

The formation of the S-shaped edge-on lamellae on the thin porous polylactic acid membrane via phase separation induced by water microdroplets

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ABSTRACT: Biodegradable polymers have received increased attention due to their potential application in the medicine and food industries; in particular, poly(lactic acid) (PLA) is a primary biopolymer because of its excellent biocompatibility and absorbability features. In this study, a porous PLA membrane was fabricated by phase separation using water microdroplets produced by an ultrasonic atomizer as a coagulation bath. The formation of S-type clusters was attributed to the slow exchange rate between solvent and coagulant, which provided time for the movement of polymer molecules. The effect of preparation conditions on the structure of S-type clusters, including polymer concentration in solution and ambient temperature was investigated. The PLA porous membrane prepared from water micro-droplets with different morphology compared with the sample fabricated from distilled water has potential application in fields of tissue engineering and artificial organ generation. © 2016 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* **2016**, *133*, 43355.

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INTRODUCTION

Poly(lactic acid) (PLA) is one of the most commonly used, biodegradable, and biocompatible polymers.¹ Produced from 100% renewable resources, PLA is degradable into CO₂ and H₂O under natural conditions.² It occurs as three different enantiomers: D, L, and meso, where D and L-poly(lactide)s are semicrystalline polymers, and meso is amorphous.^{3–5} The application of porous materials involved a large variety of fields, e.g. separation membranes, interlayer dielectrics, high surface area catalytic supports, as well as tissue regeneration scaffold.^{6–9} Rohman *et al.* produced poly(D,L-lactide)/poly(methyl methacrylate) interpenetrating polymer networks and used these as precursors to porous polymeric materials.¹⁰ Nurkhamidah and Woo investigated the use of phase separation and lamellar assembly below the upper critical solution temperature to produce a poly(L-lactic acid)/poly(1,4-butylene adipate) blend induced by crystallization.¹¹ Saracovan *et al.* studied the morphology of single-crystal lamellae grown from a dilute solution and concluded that the chiral character of a polyenantiomer is not transmitted to the lamellar morphology.¹² Lee *et al.* built a tissue regeneration scaffold made of PLA by phase-separation using a room temperature ionic liquid (RTIL) as the porogen. The scaffold was confirmed to have favorable *in vitro* cell

responses, supporting the proliferation of mesenchymal stem cells with culture time, and stimulating their osteogenic differentiation.¹³

Phase inversion is a simple and economic method to fabricate a porous membrane. When the polymer solution is exposed to the coagulation bath, a porous membrane is formed. Due to the quick exchange rate between solvent and coagulant, phase separation occurs, yielding polymer-rich and polymer-poor phases, which can be sustained until the polymer-rich phase solidifies. Generally, solidification during phase separation may occur by gelation and/or by crystallization of the polymer. After drying the polymer membrane, the coagulant is removed and micropores are formed.¹⁴ However, since the exchange rate between solvent and nonsolvent is quite high during the phase variation process, controlling the formation of the polymer membrane is a challenge. Under these circumstances, once defects or structural weak points appear on the surface of the polymer solution, the partial precipitated skin of the polymer membrane may rupture, causing water to quickly swarm into the polymer substrate, resulting in macropores in the longitudinal direction.¹⁵

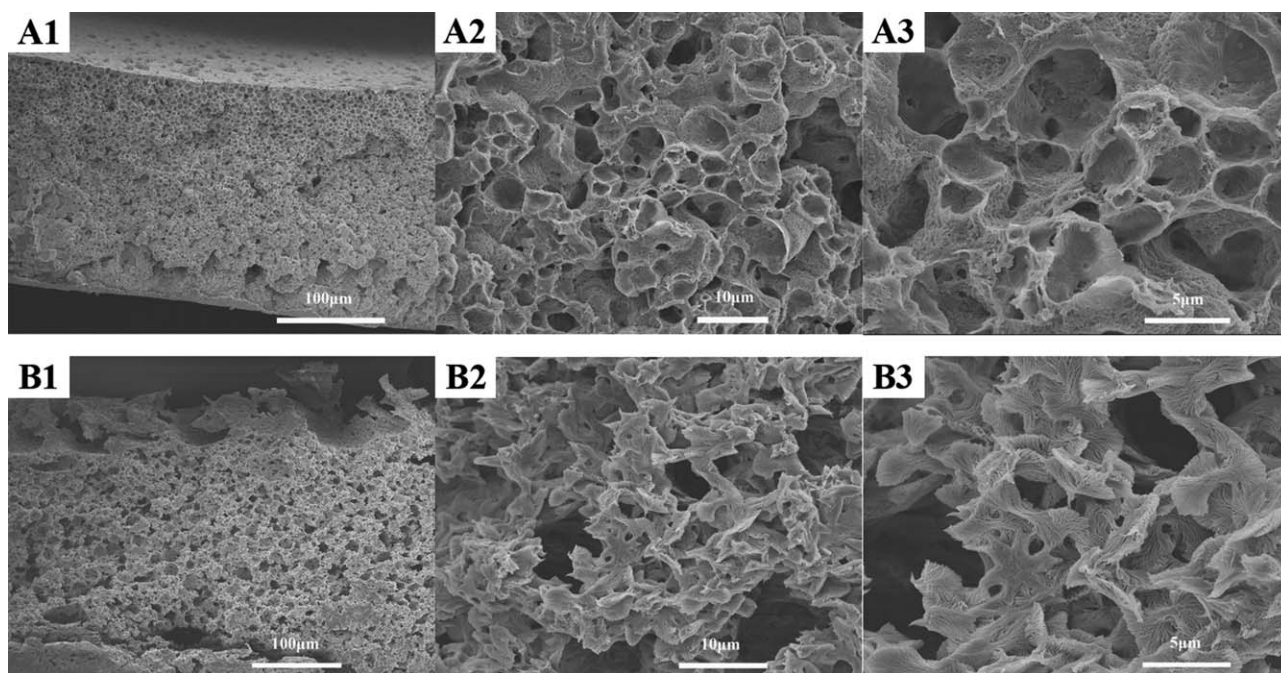


Figure 1. SEM photographs of a cross section of the PLA membrane prepared by phase inversion using liquid water (A1,A2,A3) and water microdroplets (B1,B2,B3) as coagulant. Experimental conditions: 15% PLA solution, solvent: $C_4H_8O_2$, temperature: 20°C, time to solidification: 6 h.

In order to overcome this disadvantage, a novel method of phase separation induced by water microdroplets has been developed to prepare porous biomedical polyurethane (BPU) membrane which shows excellent air permeability and mechanical properties.¹⁶ An interesting feature of the porous BPU membrane is that adjacent pores are connected by a micro-hole, as seen by scanning electron microscopy. The conditions during preparation have a significant influence on the morphology of the resulting porous BPU membrane. In this study, the PLA membrane was fabricated via a simple technique including phase inversion by water microdroplets that were produced from an ultrasonic atomizer and used as a coagulant. Additionally, the effect of various preparation conditions on the morphology was studied.

EXPERIMENTAL

Materials

Poly(L-lactide) (PLA) 6202D was purchased from Nature works LLC (United States), while analytical grade 1,4-dioxane was purchased from Aladdin. All chemicals were used without further purification. Deionized, ultrapure water was used in this study.

Preparation of PLA Membrane

An ultrasonic atomizer was used to create water microdroplets from liquid water via high-frequency vibration at approximately 1.7 to 2.4 MHz. These water microdroplets were continuously supplied and collected in a chamber for the preparation of PLA membrane. PLA and 1,4-dioxane were then added, and the mixture was stirred continuously at room temperature until a homogeneous solution was obtained, which was then placed in vacuum at -0.1 MPa to remove all air bubbles. PLA solution was directly cast onto a glass plate (100 mm long, 100 wide, and 1 mm thick) and placed in the microdroplet chamber. The suspended water microdroplets settled on the surface of the

PLA solution due to gravity, which induced phase inversion. After 6 h of precipitation induced by water microdroplets, the PLA membrane was taken out of the chamber and immersed in deionized, ultrapure water for 2 h to remove residual solvent and finally dried in an air-circulating oven at 30°C for 72 h. The resulting PLA membrane was peeled off the glass plate and preconditioned at 30°C before further analysis. A negative control was prepared by casting PLA solution on a glass plate and immersing it into deionized water.

Morphology of the PLA Membrane

The morphology of the PLA membrane was studied using a scanning electron microscope (SEM, JSM 6700F, JEOL, Japan) at an acceleration voltage of 5 kV. A cross-section of the PLA membrane was obtained by fracturing it in liquid nitrogen. Sample surfaces were sputter-coated with a layer of gold before testing.

RESULTS AND DISCUSSION

The Effect of Coagulate Bath on the Morphology of the PLA Membrane

In this study, phase inversion was used to fabricate the PLA membrane using distilled water and water microdroplets as a coagulation bath. The morphology of the PLA membrane was determined by SEM. As shown in Figure 1, the structure of the PLA membrane prepared by phase inversion clearly appears as microporous. When distilled water was used as coagulant, the structure of PLA membrane was compact [Figure 1(A1)]. In Figure 1(B1), however, the structure appears loose compared with that obtained using distilled water. Some dispersed large pores are also visible on the surface of PLA membrane. This shows that, when distilled water was replaced by water microdroplets as the coagulation bath, the compact structure of the PLA membrane was disrupted.

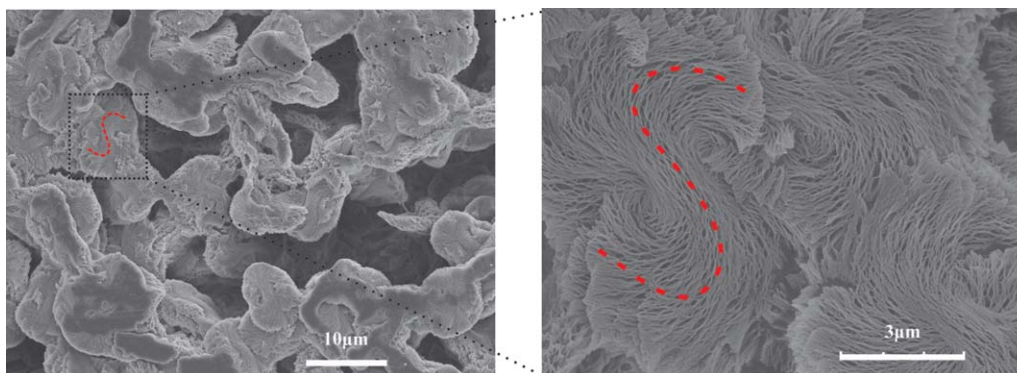


Figure 2. Typical structure of the PLA membrane prepared from water microdroplets. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

An important observation from SEM imaging was that the microporous structure of the PLA membrane prepared using water microdroplets was composed of a large number of individual units composed of fibrils with a diameter of 10 to 20 nm. A bunch of edge-on lamellae were seen radiating from the nucleus.

These lamellae exhibited an S shape and several individual units together took on a dumbbell shape. The structure of individual units was investigated further and the result is shown in Figure 2. Dendritic fibrils appear stacked together and individual units are S-shaped. Micropores are also visible between fibrils. The width

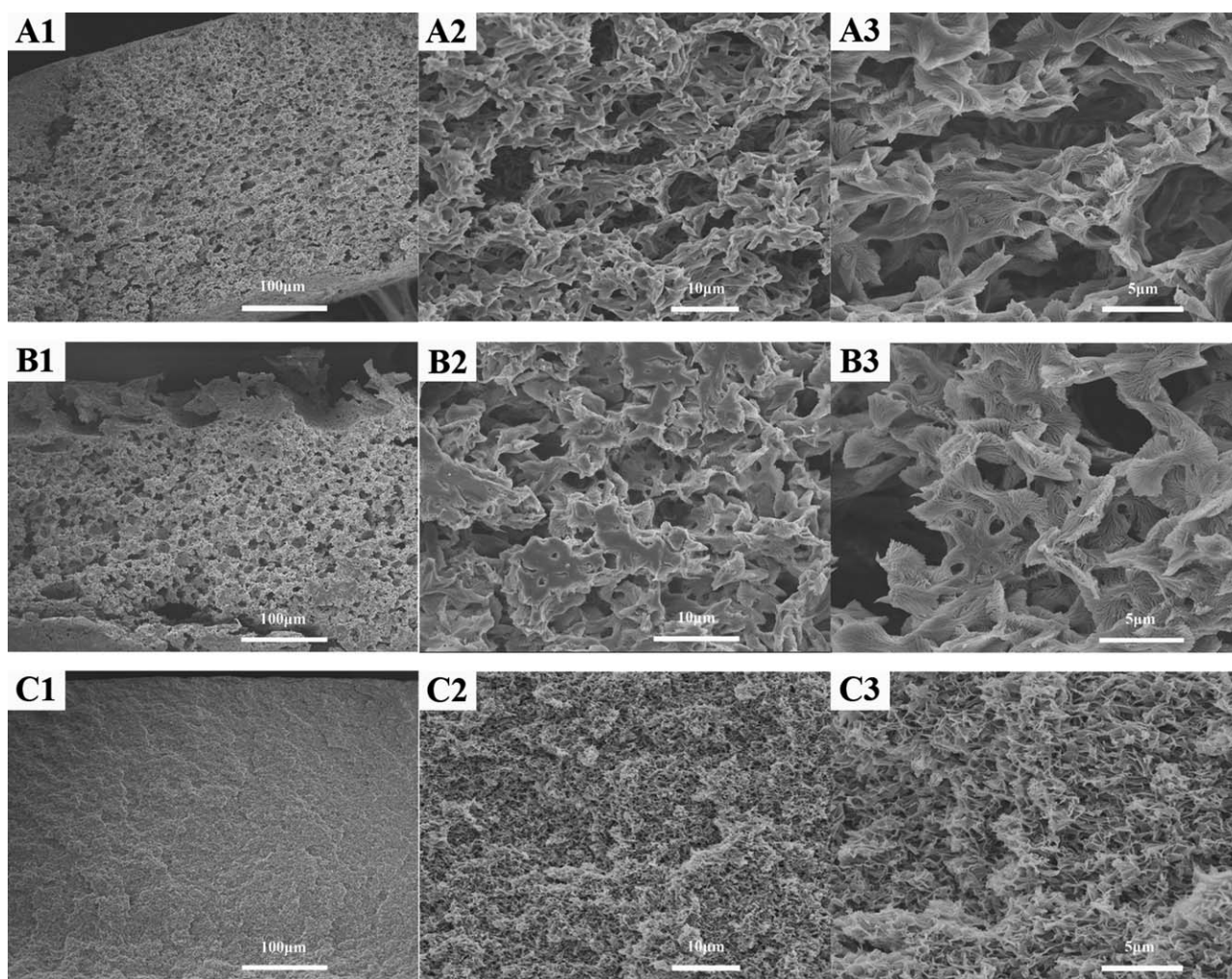


Figure 3. Cross-section SEM photographs of microporous membranes prepared from 10% (A1,A2,A3), 15% (B1,B2,B3), and 20% (C1,C2,C3) PLA solution using water microdroplets as coagulation bath. Experimental condition: solvent, $C_4H_8O_2$; solidification time, 4 h; temperature, 20°C.

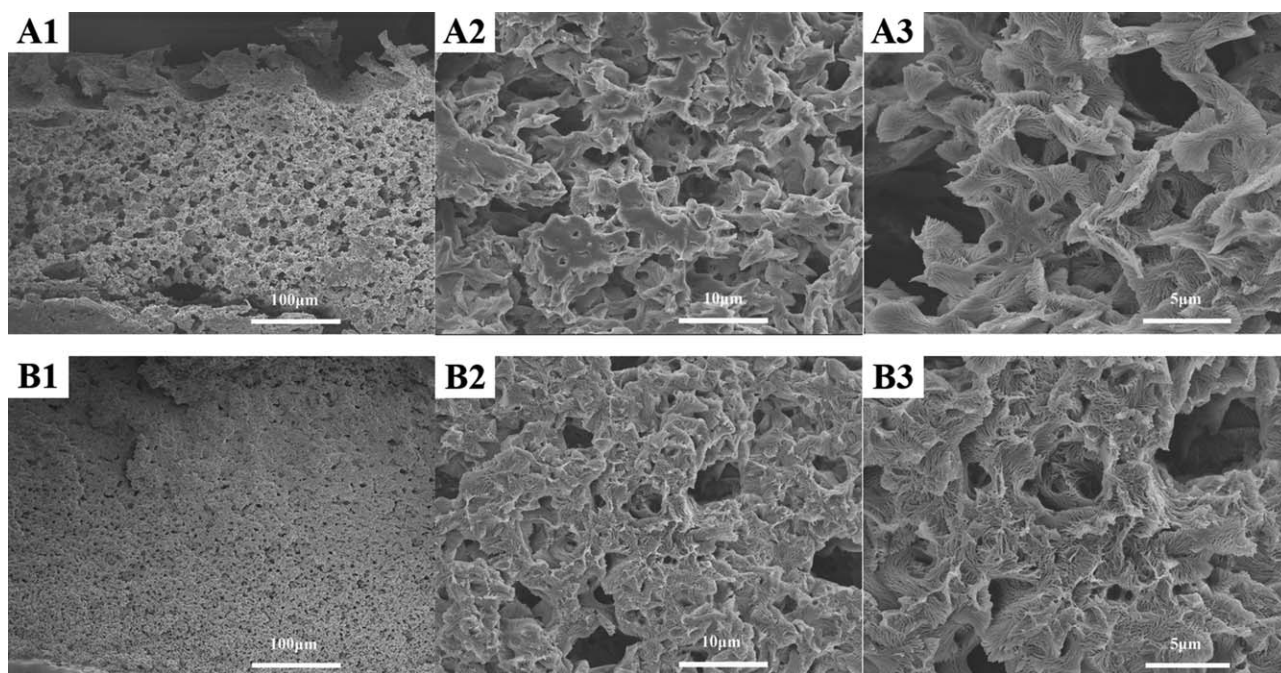


Figure 4. The effect of different temperatures on the cross-sectional morphology of the PLA porous membrane prepared using water microdroplets. (A1,A2: 20°C); (B1,B2: 40°C). Experimental condition: 15% PLA; solvent: $C_4H_8O_2$; solidification time: 4 h.

of each side appeared larger than the middle section. Additionally, individual units were linked together randomly.

Drawing on the research of Kikkawa *et al.*, the PLLAs crystal growth in thin film which was observed by *in situ* atomic force microscopy (AFM),¹⁷ we presume that the formation of the S-shaped structure included two steps: The first step was core structure formation during the initial stage of phase inversion, involving an exchange between the coagulant and polymer solvent; the second step was an increase in nucleation with time. The crystalline behavior of PLA has been previously investigated by Marubayashi *et al.* (observation of polylactide stereocomplex by atomic force microscopy).¹⁸ They showed that the radial crystal grows from a core and spans several micrometers. In their study, a chain-folding model was used to explain the lamellar curvature.

Lamellar twisting during polymer crystallization has been previously observed in several synthetic polymers. In the case of polyethylene, it has been established that the dominant lamellae adopt an S-shaped profile at the spherulite stage. This phenomenon appears to be more frequent with chiral polymers since their spherulites are frequently banded, implying that their lamellae twist.¹⁹ Lotz *et al.* suggested that the crystal twist can be correlated with the chain chirality of the peptide residue of the chain in the silk fibroin.²⁰ With further study, they indicated that changing the handedness of the chiral center produces a reversal of the lamellar twist with chiral polymers.^{21–23}

In the context of Damien Maillard and Robert Prud'homme's theory, the crystallization of thin polylactide films reveals a clear correlation between chain chirality and the macroscopic shape of edge-on crystals. Chirality results in a lamellar curvature with a specific direction,²⁴ whereas twisting of the lamellae in banded

spherulites is an intrinsic property of the material, resulting from the chain chirality. Thus, poly(L-lactide) shows an S-shape while poly(D-lactide) is Z-shaped.²⁵

The process of PLA molecular chain arrangement is very slow. Several studies on the crystalline behavior of PLA have focused on the effect of temperature, since this parameter can be accurately controlled to rise or fall as desired, which affords sufficient time for regular arrangement of the molecular chain. When water microdroplets were used in this study, to induce phase separation of the PLA solution, the suspended microdroplets settled on the surface of the solution due to gravity, immediately followed by exchange between solvent and non-solvent, resulting in the PLA solution phase separation into a polymer-rich and a polymer-poor phase. The exchange velocity of solvent and the descent of water microdroplets differ by the trace amount of atomization volume, leading to the slow crystallization and solidification process of the PLA solution, which provided adequate time for PLA molecular movement.¹⁶ The water microdroplets serve as heterogeneous matter embedded in the PLA solution that can promote PLA molecular chain facilitating ordered assembly and growth of nuclei. While growth in the PLA solution continues, surrounding molecular chains act as reservoirs to further assemble flat lamellae, creating the "S"-shaped crystalline morphology.

The Effect of PLA Solution Concentration on the Morphology of the PLA Membrane

The viscosity of PLA solution is also related to the weight concentration of polymer in the solvent. The viscosity is known to increase with a rise in polymer concentration. In this study, we investigated the effect of varying the concentration of PLA solution on morphology of the PLA membrane by SEM. As shown in Figure 3, the concentration of PLA solution appears to exert

a strong influence on membrane morphology, and the cross section became denser when the concentration increased from 10% to 20%. The cross-section of PLA membrane prepared from 10% and 15% polymer solution showed individual units composed of separate fibrils, whereas when the concentration reached 20%, the individual units were replaced by a fiber network.

The difference in morphology of the membrane indicates that higher concentrations of PLA solution result in inhibition of molecular chain movement. The formation of flat lamellae was attributed to the movement and crystalline behavior of PLA molecules. Low PLA weight concentration facilitated folding of the PLA molecule during the phase inversion process. However, with an increase in PLA weight concentration, the degree of entangled molecules increased, resulting in limited movement and crystallization of PLA molecules. To fabricate PLA membranes with flat lamellae, optimal weight concentration is necessary. We plan to identify the optimal weight concentration in future studies. However, we observed a unique, crystalline structure in the cross section of PLA membranes prepared using 15% PLA solution and water microdroplets, as shown in Figure 3(B3).

The Effect of Temperature on the Morphology of the PLA Membrane

The exchange rate between solvent and coagulation bath was related to the temperature of the PLA solution. An increase in ambient temperature is known to increase the speed of movement of the water molecules. In this study, we investigated the influence of temperature on morphology of the PLA membrane using SEM. Figure 4 shows SEM images of PLA membranes prepared from 15% PLA solution using water microdroplets as coagulant at different temperatures. The structure of the PLA membrane fabricated at low temperatures appeared less dense and the structural integrity of the flat lamellae was also better than that of lamellae in the membrane prepared at 40°C. This difference in cross sectional morphology of porous PLA membranes prepared at different temperatures was attributed to the exchange rate between solvent and the coagulation bath. The precipitation of polymer solution occurred within a shorter time due to the fast exchange rate between water and *N,N*-dimethylformamide at high temperatures. The movement of polymer molecules to form an orderly arrangement was inhibited by the precipitation of PLA, resulting in the formation of imperfect crystals.

CONCLUSIONS

The porous PLA membrane with an S-shaped crystalline structure was fabricated successfully via a simple method involving phase inversion induced by water microdroplets. The cross-section morphology of the resultant membrane investigated by SEM revealed twisted lamellae. PLA solution concentration and ambient temperature showed a significant impact on cross-sectional morphology. An increase in concentration led to an increase in viscosity, which in turn prevented the movement of PLA molecules during phase inversion.

Thus, the change in coagulating component resulted in a specific strip-type crystal shaped like an "S". This was quite different from the PLA membrane prepared using liquid water, which produces a dense upper crust and randomly porous matrix. Additionally, small voids were observed between the clusters of flat lamellae within the lamellar crystal. Therefore, we believe that the PLA membrane fabricated using water microdroplets displays excellent permeability and therefore has potential applications in the fields of tissue engineering and artificial organ generation.

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