論文の内容の要旨

論文題目

Preparation of temporary stent materials bearing phosphorylcholine groups and investigation of their role in the tissue-biomaterial interactions

(生分解性血管ステントのための細胞膜模倣型バイオマテリアルの創製及び組織適合性の評価)

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1. Introduction

Cardiovascular disease is the most common cause of human mortality in western countries; the single leading cause of human death in the USA is coronary heart disease, which is caused by atherosclerosis of the coronary arteries and produces angina pectoris or heart attack. The use of intravascular stents has revolutionized the treatment of coronary heart disease and other cardiovascular diseases by improving the blood flow in diseased blood vessels, using a less-invasive method. However, the occurrence of early and late stent thrombosis has raised serious safety concerns regarding the use of intravascular stents. Temporary stent materials might reduce the risk of late stent thrombosis because they disappear from the vulnerable tissues after the healing process is completed. Because the surface of 2-methacryloyloxyethyl phosphorylcholine (MPC) polymers resembles that of cell membranes, I hypothesized that bioabsorbable stent materials bearing phosphorylcholine groups could enhance tissue compatibility and have the potential to reduce the incidence of both early and late stent thrombosis (Fig. 1).

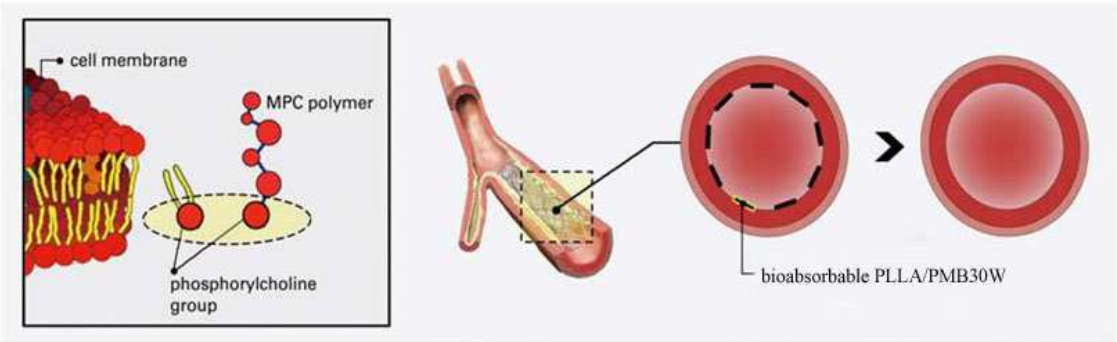

Figure 1. Scheme of temporary stent materials bearing phosphorylcholine groups

2. Methods and Results

2.1. Preparation of poly (L-lactic acid) / a water-soluble phospholipid polymer blends

A 6 wt% poly (L-lactic acid) (PLLA) solution in dichloromethane (DCM), 6 wt% Blends of PLLA and a water-soluble amphiphilic poly(MPC-co-*n*-butyl methacrylate (BMA)) (PMB30W) (weight ratio, 95/5)

blend polymer solution in DCM/ methanol (MeOH) (volume ratio, 12/1), and 4 wt% PLLA/PMB30W (weight ratio, 90/10) blend polymer solution in DCM/MeOH (volume ratio, 12/1) were prepared by stirring the solutions sufficiently until they turned transparent. The solutions were sonicated in a cold bath with ice for 30 min for homogenization. In order to prepare cast films, the polymer solutions were cast onto Teflon dishes, and the solvents were dried at room temperature overnight. In order to prepare tubing, the polymer solutions were repeated coated on the rotated Teflon rod. The PLLA/PMB30W cast films demonstrated higher breaking strengths than PLLA cast films, and their Young's modulus was similar to that of PLLA under dry conditions (Fig. 2). A high density of phosphorylcholine head groups on the inner surface of PLLA/PMB30W tubing was developed by repeated coatings with the PLLA/PMB30W blend polymer solutions (Fig. 3). The PLLA/PMB30W tubing showed stable degradation behaviors similar to the PLLA tubing. These materials are characterized by strong mechanical properties and stable degradation behaviors that are either superior or similar to those of high molecular weight PLLA.

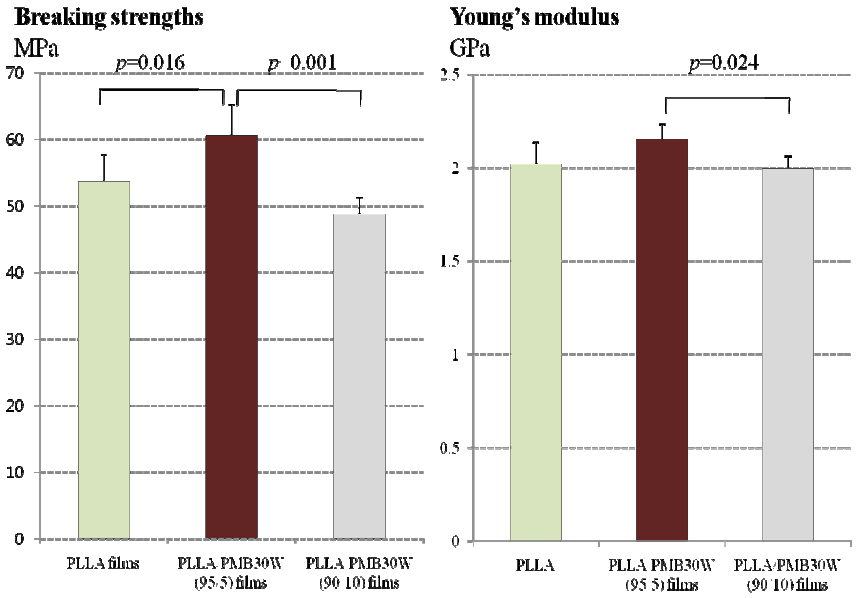

Figure 2. Mechanical properties of cast films. Columns and error bars represent means ± standard deviations for n=6. Statistical differences were obtained by ANOVA followed by Tukey's test. *p* values are given when *p* value is below 0.05.

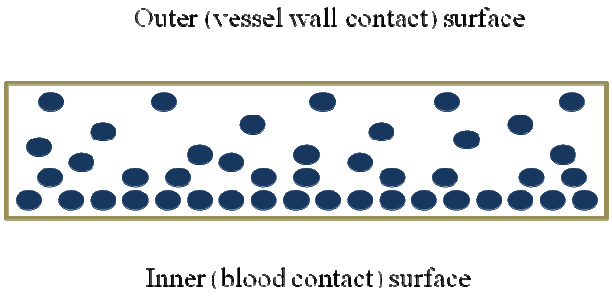

Figure 3. Schematic diagram of distribution of phosphorylcholine groups in the PLLA/PMB30W tubing. Dark blue circles indicate phosphorylcholine groups of PMB30W.

2.2. Tissue compatibility of poly (L-lactic acid) / a water-soluble phospholipid polymer blends

PLLA and PLLA/PMB30W were evaluated in vitro and small animals. A high-content automated screening assay (240 random fields per group) confirmed that PLLA induced apoptosis of a mouse

macrophage-like cell line (apoptotic population: 73.9% in 0.8 mg PLLA/mL) while PLLA/PMB30W remained biologically inert (apoptotic population: 13.8% in 0.8 mg PLLA and not quantified PMB30W/mL) (Fig. 4). Human peripheral blood mononuclear cells were cultured on PLLA and PLLA/PMB30W (n = 8 cultures per group) to compare inflammatory reactions. Enzyme-linked immunosorbent assay quantified substantial decreased concentrations of proinflammatory cytokines in PLLA/PMB30W, compared to PLLA. PLLA and PLLA/PMB30W (n = 3–6 material per group) were implanted into subcutaneous tissues of rats, rat infrarenal aortas, and the internal carotid arteries of rabbits. Subcutaneous degradation profiles showed that both PLLA and PLLA/PMB30W have not been rapidly absorbed. After intravascular implantation of polymer tubing, an average of total cross-sectional areas (CSA) exhibited that PLLA/PMB30W tubing maintained larger cross-sectional areas than PLLA in both rats (0.67 mm^2 versus 0.24 mm^2) and rabbits (0.96 mm^2 versus 0.59 mm^2) during 30 days of implantation (Fig. 5).

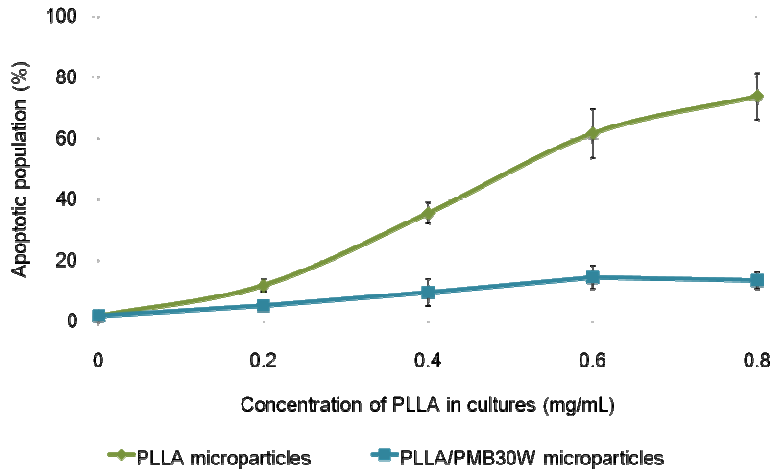

Figure 4. The apoptotic population of a mouse macrophage-like cell line after induction of PLLA or PLLA/PMB30W microparticles in the presence of serum for 24.

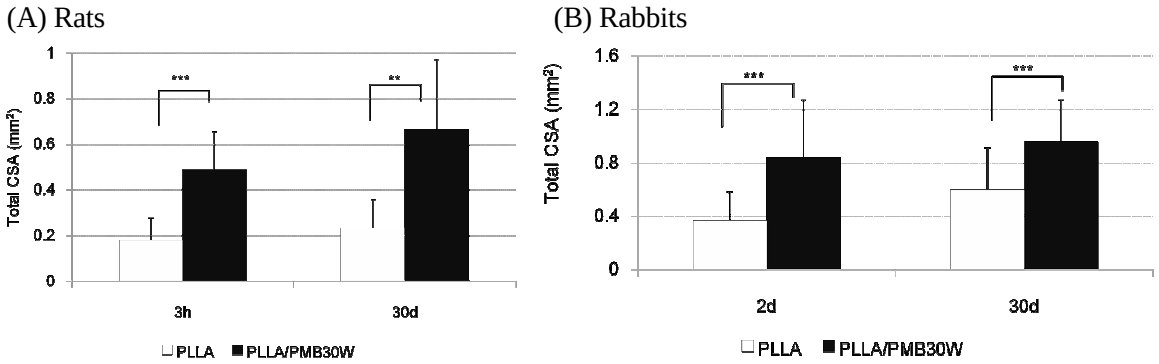

Figure 5. Cross-sectional areas (CSA) of polymer tubing tubing after the intravascular insertion. Statistical differences (**P < 0.01; ***P < 0.001) were analyzed by the Mann-Whitney U test.

2.3. Design of nanocarrier-mediated delivery of antiproliferatives

To obtain bioabsorbable sirolimus (SRL)-releasing polymer films that could release nanovehicles incorporating SRL, poly (L-lactide-*co*-caprolactone-*co*-glycolide) (PLCG), PMB30W, and SRL were blended with different formulations (Table 1.) The SRL -releasing polymer films and the released materials were characterized by differential scanning calorimeter, scanning electron microscopy, transmission electron microscopy, dynamic light scattering, and high-performance liquid chromatography. Nanovehicles were formed with PMB30W chains and could be released from the

PLCG/PMB30W/SRL films. The hydrodynamic diameter of the nanovehicles in phosphate-buffered saline was smaller than 20 nm. The PLCG/PMB30W/SRL films substantially enhanced the carrier-mediated delivery of SRL and attenuated its degradation product, 34-hydroxy SRL (Fig. 6).

Table 1. Three formulations of SRL-eluting polymer films.

	Materials	Solvent blending	Sonication	Solvent evaporation
Film X	PLCG/PMB30W/SRL (weight ratio, 90/10/1)	Acetone/EtOH (volume ratio, 7/3)	-	Casting
Film Y	PLCG/PMB30W/SRL (weight ratio, 90/10/1)	Acetone/MeOH (volume ratio, 7/3)	30 mins	Casting
Film Z	PLCG/PMB30W/SRL (weight ratio, 90/10/1)	Acetone/MeOH (volume ratio, 7/3)	30 mins	Electrospinning

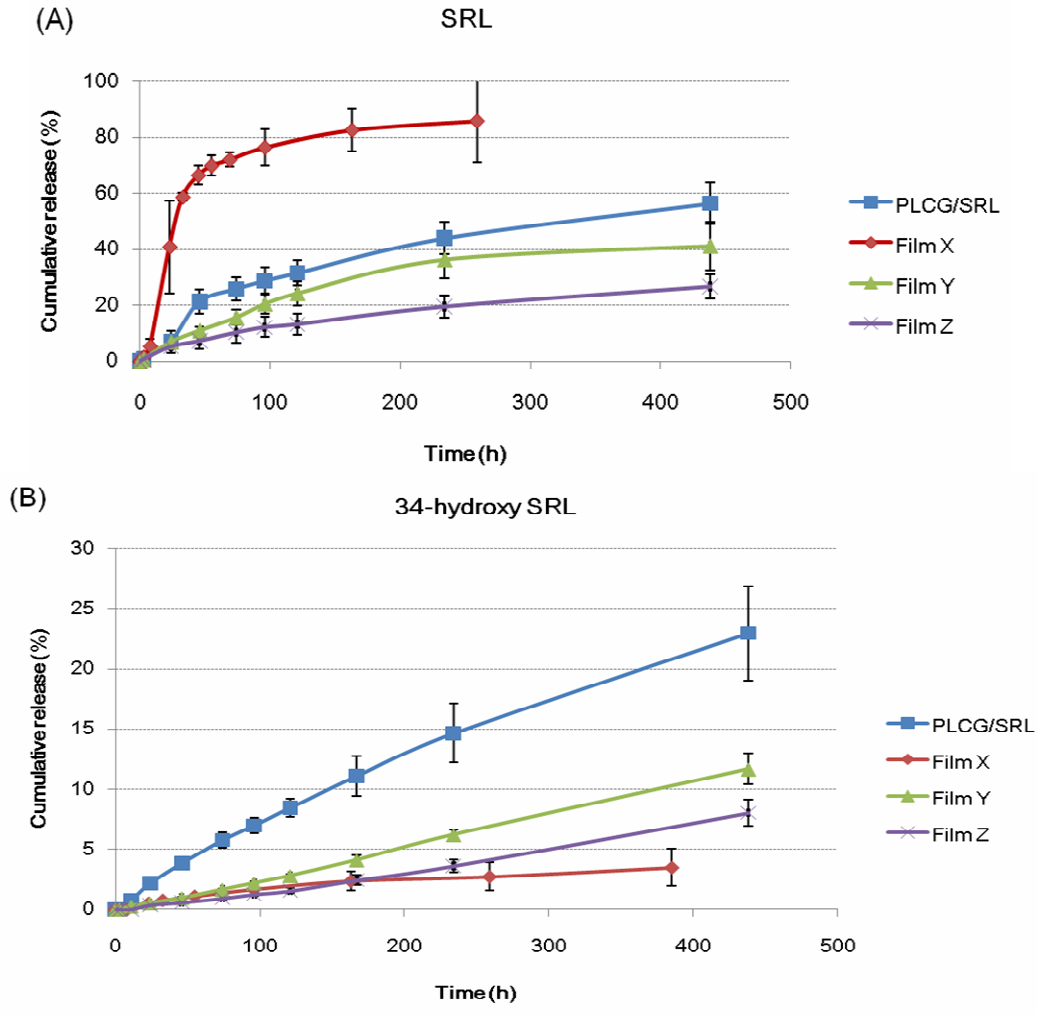

Figure 6. Release profiles of (A) SRL and (B) 34-hydroxy SRL from PLCG/SRL films (blue squares), Film X (red dimonds), Film Y (green triangles), and Film Z (purple crosses) during incubation in PBS with BSA. Data points and error bars represent mean ± SD for n=4–5.

3. Discussion

There are two possible mechanisms to explain why the PLLA/PMB30W (95/5) cast (solvents, DCM/MeOH = 12/1, v/v) films demonstrated higher breaking strengths than the PLLA cast (solvent, DCM) films. First, the cracks presented at the air contact surface of PLLA cast films could deteriorate their mechanical properties. Because the PLLA/PMB30W (95/5) cast films had a smooth surface without cracks and limited sub-micron sized pores, the polymer walls of the PLLA/PMB30W (95/5) cast films were more compact and were able to sustain deforming pressures. Second, interlocking networks were formed between the hydrophobic segments (BMA units) of PMB30W and PLLA chains. The mechanical properties of PLLA increase via solution blending with surfactants of optimum concentrations. In the PLLA/PMB30W (95/5) blend system, the homogeneous spreading of PMB30W could bridge the gap among the crystalline regions of PLLA across the amorphous regions of PLLA. Meanwhile, for the efficient surface modification, PMB30W was accumulated with MeOH-rich mixed solvents on the inner surface of PLLA/PMB30W tubing by the repeated coatings with the DCM/MeOH (12/1 by v/v) mixed solvent. Therefore, a high-density phosphorylcholine group coat was formed on the inner surface of the PLLA/PMB30W tubing even without water immersion.

The idea that phosphorylcholine groups might reduce stent thrombosis has not been resolved. Thus, the tissue compatibility of PLLA and PLLA/PMB30W was evaluated. The gravest risk associated with temporary stent materials is that they are heterogeneously degraded and release microparticles that enter circulation and may lead to the formation of thrombotic emboli. The hypothesis that microparticles covered with phosphorylcholine groups could prevent the apoptosis of phagocytes and thus remain biologically inert has been proven by a high-content screening assay. Moreover, an in vitro immunological study has revealed that temporary stent materials bearing phosphorylcholine groups can reduce the inflammatory reaction of human blood cells. Finally, animal studies have revealed that the biodegradation properties of the proposed material are appropriate for its use in stenting and can reduce thrombus formation after intravascular stent implantation in small animals. Because the formation of cell membranes in nature is highly regulated and finely tuned at the molecular level, the surface could improve the vascular tissue compatibility of these stent materials from the time of implantation to the final absorption.

For drug-eluting stent materials, a new drug delivery system was proposed to enhance and stabilize the delivery of SRL incorporated by PMB30W. SRL is an unstable agent; its degradation produces an open-chain isomer, 34-hydroxy SRL; it retains less than 10% of the antiproliferative activity of the parent SRL. An attractive approach to stabilizing bioactive agents is to incorporate them into nanovehicles. The PMB30W chains assemble themselves in an aqueous medium and form colloidal aggregates. These aggregates could be used as nanovehicles to maintain SRL inside of the nanovehicles in a stable manner. The hydrophobic part (BMA units) of PMB30W nanovehicles could inhibit the entrance of water molecules, while the hydrophilic part (phosphorylcholine groups) of PMB30W is endowed with forming the small size of nanovehicles in an aqueous medium. Thus, the shielding effect provided by PMB30W nanovehicles suppressed the hydrolysis of SRL and enhanced the carrier-mediated delivery of SRL. The release of SRL was controlled by tuning the size of PMB30W/SRL-rich domains in the PLCG/PMB30W/SRL films. This approach might reduce the in-stent neointimal hyperplasia and the drug-associated platelet activation.

4. Conclusion

In this thesis, I have proposed a new concept of clinical materials – temporary stent materials bearing phosphorylcholine groups. The preparation of the materials was efficient for the surface modification and comparable with conventional temporary stent materials in terms of bulk properties. The preliminary hypothesis that temporary stent materials bearing phosphorylcholine groups offer enhanced tissue compatibility has been proven in part. Furthermore, temporary stent materials bearing phosphorylcholine groups have been found to have potential as efficient reservoirs for enhancing and modulating an antiproliferative release.