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Tissue response to poly(L-lactic acid)-based blend with phospholipid polymer for biodegradable cardiovascular stents

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ABSTRACT

A temporary cardiovascular stent device by bioabsorbable materials might reduce late stent thrombosis. A water-soluble amphiphilic phospholipid polymer bearing phosphorylcholine groups (PMB30W) was blended with a high-molecular-weight poly(L-lactic acid) (PLLA) to reduce unfavorable tissue responses at the surface. The PLLA implants and the polymer blend (PLLA/PMB30W) implants were inserted into subcutaneous tissues of rats, the infrarenal aorta of rats, and the internal carotid arteries of rabbits. After 6 months subcutaneous implantation, the PLLA/PMB30W maintained high density of phosphorylcholine groups on the surface without a significant bioabsorption. After intravascular implantation, the cross-sectional areas of polymer tubing with diameters less than 1.6 mm were histomorphometrically measured. Compared to the PLLA tubing, the PLLA/PMB30W tubing significantly reduced the thrombus formation during 30 d of implantation. Human peripheral blood mononuclear cells were cultured on the PLLA and the PLLA/PMB30W to compare inflammatory reactions. Enzyme-linked immunosorbent assay quantified substantially decreased proinflammatory cytokines in the case of the PLLA/PMB30W. They were almost the same level as the negative controls. Thus, we conclude that the phosphorylcholine groups could reduce tissue responses significantly both in vivo and in vitro, and the PLLA/PMB30W is a promising material for preparing temporary cardiovascular stent devices.

1. Introduction

The treatment of coronary artery disease and other cardiovascular diseases has been revolutionized through the introduction of interventional procedures and the use of intravascular stents, which are inserted by a minimally invasive method and mechanically scaffold the vessel wall against elastic recoil, improving blood flow in diseased vessels [1,2]. However, current drug-eluting stents permanently remain in implantation sites with several limitations, including the risks of late stent thrombosis, hindrance of late lumen vessel enlargement, and interference with radiological imaging [3]. High-molecular-weight poly(L-lactic acid) (PLLA) has the potential to replace permanent stenting and has exhibited favorable degradation behavior in small clinical trials [3–5]. The PLLA is a biodegradable polymer whose molecular weight decreases over time due to cleavage of the ester linkage, degrading into small particles that can be phagocytosed [3,4]. Eventually, the PLLA is degraded into lactic acid and is eliminated through the citric acid cycle.

Safety concerns regarding the use of the PLLA for stenting remain, however, because foreign materials are inherently

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thrombogenic [6–11]. This is attributed to the denaturation of proteins, activation of coagulation factors, propagation of thrombi, provocation of inflammatory responses, and accumulation of debris. To control these adverse tissue reactions on the materials, the surface of the materials was covered with phosphorylcholine groups for preparing artificial cell membrane without any ligand molecules. Based on this hypothesis, 2-methacryloyloxyethyl phosphorylcholine (MPC) copolymers, which have phosphorylcholine groups in their side chain, have been synthesized [12–17]. The MPC copolymers can be incorporated by a convenient procedure such as blending with matrix polymers [18–21]. In a molecular and mechanistic approach, the biomedical function of the MPC copolymers has been explained by the free water fraction on the surface, which minimizes protein adsorption and its conformational change [22–24]. Surfaces of materials with a high density of phosphorylcholine groups significantly lower platelet activation and neointimal hyperplasia [25–30]. A water-soluble poly[MPC-*co*-*n*-butyl methacrylate (BMA)] (PMB30W) is an amphiphilic copolymer composed of hydrophobic BMA units and hydrophilic MPC units [12,13,31]. Due to its strong amphiphilic characteristics, the PMB30W plays a dual role as a biomimetic modifier of surface properties and as a surfactant for the bulk properties of polymer blends composed of PLLA and PMB30W (PLLA/PMB30W) [11].

Although it has been presumed that phosphorylcholine groups on the surface of materials can enhance biocompatibility, the critical concept that the incorporation of phosphorylcholine groups might reduce stent thrombosis has not been resolved in vitro [32], preclinically [33], or clinically [34]. This discrepancy may be due to the different dispersion state of phosphorylcholine groups on the surfaces of stent materials [11,14–17,32,33]. In the present study, we hypothesized that the PLLA/PMB30W with phosphorylcholine group-rich surfaces could exhibit favorable bulk and surface properties in vivo and may enhance tissue compatibility of temporary cardiovascular stent devices. Therefore, the objective of this study was comparison of tissue responses on the PLLA/PMB30W and the PLLA in vivo with small animal models and in vitro, and to discuss the role of the phosphorylcholine groups for reducing the tissue responses.

2. Materials and methods

2.1. Preparation of materials

The PLLA (molecular weight (Mw) = 1×10^5 for the preparation of tubing or Mw = 5×10^4 for the preparation of films) was purchased from Polysciences, Warrington, PA. The PMB30W was synthesized using a free radical polymerization technique by a previously reported method [12,13,31]. The chemical structure of PMB30W is shown in Fig. 1. The PMB30W concentrations >1 mg/mL in phosphate-buffered saline (PBS) result in the PMB30W nanoaggregates with hydrodynamic diameters <20 nm [31,35].

The PLLA and the PLLA/PMB30W (weight ratio, 92/8) tubing and films were prepared by a modified previously reported method [11]. Briefly, 6 wt% PLLA solution in dichloromethane (DCM)/methanol (MeOH) (volume ratio, 12/1) and 6 wt% PLLA/PMB30W (weight ratio, 92/8) solution in DCM/MeOH (volume ratio, 12/1) were repeatedly coated onto Teflon® rods (tubing) or cast onto Teflon® dishes (films), and the solvents were dried at room temperature. Polymer tubing and films were vacuum-dried for 1 week, and then immersed in water to equilibrate the surface overnight before the surface measurements. Polymer tubing and films were sterilized with ethylene oxide gas at 40 °C.

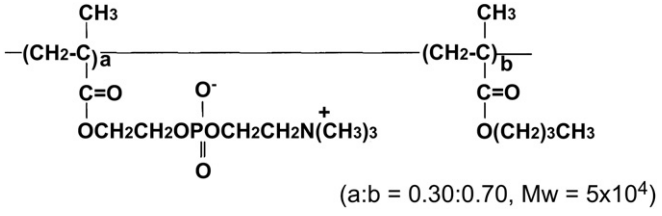

Fig. 1. The chemical structure of the PMB30W.

2.2. Surface characteristics of materials

To analyze the inner surface of polymer implants, the tubing was cut into concave membranes and pressed under 50 MPa at 60 °C for 10 min. The atomic concentration on the inner surface was measured using an X-ray photoelectron spectroscope (XPS; AXIS-HSi, Kratos/Shimadzu, Kyoto, Japan) with a magnesium Kα (energy = 1253.6 eV) source radiation. The photoelectron take-off angle was 90°. The measured phosphorous atomic concentration was attributed to the phosphorus in the phosphorylcholine groups of the PMB30W. The phosphorous/carbon atomic concentration ratios (P/C% values) were calculated by determining the relevant integral peak area and applying the sensitivity factors supplied by the instrument manufacturer (Supplementary Fig. 1).

To measure the static contact angle (SCA), a captive-bubble method was used. The polymer films were fixed horizontally, and a small air bubble was attached to the mold contact surface of polymer films in the distilled water and the SCA in water was determined by the angle between the films and the air bubble using a contact angle goniometer (CA-W, Kyowa, Saitama, Tokyo, Japan) at room temperature.

2.3. Evaluation of bioabsorption of polymer implants after subcutaneous implantation

Animal care and procedures were approved by Institutional Animal Care and Use Committee (IACUC) (No. 080929-3) at Seoul National University. The PLLA (n = 15) and PLLA/PMB30W (n = 15) polymer tubing (internal diameter [ID]: 1.6 mm; thickness: 0.2 mm; length: 2.5 cm) was implanted into the interscapular subcutaneous tissue of rats (n = 30, male Wistar, body weight: 0.3–0.4 kg) after anesthesia by inhalation of 2% isoflurane. At predetermined times (2, 4, 6 months), the polymer tubing was explanted after the animals were euthanized. The fibrous capsule surrounding the tubing was carefully removed. The polymer tubing was sonicated in 1% sodium dodecyl sulfate aqueous solution for 20 min to remove the adsorbed components and rinsed with distilled water. After vacuum drying, changes in the overall mass and molecular weight of the polymer tubing were calculated by a gravimetric method and gel permeation chromatography (Jasco system, Tokyo, Japan), respectively. The polymer tubing was dissolved in 1,1,1,3,3,3-hexafluoroisopropanol; the flow was 0.2 mL/min at 40 °C. The molecular weight of polymer was calibrated by poly(methyl methacrylate) standards.

2.4. Evaluation of acute thrombus formation after intravascular tubing insertion into small animals

Animal care and procedures were approved by IACUC (No. 08-0266) at Seoul National University Hospital.

Wistar male rats (n = 17, body weight: 0.3–0.4 kg) were anesthetized by inhalation of 2% isoflurane. After the infrarenal abdominal aorta of each animal was surgically exposed and clipped by an approximator with a surgical microscope, the vessels were carefully punctured using a taper-point needle. Then, polymer tubing (n = 17; ID: 1.2 mm; thickness: 150 μm; length: 2.0 mm) was inserted using a 20 G catheter, and the puncture sites were sutured with Ethilon 10-0 as described in Results.

For paired tests, New Zealand white male rabbits (n = 11, body weight: 3–4 kg) were anesthetized by intramuscular injection of a mixture of Zoletil (15 mg/kg) and xylazine (7.5 mg/kg) and subsequent inhalation of nitric oxide and isoflurane. Heparin (50 IU/kg per h) was administered to the rabbits as a continuous infusion during the operative procedure. With a surgical microscope, each internal carotid artery was carefully exposed and clipped by an approximator. After puncturing vessels by the aforementioned method, the polymer tubing (n = 22; ID: 1.6 mm; thickness: 150 μm; length: 3.0 mm) was inserted using an 18 G catheter. The PLLA tubing (n = 11) was inserted into the left internal carotid arteries, and the PLLA/PMB30W tubing (n = 11) was inserted into the right internal carotid arteries. The puncture sites were then sutured with Ethilon 8-0.

At predetermined times (3 h and 30 d in the rat study; 2 d and 30 d in the rabbit study), the polymer tubing-inserted arteries were surgically explanted after a heparin injection (200 IU/kg), following which the animals were euthanized. The samples were then rinsed with normal saline, placed in 2.5% glutaraldehyde PBS solution overnight, rinsed with distilled water, embedded in optimal cutting temperature compound, and cross-sectioned (thickness: 0.30 mm) while in a deep frozen state. The frozen sections were prepared by an independent researcher. For morphometric analysis, all cross-sections [PLLA from rats at 3 h (n = 13), PLLA/PMB30W from rats at 3 h (n = 12), PLLA from rats at 30 d (n = 8), PLLA/PMB30W from rats at 30 d (n = 10), PLLA from rabbits at 2 d (n = 26), PLLA/PMB30W from rabbits at 2 d (n = 20), PLLA from rabbits at 30 d (n = 32), PLLA/PMB30W from rabbits at 30 d (n = 29)] were digitally exported from a microscope camera, and the patent cross-sectional area (CSA) was analyzed using the National Institutes of Health ImageJ software (version 1.41o). Lumen area stenosis (LAS) was calculated as $[1.00 - (S/T)] \times 100$, where T is the CSA of the unimplanted polymer tubing, and S is the minimal CSA of each polymer tubing-inserted artery. In the rat study, T = 1.13 mm^2, and in the rabbit study, T = 2.01 mm^2. For observation of vessel remodeling, a PLLA/PMB30W-inserted aorta of rat (n = 1) after 50 d of implantation