

Fabrication of Macroporous PLLA Scaffold by Thermally Induced Phase Separation

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Introduction

Use of cell transplantation is investigated as the therapeutic strategy for tissue repair and organ replacement. Cell culture is related to the shape of scaffold because the scaffold plays an important role in the design of temporary substrates to be grown and specialized. Polymeric scaffolds should be porous enough to allow a high density of cells to be seeded, have sufficient mechanical stability and well-defined networks of interconnected pores[Mikos et al., 1993]. The poly-lactides (PLAs) have great potential in designing surgical implants, because of their biocompatibility and biodegradability. PLLA scaffold is currently tested for hard-tissue cell transplantation and is fabricated by gas foaming, salt leaching and freeze-drying via thermally induced phase separation (TIPS) process. TIPS method can be classified two processes, solid-liquid phase separation of PLA or PLGA binary solution was utilized to prepare macroporous scaffolds with a nonuniform ladder-like structure and liquid-liquid demixing of PLA-dioxane-water ternary system was becoming attractive to prepare well-design and open macroporus scaffold in size range from 20 to 50 μm by arresting morphology during spinodal phase separation before freeze-drying[Nam et al, 1999]. The porous morphology is determined by the final thermodynamic state and controlled by TIPS parameter. In this study, a macroporous highly-interconnected PLLA scaffold via liquid- liquid demixing in the PLLA–water-dioxane ternary solution is prepared in the presence PEG-PLLA diblock, and change of morphology is investigated.

Experiment

Cloud point temperature

Poly(L-lactic acid)(PLLA, Lacty 5000) was purchased from Shimadzu Co. The number-average molecular weight was 218,000 (PDI, $M_w/M_n = 1.55$). 1,4-dioxane and the deionized water were a good solvent and non-solvent for PLLA respectively, distilled prior to use. Weighted PLLA and PEG-PLLA diblocks (see table1) were added in 4ml-vial tube with dioxane/water mixture(87/13, w/w) as solvent, and then dissolved by heating at 63°C for 5hr with a magnetic stirrer. The homogenous PLLA solution was slowly cooled in steps of 1 °C, equilibrating the system for 10 minutes at each new temperature. The cloud point was measured by visual turbidity

Scaffold preparation

Dissolved pure PLLA solution and PLLA system with PEG-PLLA(5000-4532, 0.5wt%) was put into the water bath preheated to the quenching temperature. It was remained for 10, 60mins at 30°C, and then the annealed sample was directly immersed in liquid nitrogen to be fast frozen for 1hr. The freeze-drying was performed at -77°C and 7mmTorr for 3days, and the macroporous scaffolds were obtained.

Morphology characterization

The porous morphology of the prepared scaffold was performed by scanning electronic microscopy(SEM, Hitachi S-2400). The fracture-frozen cross sections of the scaffold in either longitudinal or transversal way was coated by gold, and examined by SEM

Table 1. Characterization of the Synthesized Diblock Copolymers

	Copolymer	$M_n^a/g \text{ mol}^{-1}$
Diblock1	PEG ₁₁₄ -PLLA ₆₂	5000-4532
Diblock2	PEG ₁₁₄ -PLLA ₄₀	5000-2848
Diblock3	PEG ₁₁₄ -PLLA ₆	5000-413
Diblock4	PEG ₄₅ -PLLA ₂₆	2000-1830

^aNumber-average molecular weight calculated from ¹H-NMR

Results and Discussion

Cloud point curves of solution in the presence of various additives(0.5wt%) are shifted to higher temperature in order of diblock1>diblock2>diblock1>diblock4 as shown in Fig 1. It has been known that phase separation was assisted by added diblock or triblock copolymer as a surfactant. This assistance is induced by the amphiphilic effect of added surfactant serving as the action of nucleus on phase separation. It is related to additive molecular structure including molecular weight, hydrophobic/hydrophilic block ratio and block lengths[Kawakatsu et al,

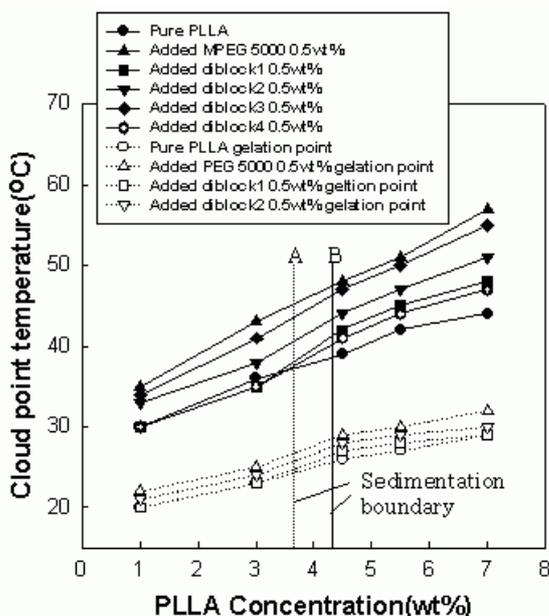


Figure 1

1995]. Obviously, an increase of PEG ratio (diblock3>diblock2>diblock1) in the ternary system shift the cloud point to higher temperature, and decrease of PEG block length (diblock4<diblock1) display lower cloud point curve in fig 1. Increasing PEG content in diblock can enhance the interaction with water because PEG exhibits the stronger solubility in water than PLLA, then liquid-liquid demixing located at higher temperature. In the preceding paper[Hua et al., 2002], because of the partial crystallization of PLLA, the pure PLLA solution generated a gelation above the concentration of

the sedimentation boundary ($<4\text{wt}\%$, dot line A in fig 1) when it was cooled below the gelation point at cooling rate ($0.1\text{ }^\circ\text{C}/\text{min}$). The gelation point temperature gradually increased with the increase of polymer concentration. Below the sedimentation boundary, the polymer solution was separated into two layers as a polymer-rich and solvent phase under phase separation temperature. Fig 1 show gelation point curves of ternary system with diblocks are small changed. It means that there are some interactions between the additives and solvent mixture during phase separation, and addition of diblocks were scarcely effect on crystallization of the PLLA. But, the sedimentation boundary ($<4.5\text{wt}\%$, solid line in fig 1) is shifted to higher polymer concentration with the increase of PEG content in solution.

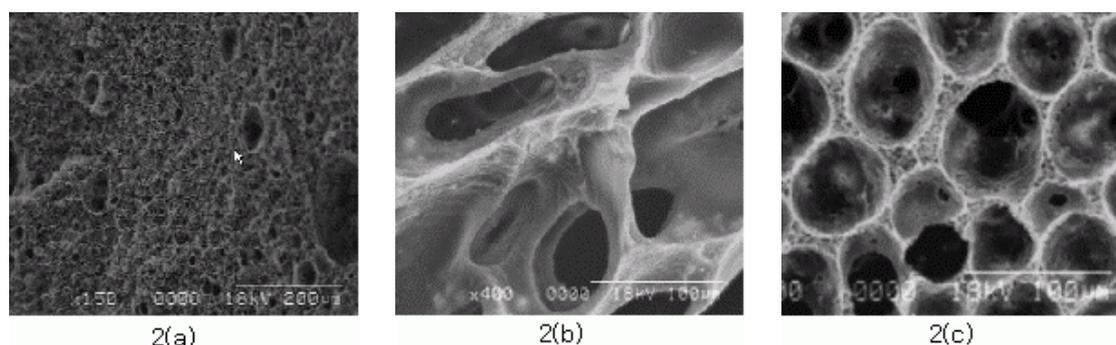


Figure 2. (a) PLLA 3wt%, 30mins, (b) PLLA 4.5wt%, 30mins, (c) PLLA 6wt%, 30mins

Fig 2 illustrates the effect of polymer concentration (3wt%, 4.5wt% and 6wt%) on scaffold morphology. The characteristic lacy structure was found for both systems soon after quenching because the quenching temperature ($30\text{ }^\circ\text{C}$) locates the system in the unstable region (see Fig 2). For the system with 4.5wt% polymer, the size of the highly interconnected pores after 30 minutes was greater than $150\text{ }\mu\text{m}$. The increase in the pore size at 4.5wt% polymer concentration was faster than for the system at the higher concentration of 6wt%, although the latter has a deeper quenching depth, as shown schematically (points A and B) in Figure 1. This is because the latter has a higher viscosity and larger polymer volume fraction. This high viscosity is induced by partial gelation at just above the gelation point due to crystallization of PLLA, combined with the viscosity increase caused by the high polymer concentration. In contrast with the scaffold from the 3wt% solution (quenching temperature $30\text{ }^\circ\text{C}$), the characteristic irregular morphology resulting from polymer sedimentation was not found for the two systems above. Furthermore, the rate of pore size development for times less than 5 minutes decreased in the order of the polymer concentration (rate at $3\text{wt}\% > 4.5\text{wt}\% > 6\text{wt}\%$), following the opposite trend to the quenching depths, as shown in Figure 1. This behavior is possibly related to the gelation effect during phase separation.

Fig 3 show the SEM micrographs gained from 4.5wt% PLLA solution (dioxane/water = 87/13, w/w) at quenching temperature of $30\text{ }^\circ\text{C}$ with different aging time (10 and 60mins). In the earlier stage (10mins), the characteristic and regular interconnected morphology is observed in Fig 3(a, c). We know, when quenching are located in the unstable region, phase separation prefer to spinodal decomposition behavior, so the interconnected and open porous structure. And, in the initial stage, morphology of scaffold depends on quenching depth. Addition of diblock could increase the phase separation temperature as shown Fig 1, it made the deeper

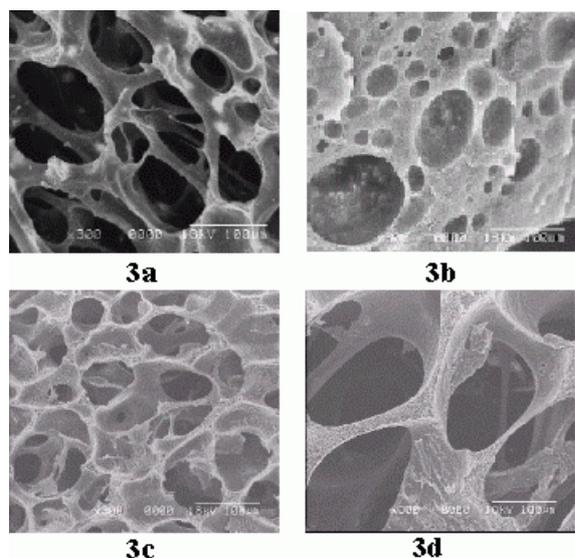


Fig 2. SEM micrographs of the scaffold. PLLA 4.5 wt %, dioxane/water = 87/13, quenching at 30°C, a) pure, for 10mins, b) pure, for 60mins, c) 0.5wt% diblock1 added for 10mins, d) 0.5wt% diblock1 added, 60mins

quenching depth at same quenching temperature. In comparison to the pure PLLA solution, the added diblock system was the larger inter-connected pore size than pure system. After aged 60mins, pure PLLA system became the irregular and closed macroporous structure as shown fig 3b, due to coalescence combination, a combination growth of larger structures at expense of smaller structure [Song et al., 1995]. But, for PLLA-dioxane-water ternary system with diblock, the regular, open and well-interconnected macro pores was observed, and pore size were fabricated in the size range of 200-250 μ m after 60mins in fig 3d. Diblock or non-ionic amphiphilic polymer such as Pluronic can play a role of lowering the interfacial tension that is important in the late stage of phase separation, when the phase separation kinetics is

controlled by the motion of interfaces driven by interfacial tension [Kawakatsu et al., 1993]. The lowering surface tension could decelerate phase separation and stabilize the high inter-connection. The hydrophilic/hydrophobic ratio can play a main factor for the decrease of the lower interfacial tension. Addition of diblock with the suitable PEG/ PLLA ratio can be obtained more effectively in the pore size and interconnection of scaffold.

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