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Development of a probucol-releasing antithrombogenic drug eluting stent

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Abstract: The success of drug eluting stents (DESs) has been challenged by the manifestation of late stent thrombosis after DES implantation. The incomplete regeneration of the endothelial layer poststenting triggers adverse signaling processes precipitating in thrombosis. Various approaches have been attempted to prevent thrombosis, including the delivery of biological agents, such as estradiol, that promote endothelialization, and the use of natural polymers as coating materials. The underlying challenge has been the inability to release the biological agent in synchronization with the temporal sequence of vascular wound healing *in vivo*. The natural healing process of the endothelium after an injury starts after a week and may take up to a month in humans. This article presents a novel DES formulation using a hemocompatible polyurethane (PU) matrix to sustain the release of probucol (PB), an endothelial agonist, by exploiting the greater difference in the solubility parameters of PB and PU. This results in the formation of crystalline PB aggregates retarding drug release from PU. The physicochemical properties of PB in PU were confirmed using differential scanning calorimetry and X-ray diffraction. Drug-polymer compatibility was examined using infrared spectral analysis. Also, *in vitro* studies using primary human aortic endothelial cells resulted in the selection of 5% w/w PB as the optimal dose, to be further tested *in vitro* and *in vivo*. This work develops and tests a promising new DES formulation to enable faster endothelial cell proliferation poststenting, potentially minimizing the incidence and severity of thrombotic events after DES implantation. © 2012 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater100B: 1068–1077, 2012.

Key Words: endothelial cells, endothelialization, hemocompatibility, sustained release, thrombogenicity

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INTRODUCTION

Recent advances in the drug eluting stent (DES) technology have been targeted toward promoting endothelialization after DES deployment. DESs have been highly effective in reducing the rates of restenosis[1–5] but have been plagued by late stent thrombosis (LST).[6] Late stent thrombosis is defined as platelet-rich thrombus occupying >25% of vascular lumen for greater than 30 days after DES implantation.[7] This complication is caused mainly by the nonselective action of the currently used DES drugs—these do not selectively inhibit the proliferation of smooth muscle cells (SMCs) but also delay endothelialization and thus precipitate a delay in vessel wall healing. Although the incidence of LST is low, and possibly no different than with bare metal stents (BMSs),[8] LST with DESs frequently results in major myocardial infarction or death.[9–12] This has dampened the initial enthusiasm for the use of DESs over the use of BMSs. Though a normally functioning endothelium is essential to prevent thrombosis, an endothelium activated by injury or by vascular interventions can become thrombogenic resulting in conditions such as LST.[7]

The normal functioning vascular endothelium, via its wide array of functions, such as barrier regulation of permeability, production of growth inhibitory molecules, thrombogenicity, and leukocyte adherence, modulates SMC proliferation and can consequently prevent restenosis after vascular interventions.[13–16] The time required by human vascular cells to reendothelialize after injury is 2 weeks and the process of healing can prolong for a month.[17] It is a widely known theory that rapid reendothelialization can preclude or, at least, decrease the incidence and severity of clinical sequelae such as thrombosis postinjury and/or intervention.[18] Though the primary cause for the delayed reendothelialization with DES culminating in LST is not completely understood, the incidence of this condition has been attributed to multiple factors. These include patient and lesion characteristics,[19] local hypersensitivity reaction from the polymer or drug,[20,21] stenting a bifurcated lesion

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TABLE I. Calculated Solubility Parameter Values of PB and Different Polymers Considered for the Tested Sustained Release Formulations

Compound	Solubility parameter (δ) $[MPa]^{1/2}$
PB	12.63
PU	25.08
PLGA	21.26
PEVA	18.56

PB: probucol, PU: polyurethane, PLGA: poly(lactic-*co*-glycolic acid), PEVA: poly(ethylene-*co*-vinyl acetate).

or stent strut penetration into the necrotic core of an atherosclerotic plaque.[22]

To develop a successful DES, two things need to be accomplished. First, one needs to incorporate one or more pharmaceutical agents that promote the regeneration of a functional endothelium while inhibiting SMC proliferation. Second, one needs to control the release kinetics of the agent(s) to maintain the optimal therapeutic dose synchronizing with the temporal healing response *in vivo*. Kang et al. described the efficacy of a novel xanthine-based derivative 1,3-dipropyl-8-cyclopentyl xanthine (DPCPX) that can specifically act on A1 adenosine receptor preventing SMC proliferation.[23] However, no report has yet been published demonstrating a therapeutic agent that in a controlled release format can simultaneously promote endothelialization and inhibit SMC proliferation.

Recently, research has involved delivering more than one biological agent concurrently from a DES in an attempt to prevent both restenosis and the occurrence of ST. Huang et al. in a recent study, developed a dual DES to release the antiproliferative drug—sirolimus and the platelet aggregation inhibitor—triflusal (2-acetoxy-4-trifluoromethylbenzoic acid) from a biodegradable polylactide-*co*-glycolide (PLGA)-coated metallic stent.[24,25] The *in vivo* study using this locally delivered combination therapy demonstrated superior restenotic inhibition at 30 days resulting from an inhibition of both inflammation and thrombus formation. Other promising approaches toward improving outcomes after stent insertion include engineering the stent surface by seeding endothelial progenitor cells[26–28] or by applying a polymeric coating, for example, of heparin[29] or phosphorylcholine,[30] or, of a biodegradable polymer, for example, polylactic acid.[8,31]

There is a definite lack of scientific evidence to show the effects of drug–polymer interaction on the release kinetics of the drugs released from the polymer-coated DESs. Drug–polymer interaction controls the physicochemical properties of the drug embedded in a polymer matrix. Physicochemical properties of the drug play a crucial role in controlling its release kinetics. Drugs can exist in either an amorphous or a crystalline form depending on its solid state solubility property (determined by its interaction with the polymer) when embedded in the polymer matrix. It is important to study the effects of this drug–polymer interaction when developing a polymer-coated DES to clearly understand the release mechanism of the drug from the surrounding matrix.

In this work, we hypothesize that the drug release from a polymer-coated DES can be engineered by controlling the physicochemical form in which the drug is distributed in the coated polymer matrix. To test this hypothesis, an endothelial regenerating agent probucol (PB) was incorporated in two different biocompatible carriers, polyurethane (PU) and polyethylene-*co*-vinyl acetate (PEVA) coated onto 316-L stainless steel stents. The effect of the physicochemical form of the drug on its release has been demonstrated by comparing the differing release rates of PB from PU and PEVA stemming from the differing solubility parameters (Table I). PB (Figure 1) is a lipophilic drug which has been shown to promote endothelialization and to inhibit atherogenesis in both animals and humans.[32–36] Specifically, PB inhibits the production of platelet-derived growth factor (PDGF)[34] and interleukin-1 (IL-1)[37] from macrophages, slowing down the generation of matrix metalloproteinases and the subsequent remodeling of the extracellular matrix. Secretion of IL-1 from macrophages has been implicated in a number of chronic inflammatory conditions and has also been postulated to contribute to the pathogenesis of atherogenesis.[38]

MATERIALS AND METHODS

Materials

The stents used in this study were provided by Boston Scientific (Minneapolis, MN). PB was purchased from Sigma–Aldrich (St. Louis, MO). Segmented polyurethane (PU, Cardiomat 610) was purchased from Polymer Technology Group (Berkeley, CA). Collagen Type I (PureCol®) was purchased from Inamed (Fremont, CA). All biological reagents and media were obtained from Cascade Biologics (Invitrogen, Carlsbad, CA). All other reagents and solvents in this study were analytical grade and used without purification.

Preparation of PB films

The PB and PU solutions (1% w/v) were prepared in tetrahydrofuran (THF). The ratio of the drug to polymer was varied from 0 to 10% w/w of the total solid. The solution was added on top of the 8-mm round coverslips (Esco, Portmouth, NH) and spin-coated at 1000 rpm. The coated films on the coverslips were dried under vacuum for 24 h to remove the residual solvent.

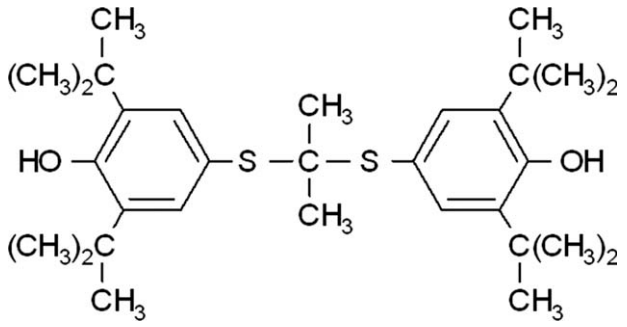

FIGURE 1. Chemical structure of PB.

Scanning electron microscopy (SEM)

The morphology and coating integrity of PB in the PU-coated stent were characterized by scanning electron microscopy (JEOL JSM-840, JEOL USA; Peabody, MA). Stents were mounted on to aluminum stubs and coated with gold palladium using a sputter coater under an argon atmosphere (Hummer I, Anatech; Hayward, CA). Coated stents were observed and imaged with an accelerating voltage of 5 kV, a probe current of 3×10^{-11} A, and a working distance of 15 mm. To achieve a cross-sectional image, a homogeneous PU film containing 10% w/w of PB was prepared by spin coating onto a 15-mm glass coverslip. The residual solvent was removed by evaporation under a chemical hood for 12 h. The samples for cross-sectional imaging were prepared by inducing free breakage of the film-coated coverslips via immersion in liquid nitrogen.

Characterization of physicochemical properties

Samples for analyzing physicochemical properties were prepared by a solvent casting method followed by powdering. A solution mixture containing 10% w/w of PB in PU was prepared in THF and casted in a porcelain mold. The PU film containing the drug was formed after evaporating the solvent in a chemical hood for 6 h. The films were then powdered manually and used for determining the form in which the drug existed in the polymer matrix. The surface chemistry of polymer films containing PB was analyzed using an FTIR spectrometer (Magna IR spectrometer 550, Spectra Tech, Oakridge, TN) in diffuse reflectance mode. Spectra were collected after 200 scans at 4 cm^{-1} resolution. The thermal analyses of the polymer film were performed using a differential scanning calorimetry (DSC; DSC 2920 TA Instrument, New Castle, DE). Samples of a mass of $\sim$ 7 mg were heated from 25 to 200°C at 1°C min^{-1} under flowing nitrogen (20 mL min^{-1}). The physicochemical form of existence of PB in the polymer film was confirmed using X-ray powder diffraction (XRPD; Diffraktometer 5000, Bruker AXS, Madison, WI) equipped with Cu Kα radiation. Film samples were analyzed in the range of 6°–40° with an angular resolution of 0.04°. The exposure time for each measurement was about 15 min.

Preparation of DES

Series of solutions containing mixtures of PB in PU were prepared in THF (total solid concentration = 1% w/v). The ratio of PB/PU in mixture was adjusted to be 5 and 10% w/w. The drug solution was coated onto the bare metal stents (BMSs) using an electrostatic spray method. BMS was mounted on a mandrel attached to a rotator and a transverse motion system. The distance between the stent surface and the spray nozzle was set at 1.5 cm. The electrostatic spray conditions were fixed at 0.025 mL min^{-1} flow rate, 8.0−10.0 kV high voltage, and 10 psi air flow. The amount of drug/polymer coated on the BMS was confirmed using weight measurements after drying the residual solvent.

In vitro drug release study

PB is an extremely hydrophobic drug with a log*P* of 10 and the solubility in water is reported to be 0.005 μg mL^{-1}.[39] Before conducting the release test, the solubility of PB was measured in PBS-Tween [0.05% Tween 20, PBS] (pH 7.4) to find a sink condition. Nearly 5 mg of PB was added to 1 mL of medium and stirred at 37°C for 48 h in Barnstead shaker at 120 rpm. The measured solubility of PB in PBS-Tween was 8.3 ± 0.22 μg mL^{-1}. PB release from the DESs was performed in PBS-Tween at 120 rpm at 37°C. The whole medium (3 mL) was replenished at every sample point. Samples were analyzed for released PB using high performance liquid chromatography (HPLC) (Agilent 1100 series; Agilent Technologies, Santa Clara, CA) using a C_{18} reverse phase analytical column (Model: W10641F 030; Waters, Milford, MA) and UV detection at 254 nm. Samples were run using a 1 mL min^{-1} flow rate at 25°C. An isocratic mode of acetonitrile and water (7:3) was used as the mobile phase.

Platelet adhesion study

The biocompatibility of the PU film coated on to the metallic stent was assessed by performing a platelet adhesion study using human blood. The supernatant containing the platelet-rich plasma (PRP) was used to conduct the platelet study. About 20 mL of human blood was obtained from healthy individuals and was centrifuged under mild conditions (0.8 × 100 cfg) for 15 min. Stents coated with various polymers (i.e., PLGA, PU, and PEVA) and BMSs (control) were incubated in PRP for 3 h. The samples were then washed with sterile 1× PBS before subjecting to glutaraldehyde fixation (2.5% v/v) for 15 min. The fixed samples were analyzed using SEM. To quantitatively measure the platelet adhesion, spin coated polymer films on coverslips were incubated with 150 μL of PRP. After 3 h of incubation, platelet count was performed using a coulter counter and the decrease in count after the experiment was expressed as a percent of platelet adhesion.

Human endothelial cell culture

Primary human aortic endothelial cells (ECs) were obtained from Cascade Biologics (now a part of Invitrogen, Carlsbad, California). EC cultures were maintained in 25 cm^2 TC-treated flasks in a 5% CO_2/95% air environment at 37°C with media specified by supplier's instructions. Specifically, ECs were cultured in M200 medium supplemented with low serum growth supplement (LSGS) and 1% antibiotics (gentamicin/amphotericin B). Cells were used within four passages of receipt for consistency. All experiments were performed on TC-treated 48-well polystyrene surfaces. For the proliferation studies, the initial EC seeding density was $2.5-3 \times 10^4$ cells cm^{-2}.

Studies on cell proliferation and morphology

The 8-mm coverslips coated with a drug eluting film were placed in each well of a 48-well plate. Collagen solution was prepared by mixing eight parts of PureCol® (3 mg mL^{-1}) with one part of 10× phosphate buffered saline (PBS). The pH of the collagen solution was adjusted to 7.4 by adding 0.01*N* HCl and 0.1*M* NaOH. The buffered collagen solution (300 μL) was added to each well containing the PB/PU-coated coverslips. The PU-collagen coated round coverslips

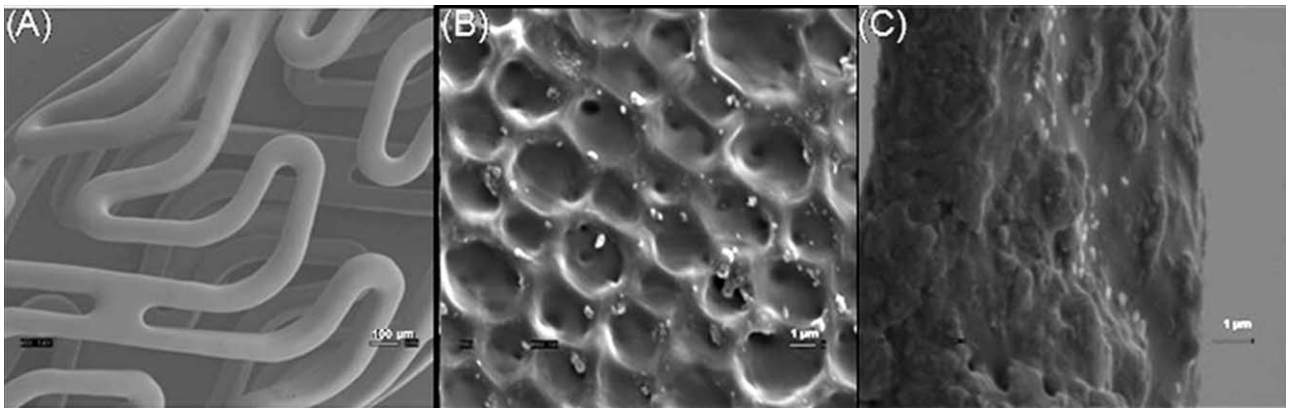

FIGURE 2. (A) Scanning electron micrograph of PB–PU-coated dilated stent, (B) Surface micrograph of PB–PU-coated film (White particles indicates the phase separated PB in PU), and (C) Cross-sectional SEM of PB–PU-coated film.

used for all studies were sterilized using ultraviolet radiation for 45 min. Next, they were rinsed three times using sterile Dulbecco's phosphate buffered saline (DPBS) prior to conditioning in appropriate serum supplemented media for at least 2 h. This treatment was done just prior to seeding the cells on the coverslips. The control (no polymer) coverslips were also similarly treated. After 48 h of seeding the ECs, the relative number of cells was determined using the MTS assay (CellTiter 96® AQueous One Solution Reagent; Promega Corporation, Madison, WI) by following the manufacturer's specifications with minor deviation. To adhere to the MTS protocol, the MTS redox reaction was carried out in the original 48-well plates using a proportionally higher amount of reagent, specifically 40 μL was added to 200 μL of freshly replenished culture medium. After 2 h of incubation in a 5% CO_2/95% air environment at 37°C, 100 μL of the reagent-culture medium mix was transferred to wells in a 96-well plate and then, the optical density at 490 nm was measured using Molecular Devices plate reader (Molecular Devices, Sunnyvale, CA). The morphology of ECs was observed daily and at different time points of culture, digitized images were captured with a Nikon phase contrast microscope (Nikon, Melville, NY) and a camera.

Statistics

The nonparametric Kruskal–Wallis equality-of-populations rank test was performed followed by pairwise *post hoc* comparisons using the Mann–Whitney test. Stata/SE 10.1 for Windows (StataCorp LP, College Station, TX) was used for the statistical analyses. A p value < 0.05 was considered significant.

RESULTS

Scanning electron microscopy

Images of PB/PU-coated stents were taken using SEM (Figure 2). SEM pictures revealed high integrity of coated films on stent without web formation. A very smooth homogeneous coating was formed, and no delamination of the film was observed after expansion indicating that the coated film could withstand the pressure associated with stent expansion [Figure 2(A)]. Figure 2(B,C) depict the surface and cross-sectional images of PB-loaded PU films by spin coating.

In vitro release study

The drug elution profile of 5 and 10% w/w PB coated stents is depicted in Figure 3. As seen in Figure 3, for both conditions there was an initial lag time after which the drug release was observed. No burst release was seen for either of the formulations used. The slope of the release profile was nearly identical as the concentration of the drug was increased indicating that drug amount did not affect the release rate. As the concentration of the drug was increased, the release was sustained for a longer period, as expected. The release from the stent containing 10% w/w PB/PU was sustained for a period of 1 month before a plateau was observed. A fresh release medium was replenished at every sampling point to maintain sink conditions for drug release. The release kinetics of PB from PU was compared to the release kinetics of PB from PEVA. In PEVA, PB existed as a molecular dispersion. Figure 3 shows the release kinetics of PB from PU and PEVA containing 10% w/w PB. An accelerated drug release was seen from PEVA compared to PU for

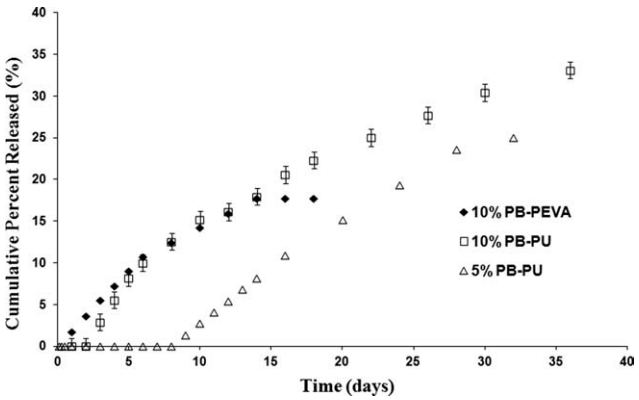

FIGURE 3. *In vitro* release profile of 5% PB/PU, 10% PB/PU, and 10% PB/PEVA-coated stents ($n = 3$).

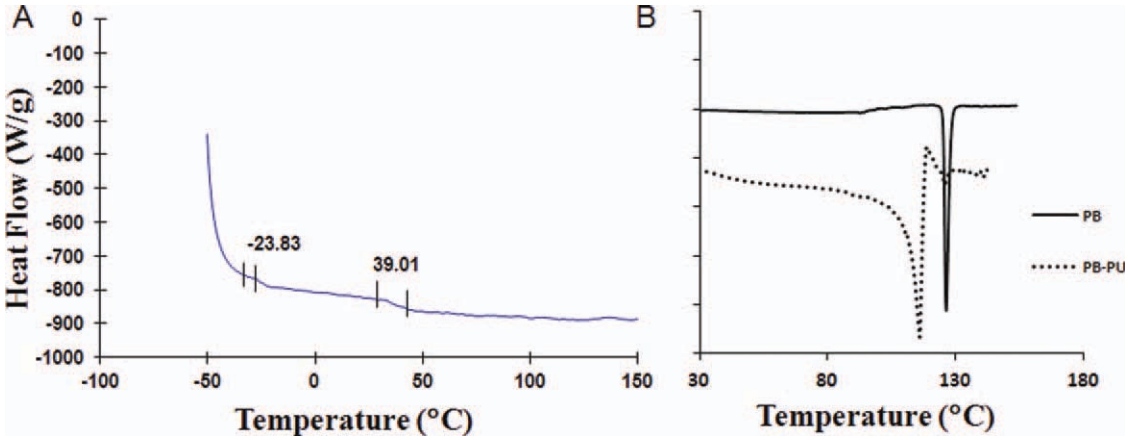

FIGURE 4. DSC thermograms of (A) segmented PU; (B) PB drug powder and PB–PU film. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the same drug loading and dose density. Also, the release of the drug was sustained for a much longer period in PU where the drug coexisted in a crystalline form, when compared to PEVA in which the drug existed as an amorphous dispersion.

Differential scanning calorimetry

The physical form of existence of the drug in the polymer film was obtained using DSC. PB/PU films containing 40% (w/w) drug was prepared by solvent casting. The film was powdered and subjected to thermal analysis. Figure 4 depicts the DSC thermograms of PU, PB, and PB-loaded PU film. Segmented PU exhibits two glass transition temperatures at -23.83°C and 39.01°C [Figure 4(A)]. The native drug exhibits a melting endotherm at 125°C while the drug loaded PU film exhibits two endotherms, one predominant peak at 110°C and another small peak at 125°C [Figure 4(B)]. PB is known to exist in two different polymorphic forms. The stable form (form I) exhibits a melting peak around 125°C, while the less stable form (form II) melts at 116°C.[40] The peak at 110°C represents the melting of form II expected at 116°C. The decrease in temperature can be attributed to the melting point depression in the presence of the second component, that is, PU. The stability of these two forms or the conversion of the less stable form II to the more stable form I in the polymer matrix is beyond the scope of this study.

IR analysis

The selection of the drug-polymer system based on the solubility parameter difference was justified qualitatively using IR analysis. The PB/PU system that has a high solubility parameter difference was compared with PB/PEVA with a low solubility parameter difference. Figure 5 depicts the IR spectrum for the two different drug polymer combinations. The IR spectrum of PU was identical to the PB/PU blend, indicating the absence of new bonds between PB and PU. In the case of PB/PEVA, a new bond was observed, as indicated by

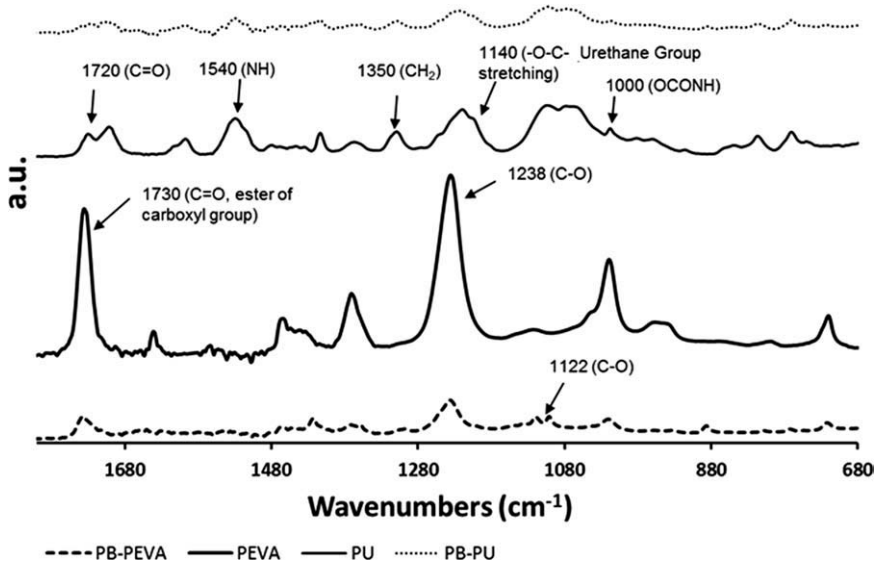

FIGURE 5. IR spectra of PEVA, PB/PEVA, PU, and PB/PU.

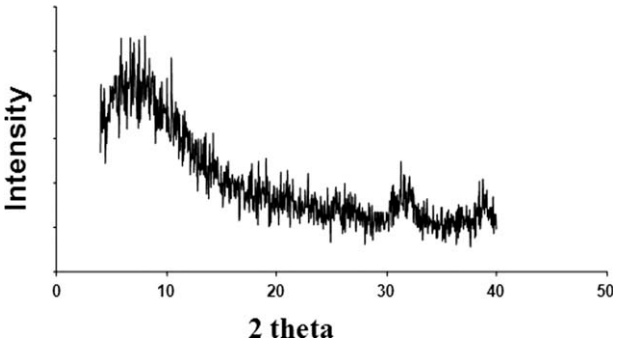

FIGURE 6. X-ray diffractogram of PB/PEVA.

a split of the peak obtained at 1122 cm^{-1}, corresponding to a C—O stretch. The emergence of a split at 1122 cm^{-1} corresponding to a C—O symmetric vibration is an indication of drug polymer miscibility due to which a one phase amorphous dispersion is formed for the system. The characteristic C—O peak, and the peak representing the vibration of the —C=O ester of the carboxyl group in PEVA appear at 1238 and 1730 cm^{-1}, respectively. The —C=O peak at 1730 cm^{-1} in PB-PEVA is much less intense than that in pure PEVA, probably as a result of the —C=O group of PEVA interacting with the O—H group of PB. For the PU system the characteristic urethane stretching was seen at 1140 cm^{-1} in addition to CH_2 and N—H vibrations at 1350 and 1540 cm^{-1}, respectively. No new peak formation was obtained for the PB/PU system, indicating immiscibility of PB in the PU matrix. This stems from the phase separation of PB in PU forming crystalline drug aggregates.

X-ray powder diffraction

The physical form of existence of PB in PEVA was confirmed using XRPD. Because PEVA melts at a much lower temperature than PB, thermal analysis could not be performed to understand the physicochemical form of PB in PEVA matrix. Figure 6 depicts the XRPD pattern of PB/PEVA. As no crystalline peak was observed, it can be concluded that drug was dispersed in an amorphous form in the polymer matrix.

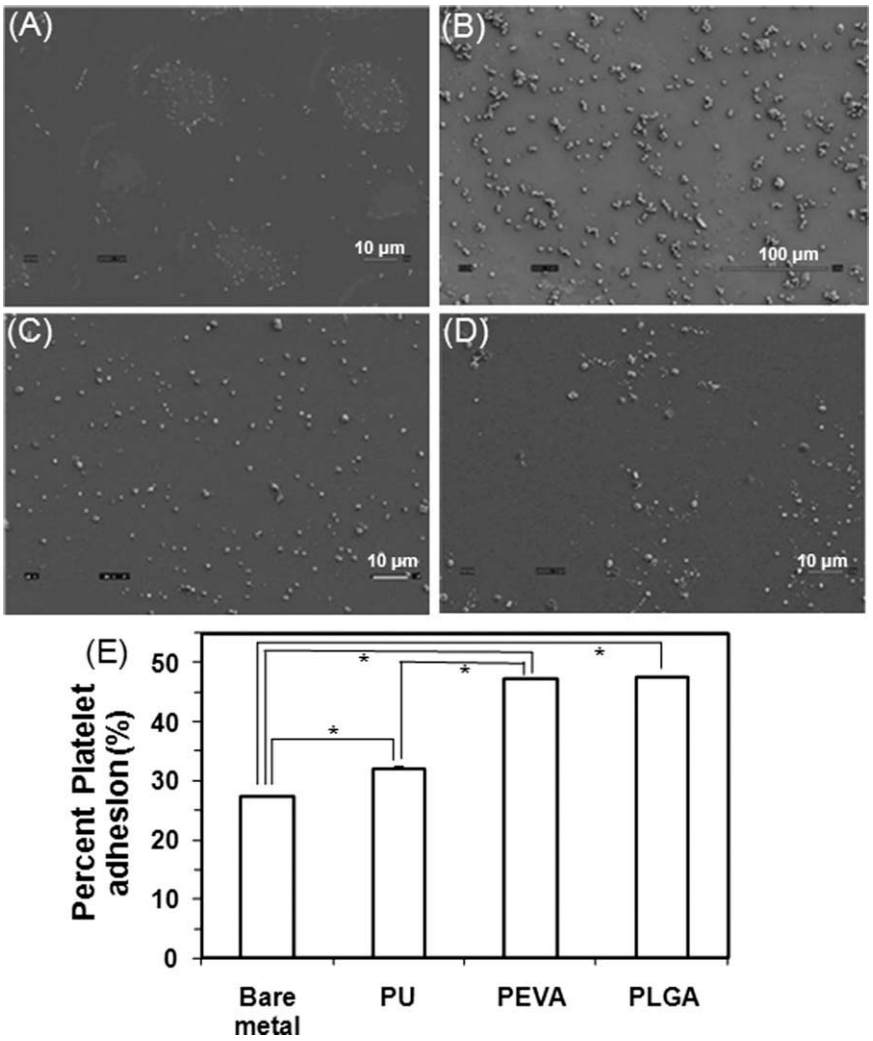

FIGURE 7. SEM of platelet adhesion on (A) Bare metal, (B) PLGA, (C) PEVA, (D) PU; (E) Quantitative estimation of platelet adhesion on various polymer surfaces ($*p < 0.05$, $n = 3$).

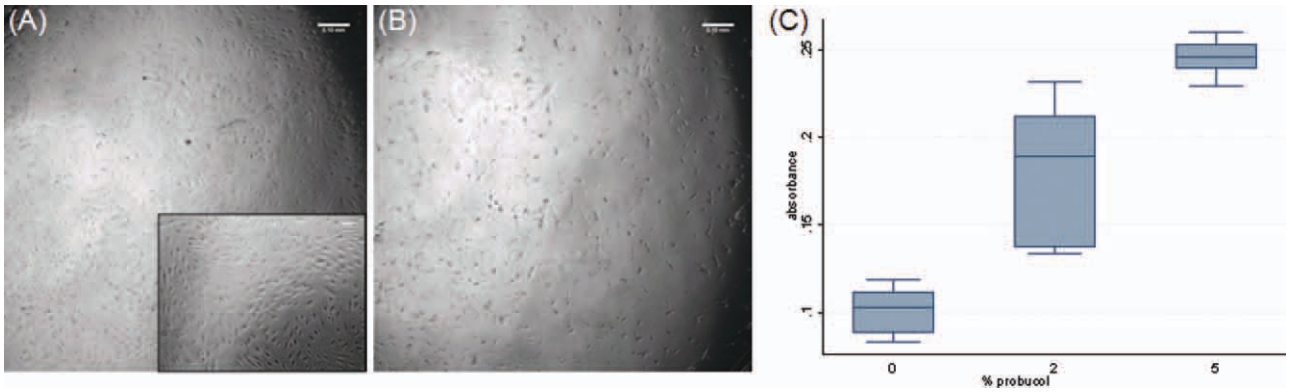

FIGURE 8. (A) Human aortic ECs on collagen I coated 5% probucol embedded PU, (B) collagen I coated 10% probucol embedded PU (Insert: Higher magnification (×10) image of ECs on collagen I coated 5% probucol). All scale bars are 0.1 mm, (C) Boxplots indicating the absorbance values obtained for the different probucol concentrations and the control (no probucol), obtained via the MTS-based proliferation assay ($n = 3$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Platelet adhesion study

In vitro platelet adhesion measurements were performed to determine the blood compatibility of the coated polymer film. PU was compared to other commonly used biocompatible polymers like PLGA and PEVA. Qualitative inference of platelet adhesion was obtained using SEM as shown in Figure 7(A–D). Further, platelet adhesion was quantified using a coulter counter and the decrease in count of platelets from PRP after incubation is expressed as percent of platelet adhesion. Figure 7(E) depicts the platelet adhesion obtained using a coulter counter experiment. Statistically significant reduction in platelet was seen for PU compared to PLGA.

Effect of probucol eluted from the collagen I coated PU films on human aortic ECs *in vitro*

The effects of PB concentration in PU matrix (i.e., 0, 2, 5, and 10% w/w) on EC proliferation were observed using phase contrast microscopy [Figure 8(A,B)]; result from 2% probucol not shown. The PU film containing 10% probucol appeared to be toxic to the cells and resulted in the cells chipping off within 2 days of seeding. Next, using the MTS-based proliferation assay, the proliferation of ECs on 2 and 5% probucol eluting PU films over a 48-h time period was quantitatively assayed. The 5% probucol eluting PU film resulted in significantly higher proliferation as compared to the 2% probucol eluting PU film and the PU control [Figure 8(C)].

DISCUSSION

A controlled release formulation of PB was developed after taking into account the drug–polymer interaction, governed by the solubility parameters of the drug and the polymer. In the developed formulation, the efficacy of PB in the polymer matrix was demonstrated by the increase in EC proliferation *in vitro* (Figure 8). Notably, the increased proliferation of ECs is not sufficient to restore vascular wall functioning; the proper functioning of ECs is intricately tied to the health of the vasculature. The fact that PB was able to exert dose-related effects in our formulation is promising because PB has been shown to restore normal EC functioning in hypercholesterolemia in animal models.[41,42] Thus, PB in the presented form appears to be an attractive drug for DESs.

The physical form in which a drug exists in a polymer matrix can play a significant role in modulating its release kinetics.[43,44] The miscibility of the drug in the polymer and the specific interaction between the drug and polymer determines the properties of the formulation. An estimation of drug miscibility in the polymer matrix can be obtained via determination of its solid state solubility in the polymer matrix by calculating its solubility parameter.[45–47] The Hildebrand solubility parameter can be calculated using the Fedors' approach as reported in literature.[48] The Hildebrand solubility parameter is the square root of the cohesive energy density. The Fedors' method of calculation involves the summation of cohesive energy and volume contributions from individual functional groups within the molecule.[49] The degree of stabilization of the polymer depends on the interaction of the drug and polymer at a molecular level and their ability to interact by hydrogen bonding.[50] It has been reported that a stable formulation of nifedipine is obtained using polymers that have solubility parameter values similar to nifedipine.[51] It is an established concept that matching the solubility parameters of the drug and the polymer could lead to a stabilizing effect without any phase separation of the drug in the polymer matrix.

In this study, we have used the above discussed concept to induce a phase separation of the drug in the polymer followed by the formation of drug crystals in the polymer matrix. A drug in crystalline form will have a high energy barrier and is more stable, retarding its release kinetics. A stabilized formulation of a drug in a polymer will be dispersed in an amorphous form in the polymer matrix, which is a higher energy state, and, hence, exhibits an accelerated release rate.

PU was chosen as the carrier matrix owing to the superior mechanical properties that can maintain its coating integrity during the dilation of the stent after insertion *in vivo*. The solubility parameter value of PU is also high compared to PB, which led to its use as the polymer matrix in this study for sustaining the release of PB. The greater difference in the solubility parameters of PB and PU may have resulted in a phase separation leading to the formation of PB crystals embedded in the PU matrix. This hypothesis was confirmed using another polymer—PEVA, where the difference in the solubility parameters between PB and PEVA was lower compared to PB and PU. This was done to confirm that a stable molecular dispersion exists when there is a lower difference in the solubility parameters of the drug and the surrounding matrix. The threshold value of the difference in the solubility parameters beyond which the phase separation initiates has not been investigated here and is beyond the scope of this study.

An initial lag time was observed for both the 5 and 10% formulation of PB in the PU matrix after which a sustained release profile was seen (Figure 3). PB was not molecularly dispersed as observed in the SEM surface and cross-sectional images (Figure 2). A clear phase separation of PB was observed and the drug aggregates were distributed homogeneously across the cross-section of the PU matrix. These aggregates are crystalline in nature as confirmed using DSC (Figure 4). PB exists in a nanocrystalline form in the PU matrix which could provide a higher energy barrier controlling its release kinetics. It can be presumed that the crystalline form of PB may modulate its release rate, with dissolution being the rate determining step followed by molecular level diffusion. In contrast to PU, no lag time was observed for release of PB from PEVA matrix (Figure 3). The slope of the release profile was high for PEVA compared to PU indicating an accelerated release from the PEVA matrix. Also, the drug release was completed in 2 weeks, while PU sustained PB release for a month. XRPD analysis revealed that PB was dispersed in an amorphous form in the PEVA matrix (Figure 6).

The physical form of existence of the drug in the polymer matrix is related to the strength, extent, and configuration of interactions of the drug molecules with the polymer matrix. The identical IR spectra for PU and PB/PU [Figure 5(A)], indicates that no new peak was formed. It is an indication of the poor interaction between the drug and the polymer due to poor miscibility of PB in the PU matrix. However, a new bond formation was seen at 1122 cm^{-1} owing to the interaction between the aromatic ring carbon of PB and the oxygen molecule from the PEVA chain forming a C—O stretch for the PB/PEVA system [Figure 5(B)]. A split in peaks at 1122 cm^{-1} is a clear indication of the interaction of PB with the carrier PEVA matrix. This interaction means that the drug was completely miscible in the PEVA matrix leading to a amorphous dispersion. This was further confirmed via an XRPD analysis (Figure 6) where no crystalline peaks were observed for the PB/PEVA sample. In the case of the PB/PU system, there is no interaction between the drug and the carrier polymer matrix leading to the phase separation and formation of nanocrystalline drug agglomerates in the PU matrix.

The adhesion of platelets to a stent surface is considered to be a major precursor of thrombosis. To analyze the hemocompatibility of the stent coated with the PU film, a platelet adhesion test was performed. Less number of platelets was noticed for PU compared to that for PLGA or PEVA [Figure 7(A–D)]. A statistically significant reduction of 20% in platelet adherence was observed for PU compared to PLGA and PEVA [Figure 7(E)]. The control bare metal sheet also showed a significant reduction in platelet adherence [Figure 7(E)]. However it is important to note that it is challenging to load a drug in a BMS and to precisely control its release kinetics. From this study, it was observed that PU, in addition to its desirable mechanical properties, also exhibits high degree of hemocompatibility for use in DES.

CONCLUSION

PB, an endothelial agonist, in a PU matrix was used to develop a sustained release DES formulation. The concept of solubility parameters was used to develop the formulation. The duration of drug release and the release rates were modulated and the spatio-temporal release profiles were obtained by modifying the physicochemical form of the drug in the carrier polymer matrix. Significant EC proliferation was observed in PB-loaded PU films indicating the efficacy of PB as an endothelial agonist in the designed formulation. Also, a high degree of hemocompatibility of PU was observed, stemming from the low platelet adhesion observed, making PU a suitable carrier for developing DESs. This study presents a sustained release DES formulation to promote endothelialization, designed using solubility parameter concepts. Future studies will be geared toward testing the optimized concentrations of PB in PU in a DES format both *in vitro* and *in vivo*.

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