBS (pH 7.4) at 37 °C in a shaking bath. Five milliliters of the release medium was taken out at desired time intervals for lyophilization, and the sample was used for HPLC analysis. Meanwhile, 5 mL of fresh buffer was replenished to keep a constant volume. For comparison, the release of free PTX was conducted under the same conditions. The concentrations of PTX were determined by HPLC analysis. All the release experiments were conducted in triplicate, and the results were the average data with the standard deviation. Finally, to determine the residual drug remaining on the stent after drug release had been terminated, the residual polymers on the stents were dissolved in CH_2Cl_2 and the residual drug contents were measured by HPLC analysis.

***In Vitro* Degradation.** The *in vitro* degradation of PLA from the stents was evaluated by immersing each stent in a 50 mL tube equilibrated with 30 mL of PBS (pH 7.4) at 37 °C in a shaking bath. To characterize the degree of degradation, the molecular weights of polymer films from the stents were investigated by ^{1}H NMR analysis (n = 3 for each time point).

***In Vitro* Cytotoxicity Assay.** A standard MTT assay was employed to evaluate the cytotoxicity of the prodrug and PLA-*b*-PIB copolymer. L929 fibroblast cells were seeded in a 96-well plate at a density of ~5 × 10^4 cells/well and cultured in DMEM with 10% serum and 1% penicillin/streptomycin in an incubator at 37 °C in a 5% CO_2 atmosphere for 24 h. The samples with different concentrations were then added to different wells and incubated with cells for an additional 48 h. Afterward, 25 μL of a MTT stock solution (5 mg mL^{-1} in PBS) was added to each well. After incubation for an additional 4 h, DMEM was removed and 150 μL of DMSO was added to each well. The optical density (OD) at 570 nm of each well was measured on a Bio-Rad 680 microplate reader. The absorbance values were normalized to wells in which cells were not treated with samples. Data are presented as the average values with standard deviations.

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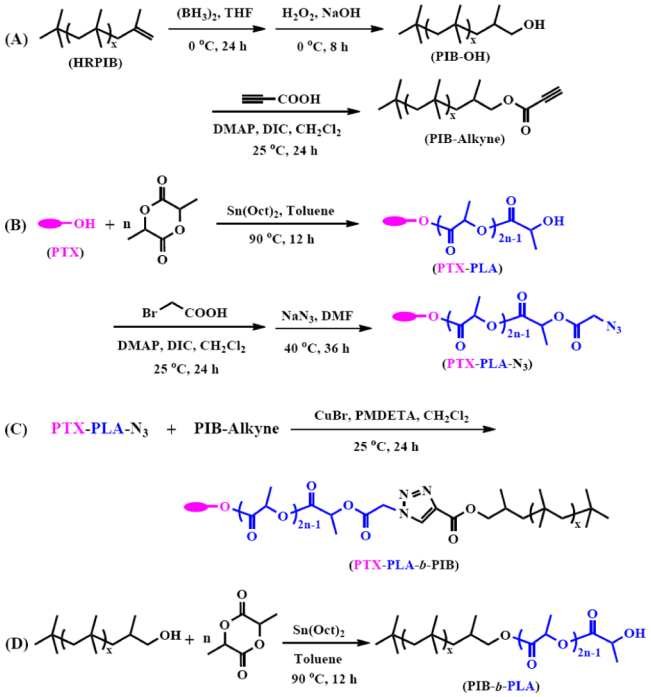
RESULTS AND DISCUSSION

Synthesis and Characterization of PTX-PLA-*b*-PIB and PIB-*b*-PLA. Over the past >10 years, "click" chemistry has become widely recognized as a highly versatile technique for the efficient synthesis of various block copolymers and functional polymeric prodrugs.[45,46] For a detailed preparation stage of PIB-OH, the hydroboration/oxidation of a commercial exo-olefin-terminated highly reactive polyisobutylene (HRPIB) has been reported by Storey et al. and our group.[43,44] In this study, the hydroxyl group at the end of the PIB chain was transferred to an alkynyl group via esterification as shown in Scheme 1A.

Scheme 1. Synthesis of the PTX-PLA-*b*-PIB Prodrug and the PIB-*b*-PLA Diblock Copolymer



The ^{1}H NMR spectra of HRPIB, PIB-OH, and PIB-Alkyne are shown in Figure 1. For PIB units, the chemical shifts at δ 1.41 and 1.11 (peaks b and c, respectively) were attributed to the protons of -CH_2- and -C(CH_3)$_2$-, respectively. The peaks at δ 4.64 and 4.85 (peaks f and f′, respectively) in Figure 1A, which were attributed to the protons of -C=CH_2 in HRPIB, disappeared in Figure 1B after hydroboration/oxidation. Besides, the new chemical shift at δ 2.78 (peak h) in Figure 1C was ascribed to the proton of -C≡CH in PIB-Alkyne, demonstrating the successful synthesis of PIB-Alkyne.

PTX can be used as an initiator to polymerize cyclic monomers as reported by Cheng et al.,[47,48] who prepared PTX-polylactide (PTX-PLA) prodrugs by a PTX-initiated ROP of LA in the presence of metal (Mg or Zn) alkoxides and an organic chelating ligand. In our recent work, we utilized PTX as an initiator for the ROP of the 2-ethoxy-2-oxo-1,3,2-dioxaphospholane (EOP) monomer with Sn(Oct)$_2$ as the catalyst to obtain the PTX-PEEP prodrug.[40] Herein, as shown in Scheme 1B, we first prepared PTX-PLA-OH via ROP of LA and then conducted the esterification reaction with bromoacetic acid in the presence of DMAP and DIC to yield PTX-PLA-Br. A nucleophilic substitution reaction with NaN_3 was

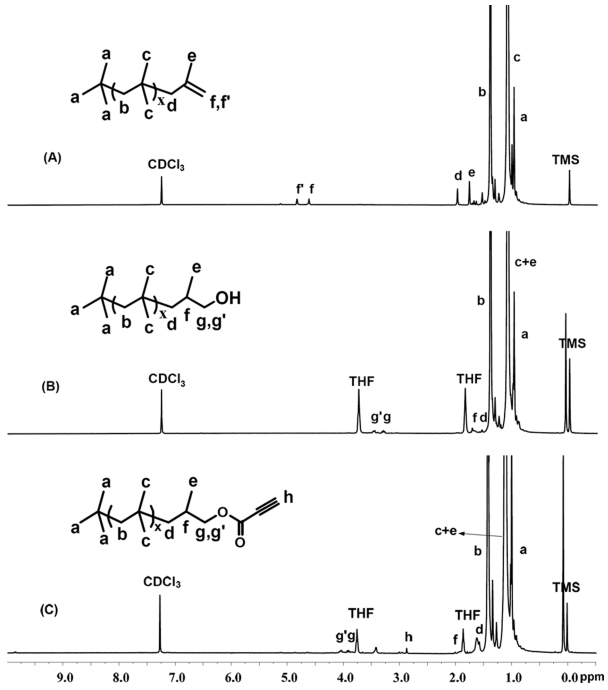


Figure 1. ^{1}H NMR spectra of (A) HRPIB, (B) PIB-OH, and (C) PIB-Alkyne in $CDCl_3$.

further conducted, and the azide-terminated PTX-PLA-N_3 was obtained. Panels A and B of Figure 2 show the ^{1}H NMR spectra of PTX and PTX-PLA-OH, respectively. All the characteristic peaks ascribed to the protons in the samples can be found in

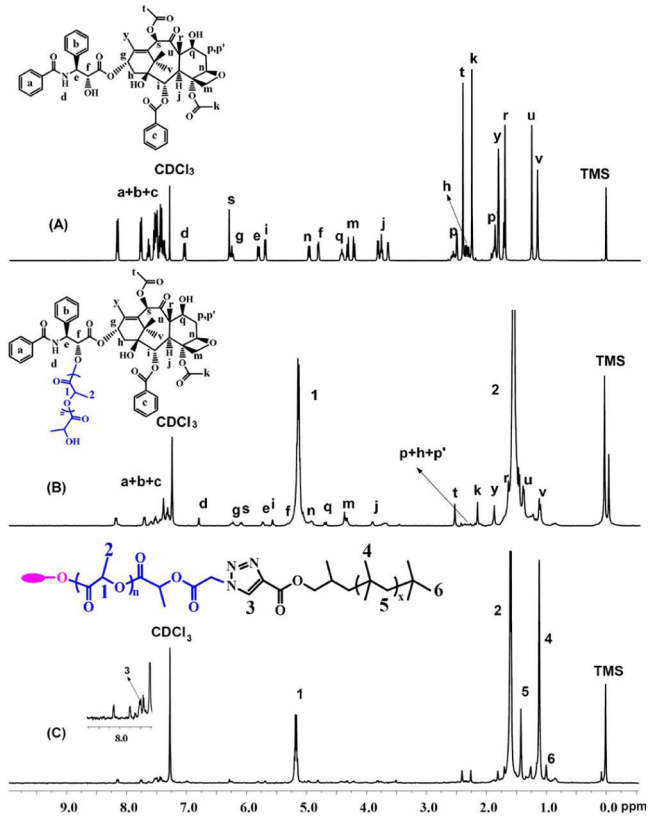


Figure 2. ^{1}H NMR spectra of (A) PTX, (B) PTX-PLA-OH, and (C) PTX-PLA-*b*-PIB in $CDCl_3$.

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the spectra. It is worth noting that in comparison with the spectrum of PTX in Figure 2A, new peaks attributed to the protons of PLA (peaks 1 and 2) are clearly observed in Figure 2B. Figure 3 shows the FT-IR spectra of PTX-PLA-Br and PTX-PLA-N_3. Compared to Figure 3A, a new strong absorption peak at 2109 cm^{-1} appeared in Figure 3B, which corresponded to the azide group.

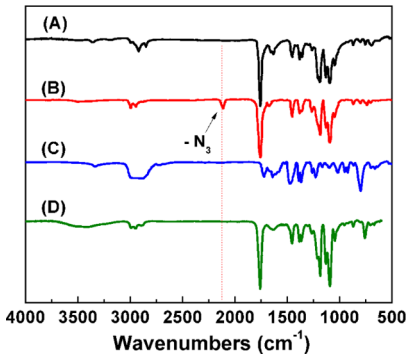

Figure 3. FT-IR spectra of (A) PTX-PLA-Br, (B) PTX-PLA-N_3, (C) PIB-Alkyne, and (D) PTX-PLA-*b*-PIB.

On the basis of the ^{1}H NMR spectrum of PTX-PLA-OH in Figure 2B, the molecular weight ($\bar{M}_{n,NMR}$) of PTX-PLA-N_3 was calculated by comparing the integrations of PTX amino -N***H***- at δ 6.83 (peak d) and PLA methylene -(C═O)-C***H***(CH_3)-O- (peak 1) at δ 5.18. The calculating equation is as follows:

$$\bar{M}_{n,NMR(PTX\text{-}PLA\text{-}N_3)} = \frac{A_1}{2A_d} \times 144.13 + 946.04$$

where A_1 and A_d are the integral values of peaks 1 and d in Figure 2B, respectively, 144.13 is the molecular weight of one repeating unit of PLA block, and 946.04 is the molecular weight of the PTX and terminal azidoacetic acid group.

Finally, the conjugation of PIB-Alkyne and PTX-PLA-N_3 was achieved by the CuAAC "click" reaction to form a new polymeric prodrug PTX-PLA-*b*-PIB as shown in Scheme 1C. Considering that it was difficult to quantitatively perform the click reaction, we used a slight excess of PIB-Alkyne in the reaction. After reaction, the residual PIB-Alkyne could be easily removed by precipitation in hexanes. Figure 2C displays the ^{1}H NMR spectrum of purified PTX-PLA-*b*-PIB, from which one can find that in addition to all the proton signals of PIB-Alkyne and PTX-PLA-N_3, the spectrum shows a new characteristic signal at δ 7.52 (peak 3), which was attributed to the protons of the triazole ring, and demonstrated the successful "click" reaction. It is clearly observed from Figure 3D that the absorption peak at 2109 cm^{-1} attributed to the azide group completely disappeared after the "click" reaction. Figure 4 displays the GPC traces of PIB-Alkyne, PTX-PLA-OH, and PTX-PLA-*b*-PIB. The GPC curve of PTX-PLA-*b*-PIB displays a major distribution that shifts toward the higher-molecular weight side compared with that of PTX-PLA, indicating that the PTX-PLA-*b*-PIB prodrug has been successfully prepared.

As shown in Scheme 1D, PIB-*b*-PLA was prepared via a ROP of LA using PIB-OH as the initiator. The compositions and molecular weights of these copolymers were similar to the values calculated from the feed ratio of PIB-OH to LA monomer, as shown in Table 1. The $\bar{M}_{n,GPC}$ of the PIB-*b*-PLA copolymers was also determined by GPC for comparison with the result obtained via ^{1}H NMR.

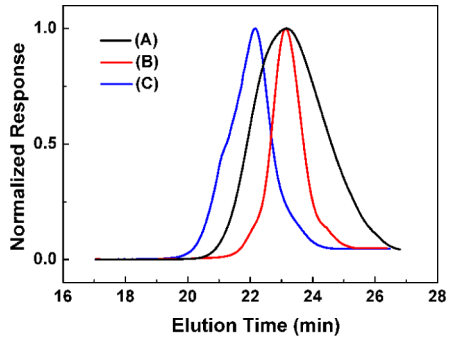

Figure 4. GPC curves of (A) PIB-Alkyne, (B) PTX-PLA-OH, and (C) PTX-PLA-*b*-PIB.

Table 1. Characterization Data of the Compositions and Molecular Weights of Various Copolymers

sample	$\bar{M}_{n,theor.}$[a] (g mol^{-1})	$\bar{M}_{n,NMR}$[b] (g mol^{-1})	$\bar{M}_{n,GPC}$[c] (g mol^{-1})	PDI[c]
PIB-OH	3600	3600	3980	1.87
PIB-*b*-PLA_{42}	9360	9650	11800	1.50
PTX-PLA_{40}-N_3	6710	6710	7200	1.12
PTX-PLA_{40}-*b*-PIB	10310	10310	12000	1.31

[a]Theoretical molecular weight. [b]Calculated from ^{1}H NMR spectra. [c]Determined by GPC with THF as the eluent and polystyrene as the standard.

Contact Angle Measurements. To evaluate the hydrophobicity of the PTX-PLA-*b*-PIB prodrug and PIB-*b*-PLA/PTX (10 wt %) mixture, the contact angles of water droplets on the polymer thin films were measured with PIB-OH and PIB-*b*-PLA films as the control samples. From Figure 5A–D, one can find that the contact angles increased from 86° (PIB-OH) to 96° (PIB-*b*-PLA) and 95° (PIB-*b*-PLA/PTX mixture film) because the PLA block enhanced the hydrophobicity. The further increase in the contact angle to 100° (PTX-PLA-*b*-PIB) could be attributed to the introduction of hydrophobic PTX.

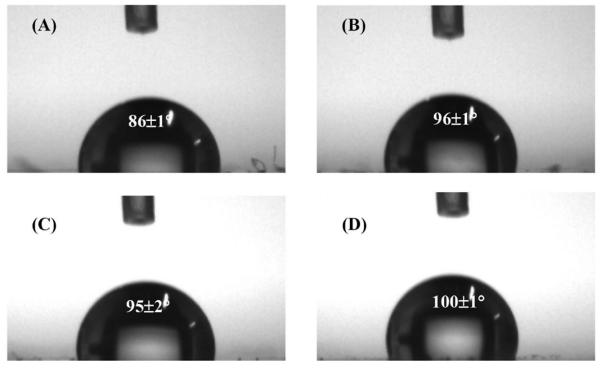

Figure 5. Photographs of water droplets and the obtained contact angle values on various polymer films of (A) PIB-OH, (B) PIB-*b*-PLA, (C) the PIB-*b*-PLA/PTX mixture (10 wt %), and (D) PTX-PLA-*b*-PIB.

Thermogravimetric Analysis. The TGA thermograms of PIB, PTX-PLA, and PTX-PLA-*b*-PIB are shown in Figure 6. Two single-step degradations at ~200 and ~310 °C were observed, which could be attributed to PTX-PLA and PIB, respectively. Two-step degradation was observed in the PTX-PLA-*b*-PIB system. The first onset degradation temperature at ~200 °C could be associated with the PLA segment. The

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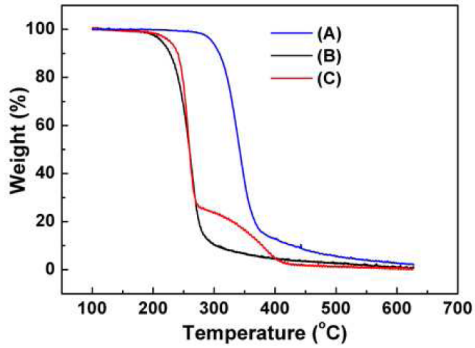

Figure 6. TGA thermograms of (A) PIB-OH, (B) PTX-PLA, and (C) PTX-PLA-*b*-PIB.

second degradation at a higher temperature (~310 °C) was attributed to the PIB segment.

Differential Scanning Calorimetry. The miscibility between the drug and each segment of a block copolymer could be demonstrated by a shift in the specific block glass transition toward the T_g of PTX (150 °C).[49] The unknown physical interaction would affect the release of the drug from DES. Figure 7A shows the thermograms with a T_g of 66 °C for

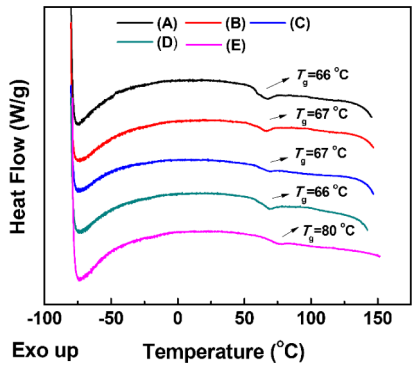

Figure 7. DSC thermograms of (A) PIB-*b*-PLA, (B) the PIB-*b*-PLA/PTX mixture (5 wt %), (C) the PIB-*b*-PLA/PTX mixture (10 wt %), (D) the PIB-*b*-PLA/PTX mixture (15 wt %), and (E) PTX-PLA-*b*-PIB with a scanning rate of 10 °C min^{-1} in the second heating run.

PLA block. When the PIB-*b*-PLA diblock copolymer was mixed with different amounts of PTX at 5, 10, and 15 wt %, based on the weights of PIB-*b*-PLA, their T_g exhibited little change as shown in spectra B–D of Figure 7, respectively. These results indicated that the PTX drug was not miscible with the PIB-*b*-PLA diblock copolymer. In contrast, the polymeric prodrug PTX-PLA-*b*-PIB displayed a changing T_g at 80 °C as shown in Figure 7E, and a shift in the T_g value of the hard block of PTX-PLA-*b*-PIB appeared, which was located between the T_g values of PTX and PLA, suggesting the possible interaction between both blocks.

Atomic Force Microscopy. The surface structure of coating on the stent plays an important role in drug release.[18,19] Panels A–C of Figure 8 show the AFM images of the film surface of stents coated with PIB-*b*-PLA, the PIB-*b*-PLA/PTX mixture (10 wt %), and PTX-PLA-*b*-PIB, respectively. For PTX-PLA-*b*-PIB, the content of PTX was 9 wt %. The AFM phase image of the PIB-*b*-PLA-coated stent showed the typical microphase-separated block copolymer morphology in Figure 8A, while there was a clear third phase in Figure 8B for the PIB-*b*-PLA/PTX mixture (10 wt %). This was because PTX was not

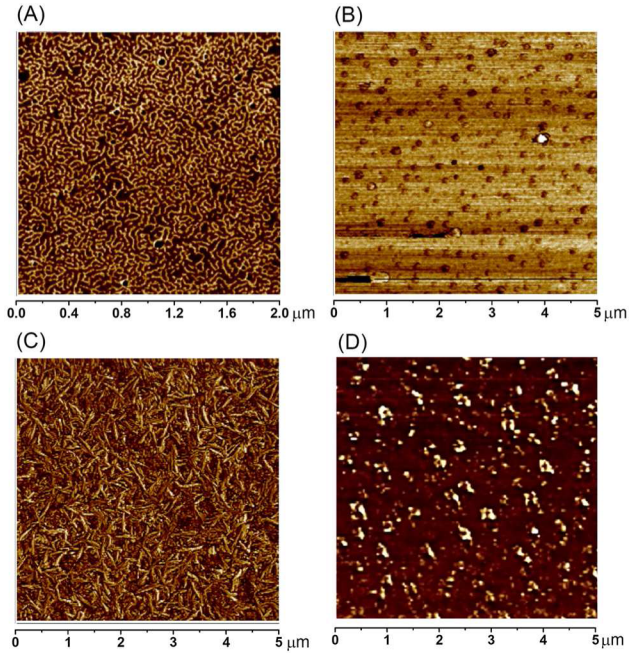

Figure 8. AFM phase images of the film surface of stents coated with (A) PIB-*b*-PLA (2 μm scan), (B) the PIB-*b*-PLA/PTX mixture (10 wt %) (5 μm scan), (C) PTX-PLA-*b*-PIB (5 μm scan), and (D) a blend of the PIB-*b*-PLA/PTX mixture (10 wt %) and PTX-PLA-*b*-PIB [1:1 (w/w)] (5 μm scan).

miscible with the PIB-*b*-PLA diblock copolymer and formed partial nanoparticles. However, no holes or voids were observed in either case. After PTX was linked with PIB-*b*-PLA through a covalent bond, the AFM phase image in Figure 8C shows well-distributed short rodlike aggregates. This phenomenon can be explained by the possible interaction between PTX and PLA, which allowed the two components to be easily miscible and resulted in the new form of the uniform microphase-separated domain. We speculated that PTX was linked to a linear polymer and presented a rodlike structure. This hypothesis was also supported by the DSC results shown in Figure 7. In addition, we have also tested the AFM phase image of a coating blended with the PIB-*b*-PLA/PTX mixture (10 wt %) and PTX-PLA-*b*-PIB [1:1 (w/w)]. The result showed that an irregular structure was found on the surface, which was probably because the introduction of the PIB-*b*-PLA/PTX mixture destroyed the regular structure of the PTX-PLA-*b*-PIB prodrug.

Scanning Electron Microscopy. Surface topography is important for the *in vivo* performance of DES.[50] Webbings and bridges between the struts may cause inflammation and VSMC proliferation, consequently resulting in higher rates of thrombosis and restenosis. Figure 9 shows the SEM images of bare metal stents and PTX-eluting stents. The stent coating in Figure 9B was smooth and conformal, and no delamination or destruction of the coating on the stent can be observed after expansion, indicating that the polymer coating possesses sufficient flexibility to allow balloon expansion of the stent without cracking or peeling from the struts.

***In Vitro* Drug Release.** The *in vitro* release of PTX from stents coated with the PIB-*b*-PLA/PTX mixture (10 wt %), PTX-PLA-*b*-PIB, and a blend of the PIB-*b*-PLA/PTX mixture (10 wt %) and PTX-PLA-*b*-PIB [1:1 (w/w)] was studied. As shown in Figure 10, 70% of PTX was released from the PIB-*b*-PLA/PTX mixture (10 wt %) in a buffer solution (pH 7.4) after

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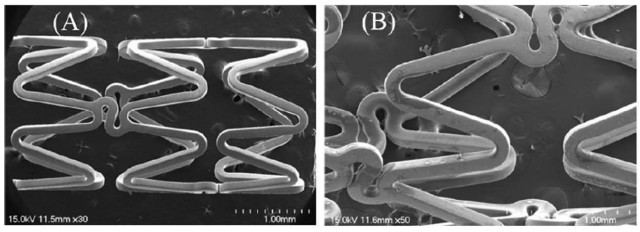

Figure 9. SEM images of (A) a bare metal stent (30×, scale bar of 1.00 mm) and (B) a stent coated with PTX-PLA-*b*-PIB (50×, scale bar of 1.00 mm).

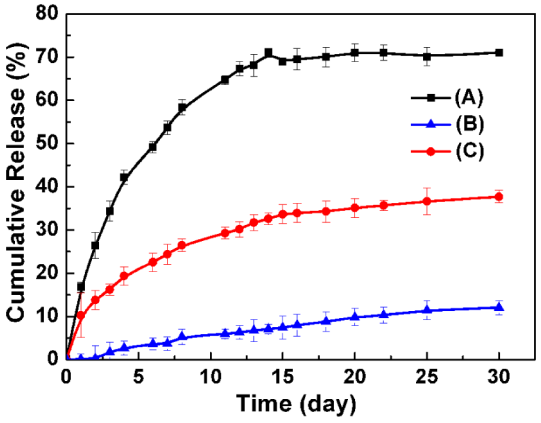

Figure 10. *In vitro* release of PTX from coronary stents coated with (A) the PIB-*b*-PLA/PTX mixture (10 wt %), (B) PTX-PLA-*b*-PIB, and (C) a blend of the PIB-*b*-PLA/PTX mixture (10 wt %) and PTX-PLA-*b*-PIB [1:1 (w/w)].

15 days, while <10% of PTX was released from the PTX-PLA-*b*-PIB prodrug under the same conditions, indicating that the prodrug has a good durability and maintains the partial drug on the coating film. Figure 10A shows a relatively high rate of release of PTX for the PIB-*b*-PLA/PTX mixture (10 wt %) in the first 15 days and then a slow rate of release within the next 15 days. Meanwhile, Figure 10B shows a slow but sustained release for PTX-PLA-*b*-PIB, which can steady release the drug for a long time. These results indicated a significantly different release mechanism between the two systems. The DSC and AFM analyses of the PIB-*b*-PLA/PTX mixture (10 wt %) showed that the polymer and PTX were not miscible with each other. Therefore, PTX was easily released from the coating mainly by physical force of elution, which caused a fast release.[18,19] On the other hand, according to the AFM images of PTX-PLA-*b*-PIB, such a coating presents a rodlike structure. Because of the covalent bonds between PTX and PLA, the release of PTX relies on the degradation of the PLA segment. The results showed that the PIB-*b*-PLA/PTX mixture (10 wt %) released relatively large amounts of PTX in the first 15 days and could not achieve a long-term and stable treatment effect. The PTX-PLA-*b*-PIB prodrug slowly released PTX but maintained a long-time and stable release, it would be suitable for preventing stent thrombosis. Considering both advantages, we fabricated a blend of the PIB-*b*-PLA/PTX mixture (10 wt %) and PTX-PLA-*b*-PIB [1:1 (w/w)] and coated it on the stent to study *in vitro* PTX release. As expected, 30% of PTX was released from the coating in ~15 days, which was greater than that of the prodrug alone but lower than that of the PIB-*b*-PLA/PTX mixture, as shown in Figure 10C. This new blend can be used as a potential coating material on the stents for maintaining a long-term drug release. In addition, to ensure that the drug was not lost by some other mechanism, the residual drug contents remaining on the stents after terminating the drug release studies were measured by HPLC analysis. The results showed that the residual PTX values of the PIB-*b*-PLA/PTX mixture (10 wt %), polymeric prodrug PTX-PLA-*b*-PIB, and a blend of the PIB-*b*-PLA/PTX mixture (10 wt %) and PTX-PLA-*b*-PIB [1:1 (w/w)] were 28.2, 85.5, and 61.1%, respectively, which proved that the release rates are correct as discussed above.

***In Vitro* Degradation.** The degradation rate of the polymer plays a key role in modulating the drug release behavior.[27,48] To study the effects of PLA degradation on drug release, the PLA degradation experiment was conducted. The results showed that 75% of PLA segments were degraded in 30 days (Figure 11). However, the rate of release of the drug from the PTX-PLA-*b*-PIB prodrug was much slower than the rate of degradation of PLA. The released drug was measured in its original form, and the analysis of drug content did not account for those PTX linked with some low-molecular weight PLA segments after degradation.

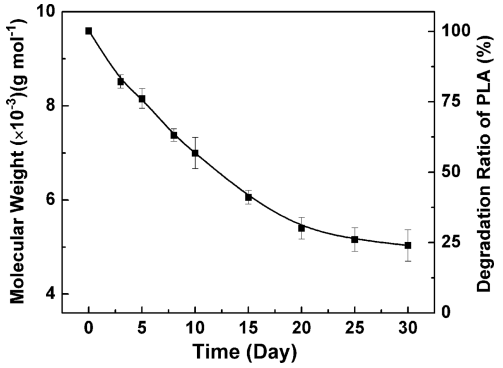

Figure 11. *In vitro* degradation profile of PIB-*b*-PLA, in which the molecular weight was calculated from ^{1}H NMR spectra.

***In Vitro* Cytotoxicity.** Biocompatibility is one of the most important properties to be considered in selecting coating materials for DES. Figure 12 shows the *in vitro* cytotoxicity of PIB-*b*-PLA, PTX-PLA-*b*-PIB, the PIB-*b*-PLA/PTX mixture (10 wt %), and the blend of the PIB-*b*-PLA/PTX mixture (10 wt

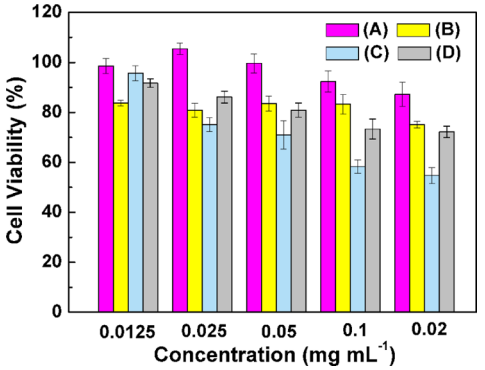

Figure 12. Cell viabilities of L929 fibroblast cells incubated with (A) PIB-*b*-PLA, (B) PTX-PLA-*b*-PIB, (C) the PIB-*b*-PLA/PTX mixture (10 wt %), and (D) the blend of the PIB-*b*-PLA/PTX mixture (10 wt %) and PTX-PLA-*b*-PIB [1:1 (w/w)] at different concentrations for 48 h.

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%) and PTX-PLA-*b*-PIB [1:1 (w/w)] against L929 fibroblast cells evaluated by the MTT assay. The viabilities of L929 cells incubated with PIB-*b*-PLA and the prodrug are all above 80% after incubation for 48 h, demonstrating that they have low cytotoxicity. Meanwhile, the viabilities of L929 cells incubated with PTX-PLA-*b*-PIB (Figure 12B) and the blend of the PIB-*b*-PLA/PTX mixture (10 wt %) and PTX-PLA-*b*-PIB (Figure 12D) are higher than that of the PIB-*b*-PLA/PTX mixture (10 wt %) (Figure 12C), indicating that the prodrug could decrease the toxicity of free PTX.

CONCLUSIONS

In summary, a PTX-immobilized biodegradable prodrug PTX-PLA-*b*-PIB was successfully synthesized and used as the coating material to fabricate a PTX-eluting stent for late-stage restenosis. These polymers were proven to be hydrophobic by contact angle measurements. AFM results exhibited typical block copolymer phase separation and different structures between the PIB-*b*-PLA/PTX mixture (10 wt %) and PTX-PLA-*b*-PIB. The *in vitro* release of PTX from a PIB-*b*-PLA/PTX-coated stent was quite different from that of the stent coated with the PTX-PLA-*b*-PIB prodrug using the same formulation. In addition, a combination of PIB-*b*-PLA, PTX, and PTX-PLA-*b*-PIB can both prevent the fast release of PTX from stents and prolong the release time of PTX. MTT assays indicated that PTX-PLA-*b*-PIB displayed a cytotoxicity lower than that of the PIB-*b*-PLA/PTX mixed layer, and the result showed that the cytotoxicity of coatings can be decreased by the prodrug strategy. Therefore, a combination of PIB-*b*-PLA and PTX-PLA-*b*-PIB can be considered as a new type of polymer coating for loading PTX. This new material can prolong the release time of PTX and reduce the risk of possible inflammation.

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Notes

The authors declare no competing financial interest.

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