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Preparation and Characterization of porous tubular scaffold made of PCL/PLCL blends for vascular tissue engineering

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ABSTRACT

Porous tubular scaffolds had been developed by physically blending poly-ε-caprolactone (PCL) and poly-(lactide-co-ε-caprolactone) (PLCL) using solid-liquid phase separation method subsequent with freeze-drying method. The effect of blending ratio on the morphology and mechanical properties of PCL/PLCL blends tubular scaffold had been investigated. The blending were confirmed using infrared spectroscopy. The microstructure behaviour were observed using scanning electron microscopy and the mechanical properties were evaluated using ring tensile test. It was concluded that the resulted tubular scaffold possessed an improved elastic modulus and enlarged pore size as the content of PLCL increased. The tubular scaffold containing 75% PLCL was found as the optimum blends ratio in terms of elastic modulus and rebound properties. The tubular scaffold made of PCL/PLCL blends has a potential for vascular tissue engineering application.

Keywords *Polymer blends, PCL, PLCL, vascular tissue engineering, phase separation.*

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Introduction

Cardiovascular disease is the major cause of death in the Western World [1–3]. It includes many kind of disease that related with heart and blood vessel such as stroke, congenital heart defect, diabetes, and atherosclerosis. In most cases, this disease causes blood vessel damage which then required a surgical intervention to replace the damaged vascular with a vascular substituted [4]. A healthy vascular taken from another part of patient's body is usually used, however, this depends on the availability of vascular that can be grafted [5]. As alternative, prosthetic graft made of PTFE or DACRON can be used. Unfortunately due to lack of tissue compatibility, these prosthetic grafts often lead to thrombus formation and hyperplasia[6–8]. Therefore, there is a crucial need of artificial vascular prosthesis that completely biocompatible and has a growth potential.

Tissue engineering have provided a new strategy to create artificial graft by combining a scaffold made of natural polymer and/or biodegradable polymer with cells[9,10]. Synthetic polymer has attracted a special interest to construct prosthetic graft as it is commercially available and reproducible. The work particularly for tissue engineering vascular (TEV) has been much focused on the use of synthetic polymer that can be hydrolyzed and degraded in the body without generating harmful products[11,12]. Such biodegradable polymers that have received much attention in developing TEV are poly glycolic acid (PGA)[13], PCL [14], poly lactic acid (PLA) [15], and PLCL [16,17].

PCL is one of the most promising candidate for biodegradable scaffold that has been widely studied. PCL has low melting point [18] with elastic and flexible characteristic [19] and regarded as soft tissue compatible material. Due to relatively low mechanical properties and long degradation time (3-5 years)[20], PCL is usually physically blended or chemically copolymerized with other polymer such PLA[18,21], resulting a controllable mechanical properties depending on the composition ratio. However, PLA has a rigid and stiff mechanical properties[22], thus limits their usefulness for soft tissue application. Blending PCL with other polymer with less rigid yet tough materials is then desired. PLCL (75:25) is both tough and rubber-like with a high elastic modulus[19]. As result, blending PCL and PLCL will produce tubular scaffolds with acceptable flexibility and wide range of tensile strength and elastic modulus.

In this study, tubular scaffold were developed with various tensile mechanical properties by controlling blending ratio of PCL and PLCL. The tubular scaffold was fabricated by solid liquid phase separation followed by freeze drying. Effect of PLCL content to the tensile mechanical properties and microstructure were examined.

Experimental Methods

Fabrication of Tubular Scaffolds

Tubular scaffold was fabricated from blending of PCL and PLCL(75/25) (Gunze ltd., Kyoto, Japan) by the solid-liquid phase separation method followed by freeze drying as described [17]. PCL and PLCL granule with various weight ratio (100:0, 75:25, 50:50, 25:75, 0:100) were dissolved in dioxane (Kishida Chemical, Osaka, Japan) with final concentration 6% (w/v). A Teflon rod of 10 mm in diameter, taken from -80ºC was vertically dipped into polymer solution and pulled out at a constant rate of 100 mm/min. After frozen again into -80º C for 2 hours, it was dried into a freeze drying machine (Tokyo Rikakikai, Tokyo, Japan) at -50ºC for 24 hours. Finally, the tubular graft was pulled out from the Teflon tubes and stored in dehumidifier chamber for further use. The schematic fabrication was shown in Figure 1c.

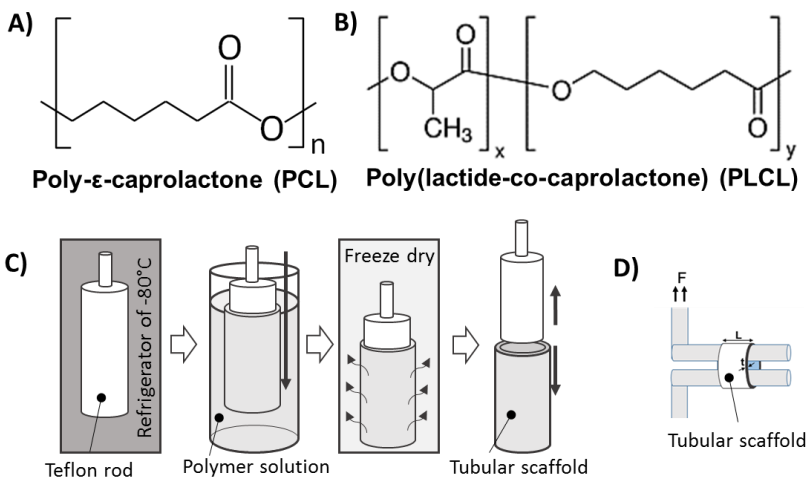

Figure 1. Chemical structure of a) PCL and b) PLCL, c) schematic procedure of scaffold fabrication, and d) ring tensile test.

Structural and Morphological Structure

Infrared spectroscopy (FTIR) was performed to confirm chemical structure of blending polymer using FTIR (JASCO 4200, Japan). The sample was sputter-coated with Pt-Pd using anion sputter coater (Hitachi, Tokyo, Japan) then the surface microstructure of the tubular scaffold was observed using field emission of scanning electron microscope (FE-SEM) (S-4100, Hitachi,

Japan). Six SEM images from each samples were taken and then analysed the pore diameter and area using Image-J software (NIH, United States). The wall thickness was measured by taking cross section images of each samples using SEM imaging.

Ring Tensile Test

Tensile mechanical testing was performed by universal testing machine (Shimadzu, Kyoto, Japan) using the ring tensile method (Figure 1d). It consisted of two small metal rods (ϕ 5mm) inserted into the lumen of the tubular scaffold. One metal rod was then moved at constant crosshead speed of 1 mm/min with 10 N load until the sample failed. The displacement and the load were recorded. Using the displacement data, the internal circumference during load was calculated using the following formula Equation (1),

$$C_{int} = D_{int} \times \pi = d_{pin}(\pi + 2) + 2\Delta s \quad (1)$$

Where C_{int} is internal circumference of tubular graft, D_{int} is internal diameter of tubular graft, d_{pin} is the diameter of metal rod, Δs = displacement. The circumferential stress (σ) was calculated using Equation (2), where F is force during testing and L and t is the length and thickness of tubular graft, respectively.

$$\sigma = \frac{F}{2Lt} \quad (2)$$

The strain was evaluated as the ratio of the internal circumference during loading with the initial internal circumference (Equation 3). The elastic modulus was calculated from the slope of linier region of stress-strain curve.

$$\varepsilon = \frac{C - C_{init}}{C_{init}} \quad (3)$$

Results

Chemical Structure

The infrared spectra of scaffold from PCL/PLCL blends with various weight ratio were shown in Figure 2. Pure PCL (PCL/PLCL 100:0) has peak absorptions at 1722 cm^{-1} corresponding to the C=O stretching vibration and at 2994 cm^{-1} due to stretching vibration of C-H bond (Figure 1a). As the

addition of PLCL, the peak intensity at 2994 cm^{-1} absorption decreased because PLCL has less C-H bond compared to PCL whose longer methylene (CH_2) chain. Moreover, new peak at 1756 cm^{-1} was appeared as the addition of PLCL in PCL/PLCL (75:25) due to stretching vibration of C=O from PLCL (Figure 1b). As the concentration of PLCL increased, the intensity of this peak became stronger, on the contrary, the peak intensity at 1722 cm^{-1} decreased. Pure PLCL did not show an absorption at 1722 cm^{-1}. The infrared spectra confirmed that PCL and PLCL were homogenously blended.

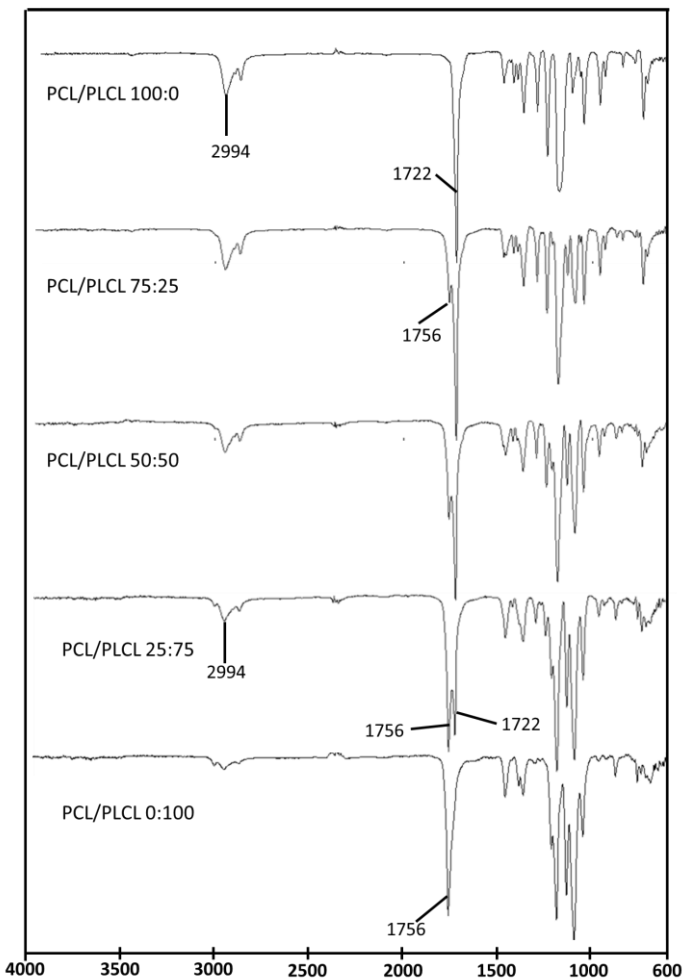

Figure 2. Infrared spectra of PCL/PLCL blends with various ratio.

Microstructural Behaviour

Figure 3 showed microstructural behaviour of PCL/PLCL scaffold at different weight ratio. All the scaffold exhibited a porous structure which oriented perpendicular to the surface. Pure PCL (Figure 3a) showed more fibrous struts compared to the pure PLCL. This because of rubbery properties of PCL. The fibrous struts was gradually disappeared as the PLCL content increased (Figure 3b,c,d,e). The pore diameter was tend to increase as the PLCL content increased, although there is no statistical difference of pore area with one another (Figure 3f). In contrast, the wall thickness of the tubular scaffolds decreased as the PLCL content increased (Figure 3g).

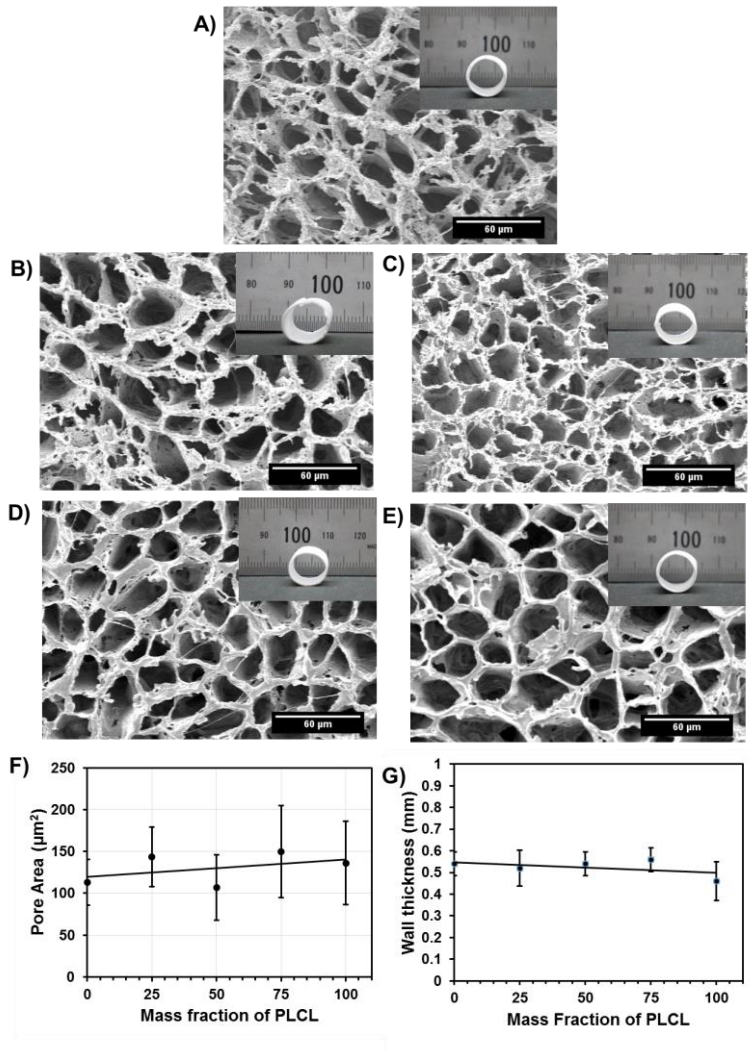

Figure 3. Macro- and microstructure of the tubular scaffolds from PCL/PLCL blends with various weight ratio. a) 100:0, b) 75:25, c) 50:50, d)25:75, e)0:100. f) Plot of pore area and g) wall thickness of the tubular scaffolds as function of PLCL weight ratio.

Mechanical Behaviour

To evaluate the effect of blending to the mechanical properties, tensile test was performed. Figure 4a showed a typical stress-strain curve of each

type of the scaffolds. As shown in Figure 4b,c, and d, PLCL has the highest tensile strength, failure strain and elastic modulus. PCL has an average tensile strength of 171.8 kPa. The tensile strength was suddenly decreased to 88.1 kPa when the PLCL content was added to 25%. This may be due to a phase separation between polymer coil of PCL and PLCL when suddenly frozen to -80°C. This phase separation affected not only to the mechanical properties but also the physical properties. The resulted PCL/PLCL (75:25) scaffold has a cracked site on it and it was easily to be torn.

However, the tensile strength was recovered as the PLCL content increased. The same behaviour was found at the failure strain. The elastic modulus increased as the PLCL content increased. Pure PCL has the lowest elastic modulus as predicted while the PLCL has the highest elastic modulus as the result of crystallisable hard and brittle properties from lactide acid monomer.

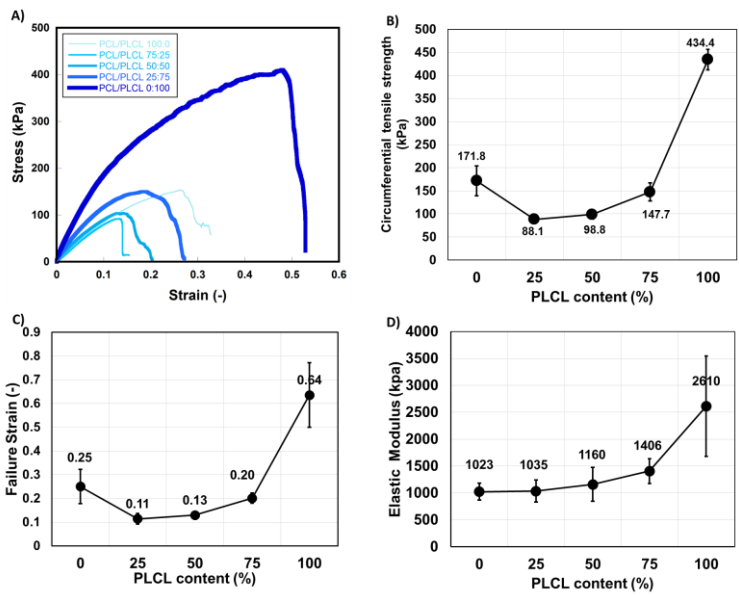

Figure 4. Mechanical properties of PCL/PLCL blends. a) Stress-strain curve. b) Circumferential Tensile Strength. c) Failure Strain. d) Elastic Modulus. Each data represented as mean ± SD (n=4).

It is important that the designated blood vessel has a rebound elasticity. To determine the optimum blending ratio, the rebound elasticity of the scaffolds were performed. As shown in Figure 5, PCL/PLCL (25:75) was

squeezed but it was able to rebound to original shape. While pure PLCL failed to rebound to its luminal shape.

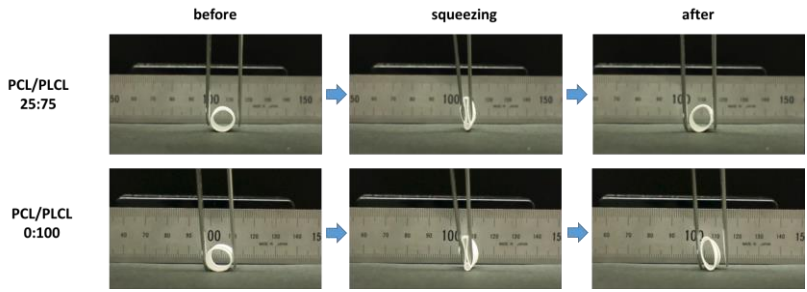

Figure 5. Photos of the rebound properties of scaffolds with PCL/PLCL ratio of 25:75 and 0:100 before and after deformed with tweezer.

Discussions

Solid-liquid phase separation method is a facile way to prepare porous scaffolds with appropriate pore diameter for cell growing. In this study polymer blends scaffold was fabricated using this method combined with freeze drying. The resulted scaffold had averaged pore diameter more than 10 µm. This size is considered to be an ideal size of cell to infiltrate and proliferate into scaffold [24]. SEM images revealed that PCL scaffold has fibrous strut morphology (Figure 3a). As the content of PLCL was increased, the fibrous structure was slowly disappeared and the pore size increased. This phenomenon is closely related with the original properties of PCL and PLCL. PCL is semi crystalline which could form a gel by crystallization, resulting a fibrous structure after freeze drying [25]. While PLCL is amorphous which hardly form a gel by crystallization, so fibrous structure was not achieved and rather to form a smooth strut morphology[26].

Blending PCL with PLCL was expected to increase the mechanical properties including tensile strength and elastic modulus. However, it was observed that as the PLCL was added to 25% of the blending ratio, a significant decrease of tensile strength was found (Figure 4b,d). This may could be related by the phase separation phenomenon between these two polymers [21,27].

It is important to design blood vessel substituted that has good compliance since the blood vessel is continuously exposed to the mechanical force from blood flow such as shear stress and burst pressure. We found that

PCL/PLCL with weight ratio of 25:75 is an optimum ratio that produce tubular scaffold with high elastic modulus and tensile strength yet flexible with a rebound properties. The biocompatibility of the fabricated scaffold is currently under investigation.

Conclusion

The effect of blending ratio on the morphology and mechanical properties of PCL/PLCL blends tubular scaffold had been investigated. It was concluded that as the content of PLCL increased, the resulted tubular scaffold possessed improved elastic modulus and enlarged pore size. The tubular scaffold containing 75% PLCL was found as the optimum blends ratio in terms of elastic modulus and rebound properties.

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