

Preparation of Porous Poly(L-lactic acid) Honeycomb Monolith Structure by Unidirectional Freezing and Freeze-Drying

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1. Introduction

Recently, the development of biodegradable porous polymeric scaffold attracts much attention. Various techniques have been developed to fabricate polymeric scaffold: progen leaching, thermally induced phase separation, gas foaming. Organs such as nerves, bones, blood vessels, and ligaments require a scaffold with an aligned porous structure for their cell proliferation. Thus, the controlled preparation of aligned porous materials in the form of 2D surface patterns or a 3D monolithic structure is critical for this purpose. A promising and novel technique for creating aligned porous structures is a unidirectional freezing method, which is relatively simple and cost effective. Mukai et al. and Nishihara et al. proposed a unidirectional freezing method to prepare a honeycomb monolith silica gel [1, 2]. Recently, several studies extended the unidirectional freezing method to polymers and prepared porous polymeric materials with aligned microtubes [3-5]. The micro honeycomb structure has great potential as catalyst support, catalyst, substrate for drug delivery, and bio scaffold due to the inherent high contact efficiency and controllability of morphology. Despite of these potentials, the structure, especially prepared by templating a cellular type solvent crystal, has the drawback of poor interconnectivity, which might lead to plugging of microtubes. When micro honeycomb monolith structure is used as a bio scaffold, it can be expected that the plugging of microtubes will easily occur by the proliferation of cell, especially from the top and bottom end of a micro honeycomb monolith structure. Poor interconnectivity can cause slow cell growth during cultivation due to the lack of oxygen and nutrient supply and waste elimination [6, 7]. One of good solutions of preventing the reduction of contact efficiency caused by the plugging is to increase interconnectivity of the structure. It could be achieved by making the wall of microtube to be open porous structure. In the case of bio scaffold made by biodegradable polymers, degradation rate is also affected by the morphology and porosity of 3D structure [8, 9].

In this study, a honeycomb monolith structured porous Poly(L-lactic acid) (PLLA) was prepared by combining unidirectional freezing and freeze-drying methods. The key factors of controlling the morphology of honeycomb monolith structure thoroughly investigated. Furthermore, to prepare a honeycomb monolith structure with micro/nanoscale porous wall, we focused on the possibility of phase separation due to the solubility change induced by decreasing temperature and the crystallization of solvent during unidirectional freezing. Poly(ethylene glycol) (PEG) was selected as a blend polymer to prepare porous PLLA structure because PEG can be easily leached out using water or relatively non-toxic hydrophilic solvent such as ethanol. The addition of PEG into the PLLA/1,4-dioxane solution enabled to prepare honeycomb monolith structures with porous wall. Phase separation of polymers induced by unidirectional freezing of 1,4-dioxane was exploited to create such a honeycomb monolith structure. The porous structure in the wall of honeycomb monolith was prepared by selective leaching of PEG. The effect of PLLA/PEG blend ratio and PEG molecular weight on the morphology was investigated.

2. Experiment

2.1 Materials

PLLA ($\overline{M}_w = 2.8 \times 10^5$, $\overline{M}_w / \overline{M}_n = 2.24$) was used as received. PEG600, PEG2000, PEG4000, and PEG6000 was purchased from Wako Chemicals Ind. Ltd. and used as received. Dehydrated 1,4-dioxane (Wako Chemicals Ind. Ltd., Japan) and Ethanol (Wako Chemicals Ind. Ltd., Japan) was used as solvent.

2.2 Preparation of porous structure

PLLA/Dehydrated 1,4-dioxane single phase solution was prepared by dissolving PLLA into dehydrated 1,4-dioxane at 323 K. After it was cooled down to room temperature, the solution was poured into a polypropylene (PP) test tube with a thickness of 1.2 mm, 100 mm in length and 10 mm in diameter. The solution was unidirectionally frozen by soaking the test tube into a liquid nitrogen bath at a constant rate. The tube soaking rate was changed in the range from 3.5 to 17.5 cm hr⁻¹. After freezing the solution completely, the solidified sample was freeze-dried at 268 K for 4 days. To investigate the effect of water on the porous structure, especially the ladder-like tube structure, distilled water was added to the PLLA/1,4-dioxane solution. The water concentration varied in the range from 0.04 wt% to 2.0 wt%. Furthermore, to prepare honeycomb monolith structure with micro/nanoscale porous wall, PLLA and various molecular weight of PEG (PEG600, PEG2000, PEG4000, and PEG6000) were co-dissolved in dehydrated 1,4-dioxane. The solutions were prepared with polymer concentrations of 7wt% and with blend ratios of PLLA to PEG of 90/10, 70/30, and 50/50 so as to see the effect of PLLA/PEG blend ratio on honeycomb monolith structure and morphology of porous wall. Then, the solutions were frozen unidirectionally at a constant rate of 2.0 cm hr⁻¹. Prepared honeycomb monolith structures were then immersed in ethanol for 7 days, and the medium was replaced every 12 hours to assure PEG leaching out. After the completion of leaching, the samples were immersed into *tert*-butyl alcohol and kept there for 1 days, and the medium was replaced after 12 hours. Next, the wet samples were freeze-dried at 273K, and finally honeycomb monolith structures with porous wall were obtained.

2.3 Morphology characterization

The microstructures of the obtained sample were observed by scanning electron microscopy (Tiny-SEM 1540, Technex Lab Co. Ltd.). For SEM observation, the freeze-dried samples were further frozen by liquid nitrogen to prevent deformation and cut with a razor blade in both parallel and perpendicular directions to the freezing direction. The prepared sample was coated with gold-palladium *in vacuo*.

2.4 Cloud point measurement

The phase diagram of PLLA/PEG/1,4-dioxane ternary system was determined using a laser scattering scheme. PLLA and PEG were dissolved in 1,4-dioxane as described in Section 2.2. About 0.85g of sample solution was then dropped to glass Petri dish, which was 16.3mm in diameter and 10.8mm in depth, and allowed to evaporate. Petri dish with reflector was mounted on an electronic balance. The laser intensity, which was reflected through polymer solution, was measured vertically using laser censor. The change of laser intensity and the weight change of solution by 1,4-dioxane evaporation were recorded. To avoid humidity effect, all experiments were carried out in a lid (overall dimensions: 18 cm by 18 cm by 10 cm) under 1L/min nitrogen (purity 99.9%) flow at 298±0.5K. Humidity and temperature in the system were measured by a

hygro-thermograph (SK-L200T II, SATO KEIRYOKI MFG. Co. Ltd.). The phase separation composition was determined by the polymer concentration at which the light intensity changed drastically

3. Result and discussion

3.1 Formation of honeycomb monolith structure

Honeycomb monolith structure was successfully prepared from a 7 wt% polymer solution without water by unidirectional freezing at a tube soaking rate of 3.5 cm hr^{-1} into a liquid nitrogen bath. After freezing the solution completely, the solidified sample was freeze-dried at 268 K (Fig. 1). Figure 1a and b respectively show a photographic image and scanning electron microscopy (SEM) image of porous PLLA prepared from a 7wt% polymer solution without water by unidirectional freezing at a tube soaking rate of 3.5 cm hr^{-1} . Figure 1b shows that the honeycomb monolith structure was established in the obtained porous PLLA. The microtube structure was created in cross section parallel to the freezing direction, and honeycomb structure was created in cross sectional area perpendicular to the freezing direction. Figure 1c clearly shows the honeycomb structure created in cross section perpendicular to the freeze direction. Figure 1d shows that micro-tubes with smooth walls were aligned in parallel to the freeze direction. The length of the aligned tube was longer than 1 cm.

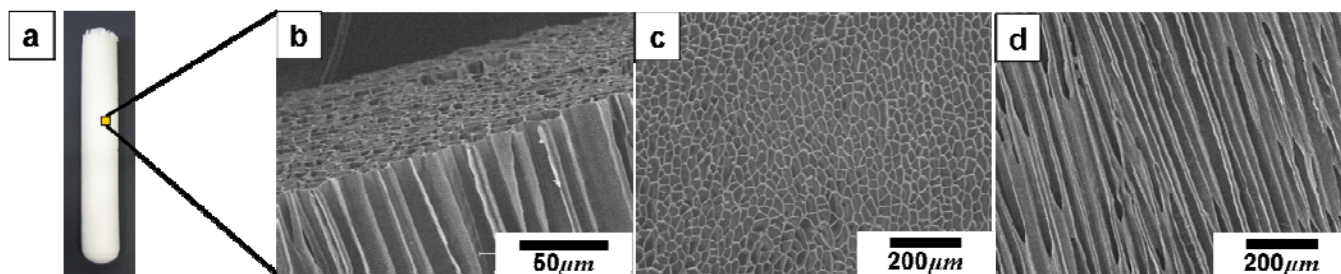


Figure 1. (a) Photographic image of porous PLLA prepared from PLLA 7 wt%/dehydrated 1,4-dioxane solution with a tube soaking rate of 3.5 cm hr^{-1} , (b~d) SEM micrographs of (b) honeycomb monolith structure taken at a tilted angle, (c) Cross sectional area perpendicular to freezing direction, and (d) Cross sectional area parallel to freezing direction.

3.2 Effect of water

To investigate the effect of water as structure modifier, solutions with different water concentrations and different PLLA concentration were frozen by soaking the PP tube into liquid nitrogen at a rate of 3.5 cm hr^{-1} . Figure 2 shows SEM micrographs of the obtained porous PLLA with 7wt% PLLA solutions. As shown in Figure 2, the porous structure was dramatically changed with the addition of water. The porous PLLA prepared from a solution with 0.04 wt% water content showed a transitional surface morphology from a smooth wall microtube structure to a ladder-like microtube with an interconnected structure (Fig. 2a and e). In the case of the solution with 0.12 wt% water content, a highly ordered interconnected structure was observed (Fig. 2b and f). For the solution with 2.00 wt% of water content, the number of interconnected pores between the tubes was drastically increased (Fig. 2c and g). The structure resembles the superdendrite structure observed by Ragnarsson et al [10]. PLLA as well as 1,4-dioxane is likely to contain moisture. The experimental results suggest that small amounts of water has dramatic effects on the porous structure and it is important to control moisture in materials used to prepare honeycomb monolith structures by the unidirectional freeze-drying method. The lack of moisture control may be a reason that honeycomb monolith structures were not created in previous study [11].

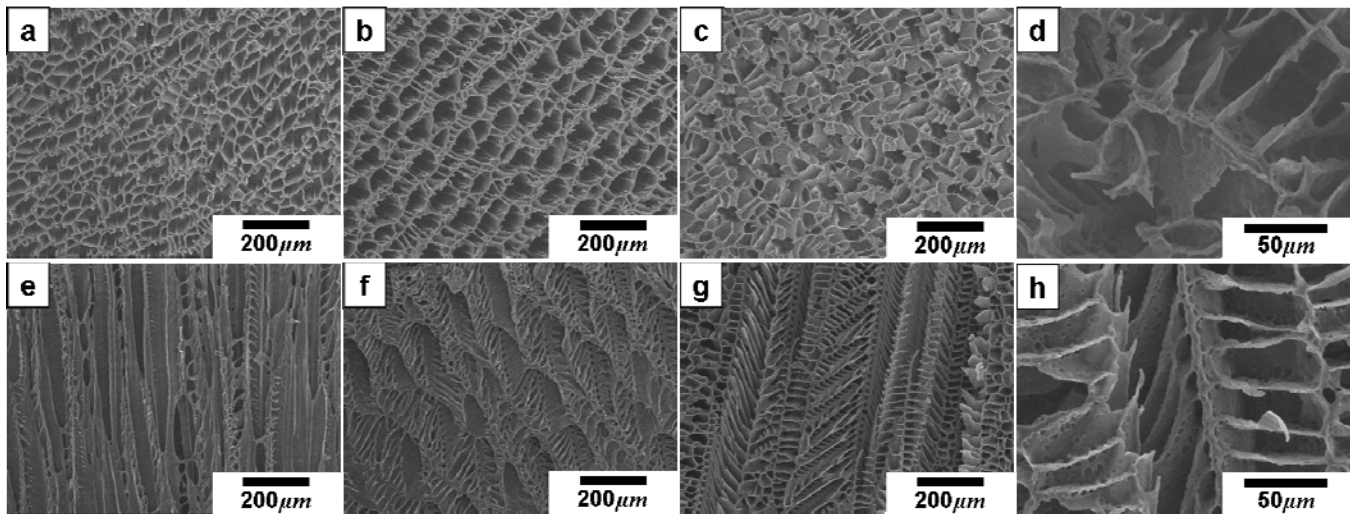


Figure 2. Effect of water on structure - SEM micrographs of porous PLLA prepared from 7 wt% of PLLA / 1,4-dioxane solution: (a~d) Cross sectional area perpendicular to the freezing direction, (e~h) Cross sectional area parallel to the freezing direction, (a and e) 0.04 wt% of water, (b and f) 0.12 wt% of water, and (c~d and g~h) 2.00 wt% of water.

3.3 Tube soaking rate

Figure 3 shows SEM micrographs of a sample cross sectioned perpendicular to the aligned microtubes of the porous PLLA prepared from a 7 wt% solution at two different soaking rates of 7.5, and 17.5 cm hr⁻¹. As can be seen, the average diameter of tubes decreased with an increase in the soaking rate, which is equivalent to the effects of the cooling rate. This trend coincides with the result of Zhang et al [4]. They measured average spacing of ice crystal of poly(vinyl alcohol) aqueous solution with temperature gradient plate and microscope. The average diameter and wall thickness decreased with an increase in the soaking rate. This is because rapid cooling increases the degree of super-cooling and leads to the enhancement of crystal nucleation of 1,4-dioxane. This result shows that tube diameter and the size of the honeycomb can be controlled by the cooling rate.

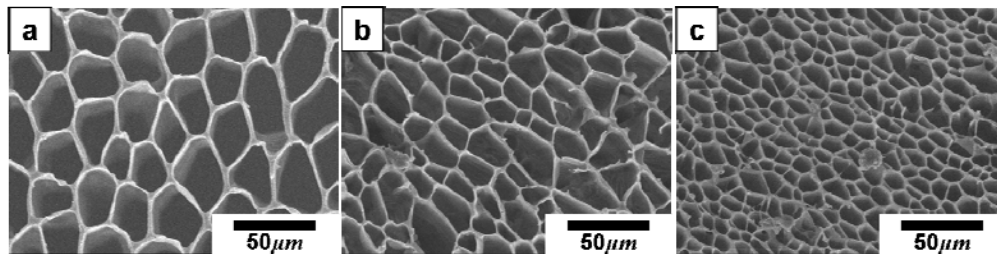


Figure 3. Effect of tube soaking rate (cooling rate) on honeycomb structure- SEM micrographs of porous PLLA prepared from 7 wt% of PLLA/dehydrated 1,4-dioxane solution at the cross sectional area perpendicular to the freezing direction: (a) soaking rate =3.5 cm hr⁻¹, (b) soaking rate =7.5 cm hr⁻¹, and (c) soaking rate =17.5 cm hr⁻¹

3.3 Phase diagram

The phase diagram of PLLA/PEG/1,4-dioxane ternary system was determined using the method described in Section 2.4. The compositions of solutions at which cloud point were measured are displayed in the phase diagram (Fig. 4). We adopted solvent casting method instead of controlled cooling of solution due to the very high viscosity of solution. Even though solvent cast solution shows solvent concentration profile during evaporation of solvent, the result data set

had relatively good tendency. The solubility of PLLA/PEG blend in 1,4-dioxane decreased with the increase of PEG molecular weight. This result agreed with the data acquired by PEG/1,4-dioxane binary solution.

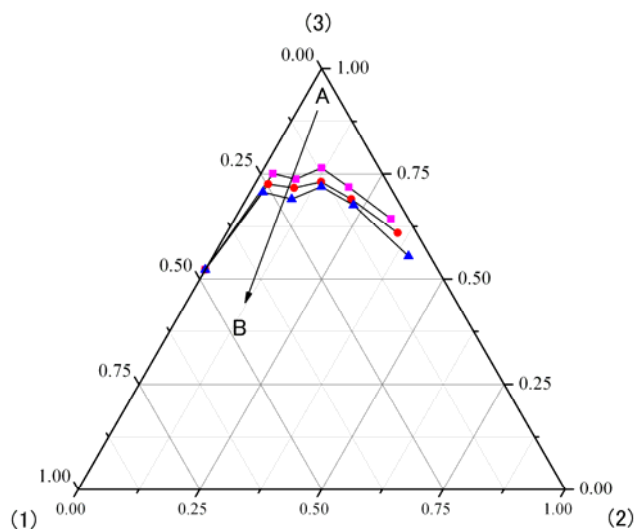


Figure 4. Phase diagram of PLLA (1)/PEG (2) /1,4-dioxane (3) solutions at 25°C.
 (▲) PEG600, (●) PEG2000, and (■) PEG6000

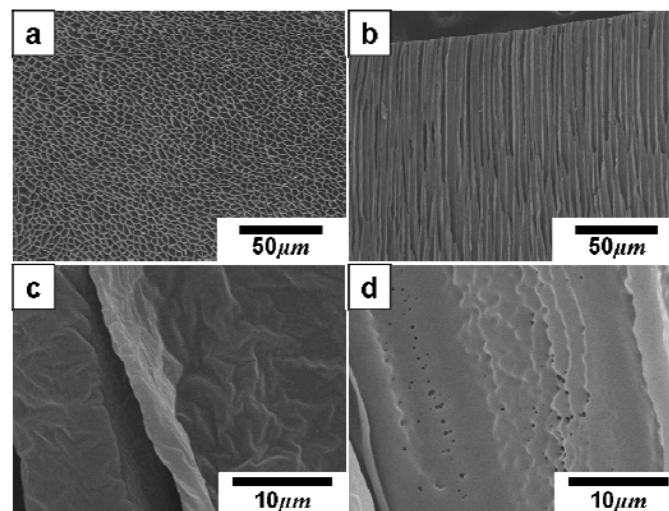


Figure 5. SEM micrographs of honeycomb monolith structure prepared from PLLA/PEG6000 blend (90/10) and dehydrated 1,4-dioxane solution (a) cross sectional area perpendicular to freezing direction, (b)-(c) cross sectional area parallel to freezing direction, (d) cross sectional area parallel to freezing direction after leaching with ethanol

3.4 Preparation of honeycomb monolith structure with porous wall

A mixture of PLLA/PEG 6000 blend (90/10) and dehydrated 1,4-Dioxane solution (93wt% solvent concentration) was prepared and unidirectionally frozen at a tube soaking rate of 2.0 cm hr⁻¹ into a liquid nitrogen bath. After freezing the solution completely, the solidified sample was freeze-dried at 268 K. As shown in Fig. 5, Honeycomb monolith structure was successfully prepared. Figure 5a-b show scanning electron microscopy (SEM) images of PLLA/PEG blend honeycomb monolith structure. Figure 5a clearly shows the honeycomb structure created in cross section perpendicular to the freeze direction. Figure 5b shows that micro-tubes with smooth walls were aligned in parallel to the freeze direction. To prepare micro honeycomb monolith structures with porous channel wall, honeycomb monolith structure was leached with ethanol to remove PEG. Figure 5c and d respectively shows the SEM micrographs of cross sectional area parallel to the freeze direction before and after leaching. As can be seen, small pores are successfully prepared by leaching.

3.5 Effect of PEG molecular weight on porous channel wall

The effect of PEG molecular weight in the polymer blend solution on the morphology of channel wall was investigated. Four PLLA/PEG blend (70/30) 1,4-dioxane solutions, of which the PEG molecular weight were changed as PEG600, PEG2000, PEG4000, and PEG 6000, were unidirectionally frozen and freeze-dried. The freeze-dried honeycomb monolith structures were leached with ethanol to remove PEG and freeze dried again using the procedure which was described in experiment section. Figure 6, respectively, shows SEM micrographs of porous

channel wall of micro monolith honeycombs prepared from these four different PEG molecular weight solutions. As shown in Figure 6, pore was created on the channel wall of honeycomb monolith structure by leaching out PEG. The pore size increased and the number of pore decreased with increase of the PEG molecular weight.

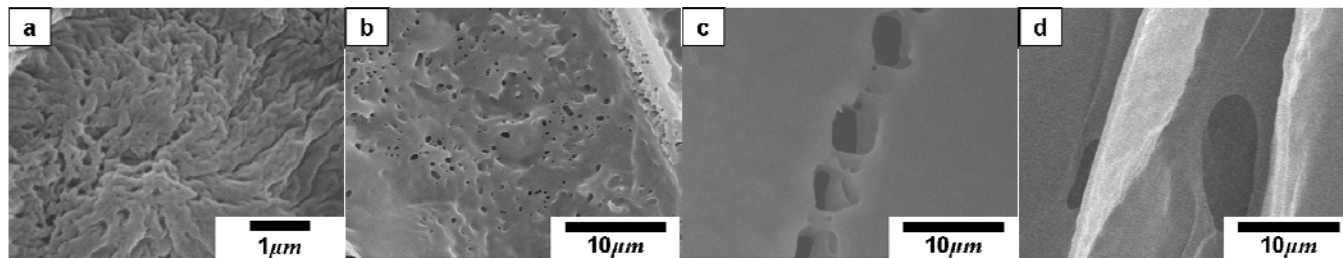


Figure 6. Effect of PEG molecular weight on morphology of channel wall - SEM micrographs of ethanol leached cross sectional area perpendicular to freezing direction prepared from PLLA/PEG blend (70:30) and dehydrated 1,4-dioxane solution (93wt% solvent concentration). (a) PEG600, (b) PEG2000, (c) PEG4000, and (d) PEG6000

3.6 Effect of PLLA/PEG blend ratio on porous channel wall

Figure 7 respectively shows the SEM micrographs of ethanol leached samples cross sectioned parallel to the aligned microtubes of the porous PLLA materials prepared from PLLA/PEG2000/1,4-dioxane solutions with different PLLA/PEG blend ratio. As can be seen, the pore size on channel wall increased with the increase of PEG blend ratio due to the high content of PEG in honeycomb monolith structure.

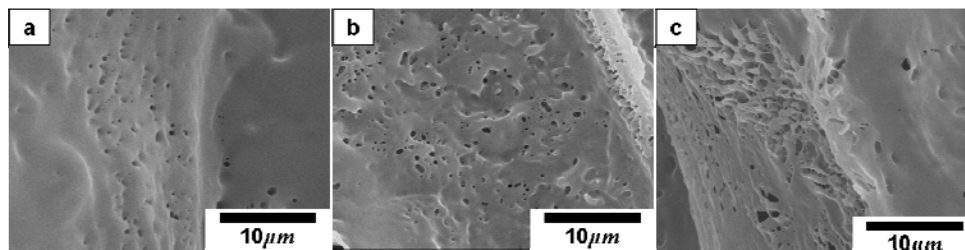


Figure 7. Effect of PLLA/PEG blend ratio on morphology of channel wall - SEM micrographs of ethanol leached cross sectional area perpendicular to freezing direction prepared from different PLLA/PEG6000 blend ratio and dehydrated 1,4-dioxane solution (93wt% solvent concentration). (a) PLLA:PEG=90:10, (b) PLLA:PEG=70:30, and (c) PLLA:PEG=50:50.

3.7 Formation mechanism of aligned structures

The porous structure was created by templating from the aligned solvent crystals that were formed during unidirectional freezing. The solvent was crystallized and grown in the freezing direction due to the constitutional supercooling. When the degree of supercooling was large during the crystallization process, Mullins-Sekerka instability occurred and created crystals with cellular (microtube like) or dendrite (ladder like) structures [10, 12]. In the course of directional freezing, polymer was expelled from solvent crystals to the liquid phase and the constitutional supercooling condition was established at the interface. Water and excess polymer chains acted as impurities. Therefore, when water existed, the degree of supercooling increased and the degree of Mullins-Sekerka instability increased due to an impurity-induced reduction of the freezing point of the solution. The instability created dendrite-type branched crystals. As a result, the unidirectional freezing created ladder-like tubes in the presence of water. The interconnectivity of tubes increased as the instability increased. There are some differences in the porous structures of ladder-like tubes prepared in the presence of water than structures produced with a high-concentration of polymer solution. As shown in Figure 2d and h,

small pits, 2 μm in diameter, could be observed on the tube wall. The formation mechanism of these small pits can be speculated as follows: water was expelled from 1,4-dioxane crystal and concentrated on the solid-liquid interface due to the lower freezing point of water than that of 1,4-dioxane (m.p. 11.8°C). An increase in water caused a liquid-liquid phase separation of polymer solution on the interface before the solution was completely frozen. Since water acted as an anti-solvent to PLLA [13], the liquid-liquid phase separation created small pits on the tube wall.

3.8 Formation mechanism of pores on the channel wall

The formation mechanism of PEG phase was speculated as follows. As illustrated in Fig. 8a and b, the solvent of polymer blend solution crystallized and grown in the freezing direction. In the course of unidirectional freezing, PLLA and PEG concentration of liquid phase surrounded by 1,4-dioxane crystal increased gradually and 1,4-dioxane content decreased (Fig. 8c and d). This change in solvent concentration caused liquid-liquid de-mixing to PEG rich phase and PLLA rich phase and coalesced until it could not move due to the increase of viscosity by the decrease of 1,4-dioxane in the liquid phase. As explained in Section 3.6, the pore size on the channel wall increased and the number of pore decreased with the increase of PEG molecular weight. This can be explained with the decrease of PLLA/PEG solubility in 1,4-dioxane by increase of PEG molecular weight which was illustrated in Figure 4. In the case of high molecular weight PEG, liquid-liquid phase separation occurs at the relatively high 1,4-dioxane concentration and PEG rich phase has relatively long time to coalesce to large size compared with low molecular weight PEG. The increase of pore size by the increase of PEG blend ratio can be easily expected with increased PEG phase. The decrease of solution viscosity due to the high PEG blend ratio could be another factor of coalescence of PEG domain to large size.

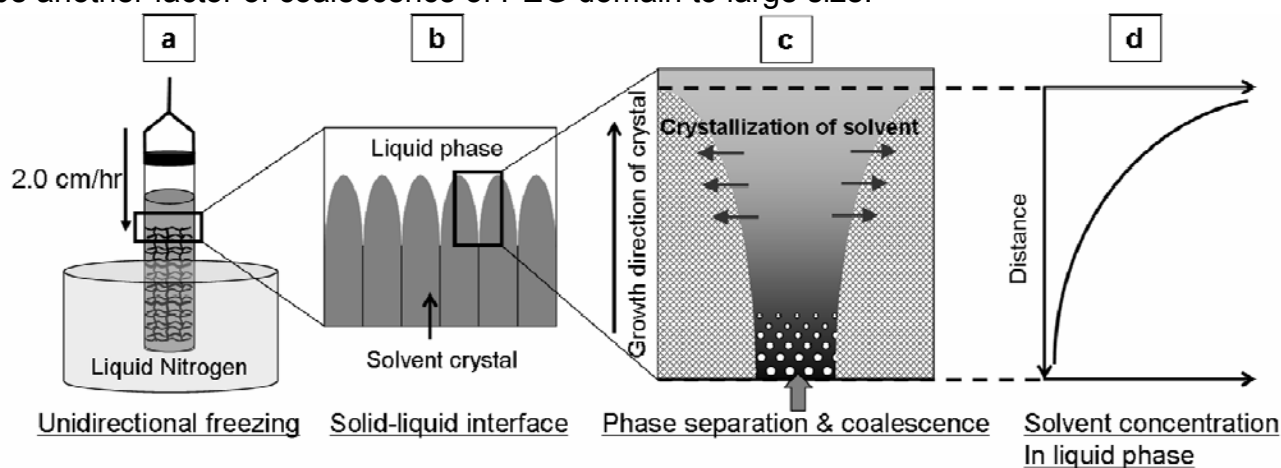


Figure 8. Schematic illustration of the unidirectional freezing and the phase separation

4. Conclusion

In this study, honeycomb monolith structures of PLLA were successfully prepared by thermally induced solid-liquid phase separation and subsequent solvent sublimation. Water content is key factor of creating ladder-like structures in aligned microtubes. Tube diameter and the density of tubes per unit cross section perpendicular to the aligned direction were controlled by tube soaking rate. Furthermore, honeycomb monolith structures of PLLA with micro/nanoscale porous wall were successfully fabricated by combining unidirectional freezing

technique and phase separation of polymer blend solution. PEG was used as a blend polymer to prepare phase separated morphology. Porous structure in the channel wall of honeycomb monolith structure was successfully prepared by leaching out PEG domain. Porous structures in channel wall of honeycomb monolith structure were controlled by PEG molecular weight and polymer blend ratio. Based on the PLLA/PEG/1,4-dioxane ternary phase diagram, we speculated the formation mechanism of porous structure on the channel wall as a crystallization induced phase separation. We also suggested that the porous structure changed by PEG molecular weight because PLLA/PEG/1,4-dioxane ternary phase diagram affected by PEG molecular weight. We expect that this method has a potential to improve the contact efficiency of aligned porous materials for tissue, catalyst, organic electronics, micro fluidics, and membrane engineering.

5. Reference

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