

## 비용매 휘발법을 이용한 생체모사 혈액친화성 폴리락티드-ε-카프로락톤 공중합체 필름의 제조

임진익 · 김수현<sup>\*,\*\*,\*†</sup>

단국대학교 화학공학과, \*한국과학기술연구원 생체재료연구단, \*\*고려대학교 KU-KIST 대학원  
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## Blood-compatible Bio-inspired Surface of Poly(L-lactide-co-ε-caprolactone) Films Prepared Using Poor Co-solvent Casting

Jin Ik Lim and Soo Hyun Kim<sup>\*,\*\*,\*†</sup>

Laboratory of Biointerfaces/Tissue Engineering, Department of Chemical Engineering, Institute of Tissue Regeneration Engineering,  
College of Engineering, Dankook University, Jukjeon-dong, Yongin-si, Gyeonggi-do 448-701, Korea

\*Division of Life and Health Sciences, Biomaterials Research Center, Korea Institute of Science and Technology, Seoul 136-791, Korea

\*\*KU-KIST Graduate School of Converging Science and Technology, Korea University, Seoul 136-701, Korea

**초록:** 혈항혈전성 표면의 제조를 위해 간단한 비용매 휘발 방법을 통하여 고탄성체이면서 생분해성 폴리 락티드-ε-카프로락톤 공중합체 필름의 표면에 연잎 구조물과 유사한 마이크로 돌기를 만들어 주었다. 표면 구조와 소수성도, 항혈전 효과 등을 시험했으며, 결정화도와 탄성회복률 등의 물리적 특성도 분석하였다. 그 결과 비용매와 메틸렌클로라이드의 혼합 부피비 1:2에서 연잎표면과 유사한 최적의 효과를 얻었으며, 이때 수접촉각은 124°였다. 혈소판 부착시험에서는 처리하지 않는 군에 비해 약 10%만 부착되는 효과를 확인할 수 있었다.

**Abstract:** Simple poor-cosolvent casting was used to surface treat biodegradable elastic poly(L-lactide-co-ε-caprolactone) (PLCL; 50:50) copolymer films that presented lotus-leaf-like structures. We evaluated whether the lotus-leaf-like-structured PLCL (L-PLCL) films could be used as a biomaterial for artificial vascular grafts. The surface morphology, hydrophobicity, and antithrombotic efficiency of the films were examined while immersed in platelet-rich plasma (PRP) using scanning electron microscopy (SEM) and a contact angle meter. The recovery and crystallinity of the films were measured using a tensile-strength testing machine and an X-ray diffractometer, respectively. The solvent containing acetic acid, as a poor co-solvent, and methylene chloride mixed in a 1:2 ratio produced an optimal PLCL film with a water contact angle of approximately 124°. Furthermore, the surface of the L-PLCL films immersed in PRP showed a lower rate of platelet adhesion (<10%) than that of the surface of an untreated PLCL film immersed in PRP.

**Keywords:** antithrombotic material, lotus-leaf-like structure, co-solvent system, blood vessel, surface modification.

### Introduction

Synthetic biodegradable polymers, such as polylactic acid (PLA), polyglycolic acid (PGA), poly(lactide-co-glycolide) (PLGA), poly(ε-caprolactone) (PCL), poly(glycolide-co-ε-caprolactone) (PGCL), and poly(L-lactide-co-ε-caprolactone) (PLCL), have been developed for use in vessel, bone, and skin tissue engineering.<sup>1,2</sup> Unfortunately, the use of these polymers has been found to give rise to adverse effects, such as blood clots and tissue capsulation, by having an interface reaction

between the artificial surface and the biomolecules.<sup>3-6</sup> In particular, the interaction of blood with biopolymers as foreign materials is very complicated. The first event that occurs is that plasma proteins are adsorbed on the biopolymer surface when the material comes into contact with blood, and the platelets then adhere, contributing to surface-induced thrombosis. Furthermore, longer exposure of the biopolymer to blood can lead to embolization, calcification, and changes in the properties of the biopolymer.<sup>7-14</sup> To overcome these problems, various approaches have been adopted that improve blood compatibility of the polymeric materials. These include endothelial cell culture or immobilization,<sup>15</sup> chemical modification,<sup>16,17</sup> and the use of biological anticoagulants such as heparin, pros-

<sup>†</sup>To whom correspondence should be addressed.  
E-mail: soohkim@kist.re.kr

taglandin, and urokinase.

Unfortunately, these approaches are likely to be more applicable to non-degradable systems with stable surfaces. Localized heparin release has also been evaluated as a preventive measure for cardiovascular thrombosis and other effects of vascular repair, but most studies so far have involved the use of coatings or microspheres<sup>18,19</sup> that do not solve the problems of low drug loading and lack of extended release period.

Therefore, the most desirable method to improve the blood compatibility of the material is to control the interface properties through physical rather than chemical modification of the biopolymer surface. The superhydrophobic effect is well known in nature, and it results from the entrapment of air within surface asperities beneath the contacting wetting fluid. The trapped air greatly enhances the net water repellency, and the condition is thus termed superhydrophobicity.<sup>20</sup> The water repellent properties of lotus leaves and bird feathers are commonly-cited examples.<sup>21,22</sup> Recently, many researchers have reported on antithrombotic effects of superhydrophobic surfaces. Various methods have been developed to fabricate superhydrophobic surfaces, such as the template tool, a sol-gel process, solution evaporation, layer-by-layer self-assembly, plasma treatment, and electrospinning.<sup>23,24</sup> Furthermore, superhydrophobic surfaces, such as the lotus-leaf-like structure, have been extended to an increasing number of applications, including contamination prevention (self-cleaning windows, windshields, and exterior paints for buildings), microfluid electromechanical systems, and enhanced lubrication.<sup>23,24</sup>

The objectives of this study were as follows: (1) to determine the optimal surface treatment conditions to achieve a lotus effect; (2) to evaluate the antithrombotic efficiency; and (3) to gauge the applicability of these materials in artificial vessels in future studies.

## Experimental

**Chemical Reagents.** In this study, *L*-lactide (Purac Biochem, Birmingham, UK) was used to synthesize PLCL.  $\epsilon$ -Caprolactone, stannous octoate, chloroform, acetic acid, methylene chloride, and methanol from Sigma-Aldrich (St. Louis, MO, USA) were used without further purification.

**Fabrication of Lotus-leaf-like Structured PLCL (L-PLCL).** PLCL was prepared by a ring opening reaction of *L*-lactide and  $\epsilon$ -caprolactone as previously described.<sup>25</sup> PLCL (0.5 g; Mw: 211000) with a molar ratio of 50:50 (*L*-lactide:  $\epsilon$ -caprolactone) was dissolved in a series of 10 mL mixtures of acetic

acid (as a poor co-solvent) and methylene chloride (as a good solvent) at concentrations of methylene chloride (%) of 0, 37.5, 50, 66.7, and 80 (Table 1). The PLCL solutions presented states from transparent to weak colloid depending on the concentration of the poor co-solvent concentration. The PLCL solutions were then cast into a clean glass mold (50×50×3 mm<sup>3</sup>) for three weeks at room temperature. Then the dried peaks that formed on the films were observed and compared to the colloid particles in the PLCL solutions.

**Colloidal Property of PLCL Solution by Poor Co-solvent.** PLCL was dissolved in a series of mixtures of acetic acid (as a poor co-solvent) and methylene chloride at concentrations of methylene chloride (%) of 0, 37.5, 50, 66.7, and 80. The size of the micro particles of each solution was measured using a dynamic light scattering spectrophotometer (DLS-7000, Otsuka Electronics, Osaka, Japan) and were compared with the peaks that formed on the dried films of the mixtures.

**Characterization of L-PLCLs.** The surface morphology and diameter of the lotus-leaf-like structures were determined using field emission scanning electron microscopy (FE-SEM: S-4700; Hitachi, Tokyo, Japan).

To evaluate the effects of the surface modification on the hydrophobicity of the L-PLCLs, the water contact angles of the PLCLs were measured using a contact angle meter (Phoenix 150; Surface Electro Optics, Seoul, Korea).

The crystal intensity diagrams of the L-PLCL films were obtained using X-ray diffraction (XRD: D8 advance; Bruker AXS, Karlsruhe, Germany) operating with CuK $\alpha$  radiation ( $\lambda$  = 0.15406 nm) at 40 kV with a current of 100 mA at a speed of 1°/min.

The samples were prepared to have dimensions of 20×10×1 mm<sup>3</sup>. Recovery testing was performed using a universal testing machine (Instron model 4467; Canton, MA, USA), and a 10 N load cell with a crosshead speed of 10 mm/min (strain = 0, 5, 50, 100, and 150%) was used. The recovery was calculated as follows

$$\text{Recovery}(\%) = 100 - [(L_2 - L_0)/(L_1 - L_0)] \times 100 \quad (1)$$

where  $L_0$  indicates the original length,  $L_1$  indicates the extended length, and  $L_2$  indicates the final length after releasing the stress.

**In Vitro Platelet Adhesion.** A platelet-rich plasma (PRP) test was performed by using the previously reported method.<sup>26,27</sup> PLCL and L-PLCL films were equilibrated overnight in syringes containing 2 mL of PBS. Prior to the adhesion studies, the buffer was removed and 2 mL of PRP were

introduced into each syringe. The syringes were tapped to remove air bubbles, sealed, and rotated in a shaking incubator at 37 °C. After an adhesion time of 60 min, the films were quickly removed from the syringes and were then fixed in a formaldehyde solution for 24 h. The platelets that had adhered to the surface of the film were detected using field emission scanning electron microscopy and were counted using a hemacytometer.

## Results and Discussion

**Colloidal Property of PLCL Solution by Poor Co-solvent.** In the case of composition A (acetic acid as a poor co-solvent, Table 1), the micro-sized PLCL particles (with sizes of around 2  $\mu\text{m}$ ) were observed and decreased in size or were completely solved as the methylene chloride concentration increased (Figure 1) (acetic acid/methylene chloride: particle size, 5/0:  $2\pm0.07$ , 5/3:  $1.8\pm0.1$ , 5/5: 0, 5/10: 0, and 5/20: 0  $\mu\text{m}$ ). On the other hand, the size of the peaks on dried films increased as the methylene chloride concentration increased (acetic acid/methylene chloride: peak size, 5/0: 0, 5/3: 0, 5/5:

$6.4\pm1.6$ , 5/10:  $7\pm2.8$ , and 5/20:  $52\pm12$   $\mu\text{m}$ ). We inferred that the decrease in the particle size was the result of having methylene chloride as a good solvent for PLCL, whereas the increase in the peak size was attributable to thermodynamic instability during the recrystallization process of the particles. In the case of the film dried for a long time under a stable solution state by increasing the concentration of good solvent rather than that of the poor co-solvent, the particle size commonly increased due to the aggregation between the particles due to the stable thermodynamic properties.<sup>28</sup> Therefore, we suggest that the peak size can be changed by controlling the ratio of the good solvent to the poor solvent.

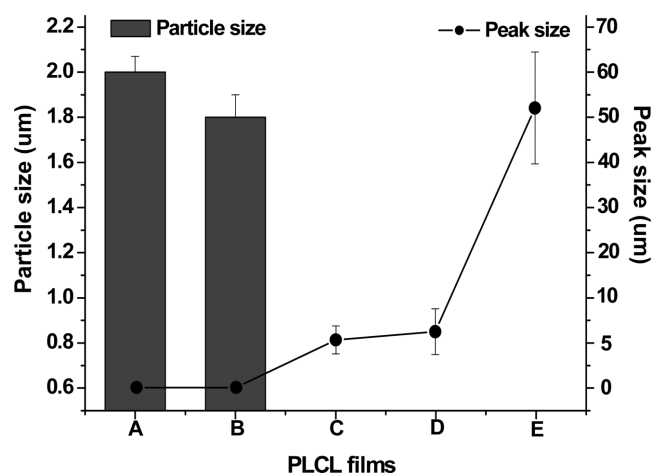
**Morphology and Contact Angle of L-PLCL Films.** The surface morphology of the films prepared using composition A and composition B showed no difference. In addition, peaks of around 2-50  $\mu\text{m}$  in diameter were observed on the L-PLCL films prepared using the solution compositions C, D, and E (Figure 2). The surface morphology of the film prepared using composition D showed uniform peaks of 5-10  $\mu\text{m}$  in diameter, which created a morphology similar to that of a naturally occurring lotus-leaf structure.<sup>29</sup> However, the surface morphology of the film prepared using composition E was not uniform. As indicated in Figure 2(F), the surface morphology of the film prepared using composition D showed uniform lotus-leaf-like peaks with diameters that were quantitatively measured to be of around 5-10  $\mu\text{m}$ . Therefore, we anticipate that this film would present an improved antithrombotic effect as a result of the lotus-leaf-like structure achieved using a solution with a 5/10 ratio of the poor cosolvent to the good solvent (composition D).

The hydrophobicity of the PLCL film surfaces was determined by measuring the water contact angles for the films (Figure 2). The water contact angle for the L-PLCL film prepared using composition D was of  $124\pm2.1^\circ$ , which was higher than that of the original PLCL film ( $78.9\pm1.5^\circ$ ). We believe that the enhanced hydrophobicity of the L-PLCL film surface can be attributed to the lotus-leaf-like microstructures on the film surface. Although superhydrophobic surfaces in self-cleaning applications are commonly reported to have water contact angles of over  $150^\circ$ , we used a highly hydrophobic surface with a contact angle of around  $124^\circ$  to try to achieve an antithrombotic effect.

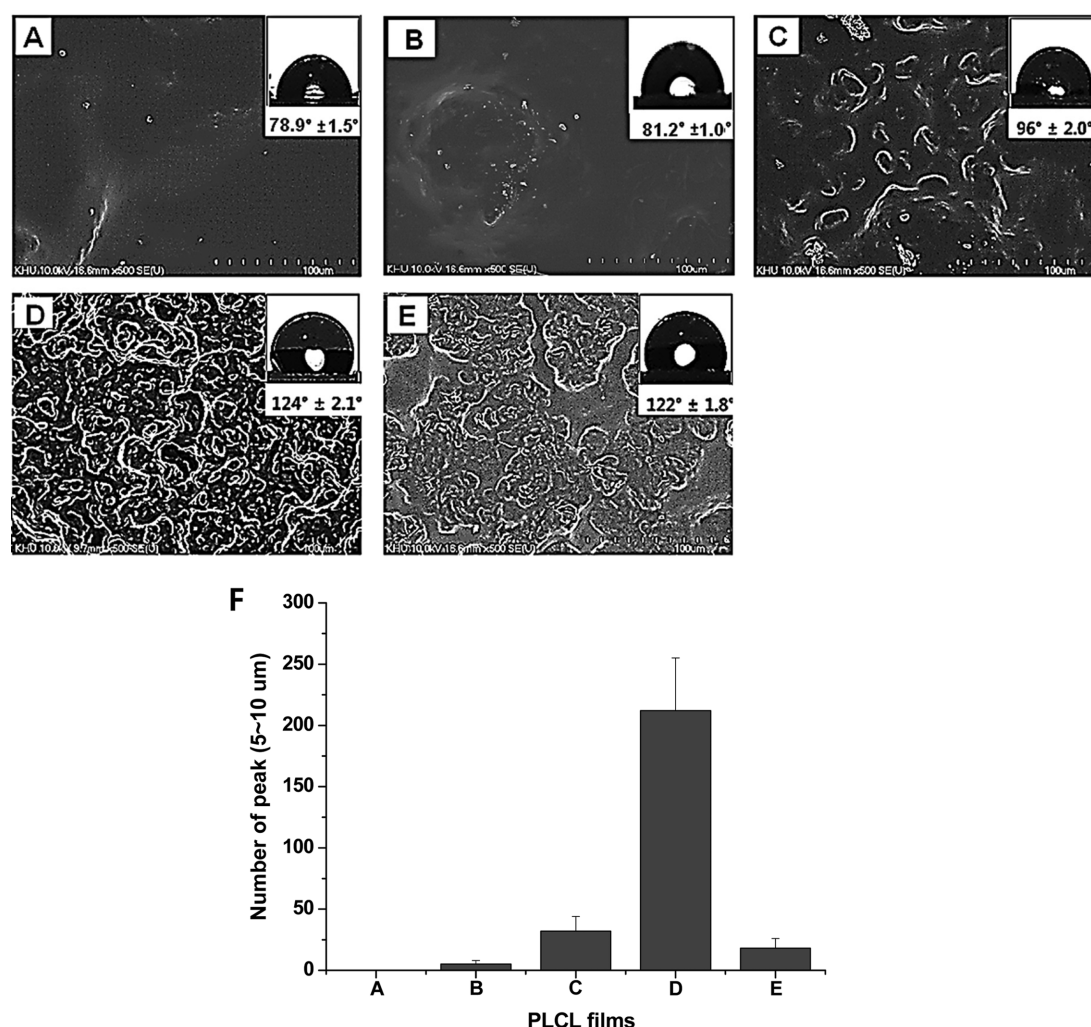
**Crystallization Intensity of L-PLCL Films.** The crystallinity of the L-PLCL films and some of the X-ray diffraction patterns for the films varied according to the concentration of the poor co-solvent in the solutions used to prepare the films.

**Table 1. Composition for L-PLCL Film**

Composition	PLCL (g)	Acetic acid (mL)	Methylene chloride (mL)
A	0.5	10	0
B	0.5	6.25	3.75
C	0.5	5	5
D	0.5	3.33	6.67
E	0.5	2	8



**Figure 1.** Particle and peak sizes of each solutions and of the dried films.



**Figure 2.** SEM images and contact angles for PLCL films prepared using solutions containing co-solvents at ratios of (A) pure PLCL,  $\times 500$ ; (B) PLCL 5/0; (C) L-PLCL 5/5; (D) L-PLCL 5/10; (E) L-PLCL 5/20; (F) quantitative analysis of peak size.

Three main diffraction peaks at around  $2\theta = 16$ ,  $19$ , and  $23^\circ$  were observed in the small-angle region of the diffraction profiles for the PLCL films (Figure 3). The  $2\theta = 16$  and  $19^\circ$  diffraction peaks were clear, indicating a high degree of crystallinity in a hard-segment region of the elastomeric PLCL. The intensity of the diffraction peaks at  $2\theta = 16$  and  $19^\circ$  decreased in intensity from 14400 to 9400 at  $2\theta = 16^\circ$  and from 11100 to 6100 at  $2\theta = 19^\circ$  as the crystallinity decreased with increasing concentration of the poor co-solvent in the solution, suggesting that the concentration of the poor co-solvent in the solution affects the crystallinity of PLCL films prepared from the solution. We believe that the decrease in crystallinity implies a change in the mechanical properties, such as the elasticity, of the L-PLCL films.

**Elastic Recovery Properties of L-PLCL Films.** The elas-

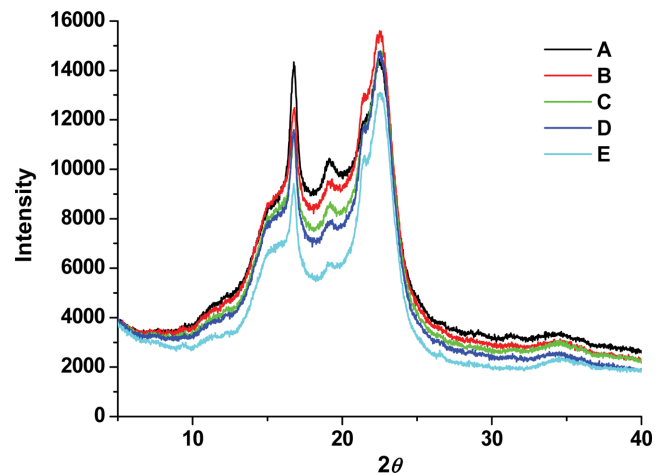
**Table 2.** Elastic Recovery Property of PLCL Films according to Co-solvent Contents

Co-solvent ratio	5/0	5/3	5/5	5/10	5/20
Strain (%)					
0	100	100	100	100	100
5	100	100	100	100	100
50	100	100	100	100	100
100	100	95	94	95	96
150	97	91	89	92	91

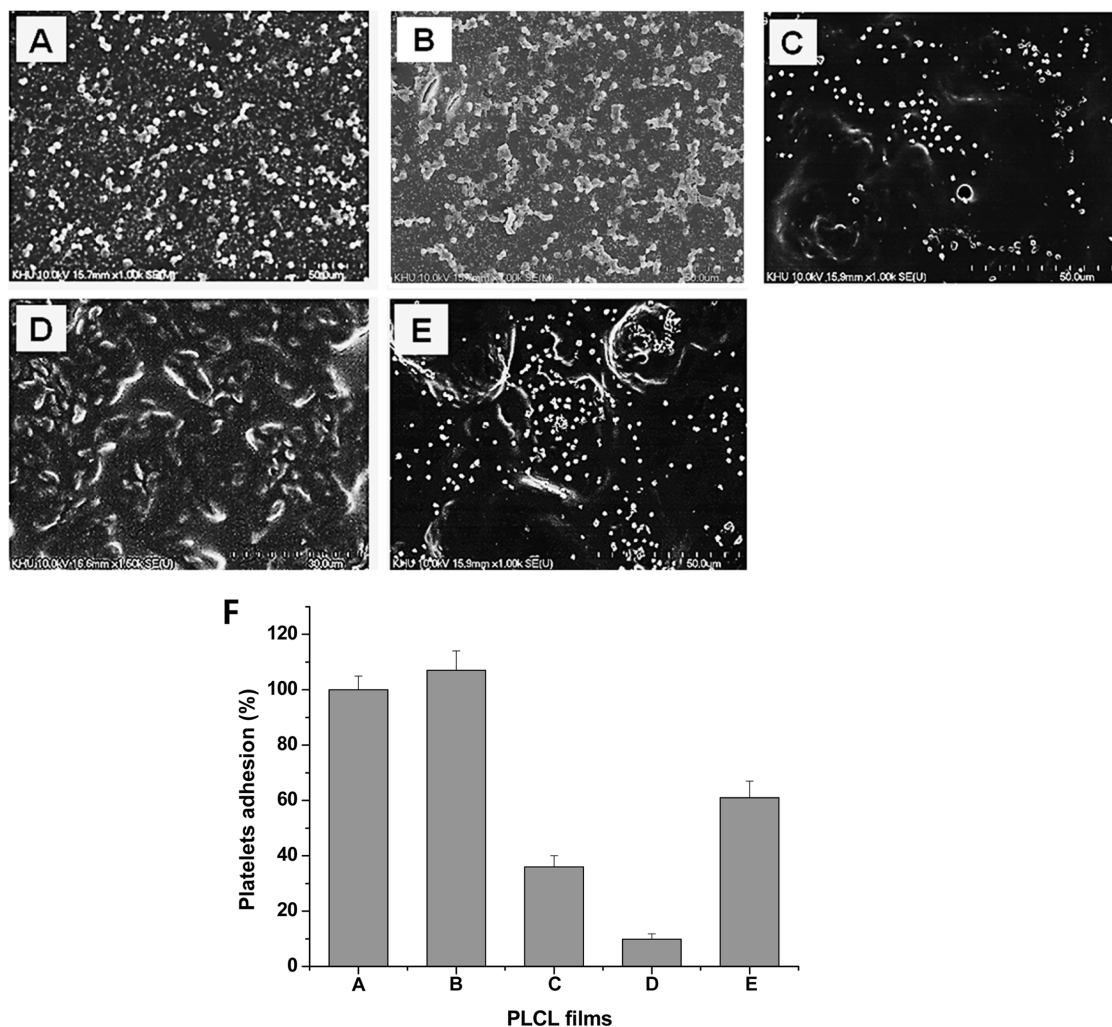
tic recovery of each of the PLCL films were compared. The pure PLCL and L-PLCL films elongated to 100% showed different elastic recoveries (Table 2). We suggest that the somewhat low recovery efficiency for the L-PLCL films was due to

smaller hard-segment regions in the L-PLCL films resulting from the cosolvent in the solutions. Despite the low elastic recoveries of the films elongated at 100%, we assert that blood vessels only require about 10% elastic recovery. Therefore, these properties of L-PLCL make it a material suitable for building scaffolds for blood vessels and for other tissue-engineering applications.

**Platelets Adhesion Test.** When biomaterials are in contact with blood, proteins are first adsorbed instantaneously onto the surfaces and then become deformed. Then, the platelets adhere to the surfaces and are subsequently activated and aggregated, which may play a major role in the formation of thrombus. Figure 3 shows the behavior of the platelets adhering onto the surfaces the PLCL films *in vitro*. Fewer platelets adhered to the surfaces of all of the L-PLCL films than to the surface of the



**Figure 3.** XRD patterns for PLCL films prepared using solutions containing various solvent contents.



**Figure 4.** SEM images of platelets adhered onto the PLCL films prepared using solutions containing co-solvents at ratios of (A) pure PLCL,  $\times 500$ ; (B) PLCL 5/0; (C) L-PLCL 5/5; (D) L-PLCL 5/10; (E) L-PLCL 5/20; (F) quantitative analysis of adhered platelets.

control PLCL. Furthermore, the surface of the L-PLCL film prepared using composition D showed a morphology more like that of a lotus leaf than the surfaces of the other L-PLCL films did. Fewer platelets adhered to the surface of this film (approximately 10%, Figure 4(F)) than to the surfaces the other L-PLCL films (Figures 4(B), (C), and (E)) probably because the other L-PLCL films showed a negative morphology. That is, the film surface prepared using composition D contained irregular peaks and puddles that prevented the platelets from adhering onto the surface.

## Conclusions

Blood-compatible L-PLCL film was fabricated using a poor co-solvent by using a casting method with poly(*L*-lactide-*co*- $\epsilon$ -caprolactone) (50:50) to achieve a lotus-leaf-like structure. We conducted various tests to assess the usefulness of these films as artificial blood vessels with antithrombotic properties, characterizing the chemo-physical properties of L-PLCL according to the co-solvent content, elastic recovery properties, and lotus-leaf-like structure. The antithrombotic effect was improved by a more defined lotus-leaf-like structure. Although further studies including more detailed evaluations of antithrombotic and biodegradable properties are still required, this novel L-PLCL film has potential to be applied as a surface treatment method for blood compatible biodegradable materials.

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