Unique Ivy-Like Morphology Composed of Poly(lactic acid) and Bacterial Cellulose Cryogel

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ABSTRACT: This study examines the unique morphology and properties of enhanced poly(l-lactic acid) (PLLA) monoliths having a bacterial cellulose (BC) framework. Open-porous BC/PLLA monoliths were successfully prepared using thermally induced phase separation (TIPS) and a freeze-drying technique. The BC/PLLA monoliths exhibited a unique ivy-like structure composed of leaf-like PLLA units and a BC fiber network. We demonstrated for the first time that the interpenetrating BC fiber gives PLLA monoliths four times higher compressive strength than the pristine PLLA. Scanning electron microscopy observation and the N$_2$ adsorption test revealed that the size of PLLA units and the surface area of the monoliths can be manipulated by varying the starting PLLA concentration during the TIPS process. Moreover, the hydrophilicity of the PLLA monoliths was easily controlled by incorporating BC; the neat PLLA monoliths showed a high static water contact angle of as high as 128.8° ± 1.1°, whereas the BC/PLLA monoliths exhibited a much lower contact angle (102.1° ± 1.7°) and greater absorbability to water.

INTRODUCTION

Poly(lactic acid) (PLA) is a representative bioplastic with biodegradability, biocompatibility, and nontoxicity, and can be produced from renewable resources such as sugarcane. In addition to these fascinating properties, PLA also has a higher transparency, unique mechanical properties, and a versatile processing ability when compared to other biomass-derived plastics. As a result, PLA has attracted considerable attention as a biomaterial and as an alternative to petroleum-based polymers. Because of the recent industrialization of PLA and its environmental adaptability, it has been applied to various fields such as biomedicine, food packaging, commodities, and even 3D printing technology based on the increasing awareness of sustainable societies.

Recently, PLA monoliths have been in the spotlight as a functional material because of their high surface area and interpenetrating 3D network. Owing to these unique characteristics, monolithic PLA has been utilized as scaffolds for human cells, artificial bones, supports for controlled release of drugs, and biodegradable filters. However, the practical use of PLA monoliths remains challenging because their mechanical properties considerably deteriorate because of the development of porous structures. Furthermore, hydrophobic surfaces of monoliths that are notably high limit their applications, particularly in scaffolds and filters because the loss of hydrophilicity produces insufficient medium osmosis and a high back pressure during the flow. To overcome these drawbacks, numerous studies have been conducted to utilize the modifier agents. However, no studies so far have achieved sufficient properties or were able to control the pore size. In particular, the precise control of the pore size is critical to meeting requirements for usage because the morphology of monoliths affects several factors such as cell adhesion, biodegradability, and filtration ability. Thus, producing hydrophilic PLA monoliths that have high mechanical properties and have the ability to control the morphology is desirable.

Bacterial cellulose (BC) gel can be a promising means to address the physical properties and hydrophobicity of PLA monoliths. BC gel is an extracellular byproduct of acetic acid bacteria, which exhibits biodegradability and biocompatibility similar to those of PLA. Compared to general plant-based cellulose, BC gel has considerable superiority in terms of 3D network, porosity, purity, crystallinity, and water content, making it an attractive biomaterial in numerous fields. In addition, a layered structure is formed in a BC gel during cultivation, which is the cause of its unique mechanical anisotropy. Because it exhibits a high Young’s modulus, tensile, and compressive strength, the BC gel has also been used to reinforce polymers. Therefore, the use of the BC gel would change PLA monoliths to a next-generation porous material, storing its environmental adaptability.

Few studies have been conducted that examine PLA monoliths that use a single crystal of BC (i.e., BC nano-whisker; BCNW) instead of the BC gel. In contrast to a BC...
gel with a 3D structure, BCNW can be easily introduced into the PLLA monoliths through a variety of methods. For instance, Luo et al. reported that poly(L-lactic acid) (PLLA) monoliths with 5 wt % of BCNW were successfully produced using a solvent casting method and showed improved mechanical strength and hydrophilicity. However, mechanical properties showing an increase in tensile strength, tensile modulus, and compressive strength were limited to less than 64% from the neat PLLA monolith. Furthermore, in contrast to BC gel, the addition of considerable BCNW generally causes self-aggregation, which causes poor dispersion. Thus, the amount of BCNW can be manipulated within a very narrow range to control physical properties and wettability. In addition, until now, no studies have been conducted that achieved both the reinforcements previously mentioned and the control of PLA porous morphology.

In this study, we attempted to synthesize a monolithic composite of PLLA and BC gel having good mechanical strength and hydrophilicity using a simple phase separation technique while controlling the porous morphology during the process. Moreover, we measured the fundamental physical properties of the monoliths through a compressive test and water contact angle examination. The mechanical anisotropy of the BC/PLLA monoliths was also investigated, focusing on the directions of BC layers.

■ MATERIALS

PLLA was purchased from NatureWorks LLC (Ingeo Biopolymer 4032D). 1,4-Dioxane and 2-butanone were obtained from Nacalai Tesque and were used as received. BC gel was purchased as a Nata de coco from Thailand and exchanged with sufficient amount of water before use.

■ RESULTS AND DISCUSSION

BC/PLLA monoliths were prepared through the formation of a porous structure of PLLA within the BC gel using a thermally induced phase separation (TIPS) method. Utilizing TIPS has remarkable advantages in terms of process flexibility, reproducibility, and controlling the pore size when compared to the traditional nonsolvent-induced phase separation (NIPS). For this study, the NIPS technique could not be used because its fast phase separation after the addition of nonsolvents halts the diffusion of nonsolvents in the BC gel, thereby preventing PLLA from forming inside the gel. This suggests that TIPS can be a limited means to produce a monolithic composite of PLLA and BC gel, while maintaining the morphology of each. TIPS was performed using 1,4-dioxane as a good solvent, water as a nonsolvent, and 2-butanone as a mid-solvent (Scheme 1). This recipe for phase separation was based on the previous investigation conducted by our research group. Briefly, this ternary system that includes a mid-solvent easily enables PLLA morphology to be manipulated simply by changing the ratio of solvents or the concentration of PLLA.

First, the BC gel (water content = 99.5%) was immersed in 1,4-dioxane/2-butanone mixture at room temperature, while fixing the solvent ratio (1,4-dioxane/2-butanone/water) to 20/0/80 (v/v%), under stirring overnight. PLLA was added to the solution in the range of 50–100 mg/mL (Table 1) and heated at 75 °C for 20 h to produce a BC gel saturated with the PLLA solution. Then, the BC gel that includes the PLLA solution was immediately cooled to 0 °C for 1 h using an ice bath to induce phase separation of the PLLA. The resultant BC/PLLA wet gel was exchanged with sufficient amount of water and was freeze-dried under 10 Pa for 2 d to yield BC/PLLA dry monoliths. Pristine PLLA monoliths listed in Table 1 were also prepared using this same method, with fixed (1,4-dioxane/2-butanone/water) 20/70/10 (v/v%) as the solvent. Each sample was named BC/PLLAx or PLLAx, where x denotes the starting concentration of PLLA used in the process (Table 1).

BC/PLLA monoliths were successfully prepared without undergoing shrinkage through freeze-drying. The overall BC content in the monolith was changed from 8.6 to 4.4 wt % as a result of the starting PLLA concentration (Table 1). The PLLA concentration of less than 50 or more than 125 mg/mL resulted in a polymer precipitation without gelation or a clouded heterogeneous solution. In addition, PLLA50 and PLLA75 were sufficiently fragile to collapse before the formation or measurements of the sample.

Scanning electron microscopy (SEM) observation demonstrated that the open-porous BC/PLLA structure was formed without discontinuous pores (Figure 1), thus storing the original anisotropy of BC (Figure S1). It is noteworthy that the BC/PLLA monoliths exhibited an irvy-like double network (Figure 2) composed of the BC fiber (Figure 1a) and leaf-like PLLA units (Figure 1b,c). Furthermore, a single PLLA unit was also formed and attached to the BC framework, which is clearly seen in BC/PLLA50 (Figure 1d). This suggests that crystallization of PLLA occurred in the BC gel through solid–liquid phase separation during the TIPS process and is the main mechanism of the formation. In general, TIPS employs two mechanisms until demixing is completed: liquid–liquid (L–L)
and solid–liquid (S–L) phase separation. L–L phase separation induces demixing between the polymer-rich and the lean phases through spinodal decomposition, which results in a continuous polymer morphology. By contrast, S–L phase separation is driven by crystallization of polymers, which generates a sea-island structure with polymer crystals.

Considering the formation of a leaf-like PLLA 3D network and the existence of an independent PLLA unit, the crystallization of PLLA (S–L phase separation) can occur during the polymer-rich phase of initial L–L phase separation induced by quenching. This tendency can be found in studies on PLLA monoliths (e.g., Rezabeigi et al. also successfully fabricated noncontinuous porous PLLA through S–L phase separation through NIPS). The leaf-like PLLA unit discovered in our study may be explained by the single PLLA crystal formed at the beginning of the S–L phase separation. It is known that the PLLA monoliths consisting of leaf- or lozenge-like crystals can be prepared through both the TIPS and the NIPS under limited conditions. Per the previous discussion, differential scanning calorimetry (DSC) measurements (Figure S2) were conducted to calculate the crystallinity (Xc) of PLLA, assuming a theoretical heat of fusion of 93.1 J/g. The Xc of BC/PLLA100 (66.4%, PLLA section) and PLLA100 (68.3%) monoliths were much higher than that of the starting PLLA pellet (38.9%), which clearly suggests that the crystallization of PLLA was promoted by S–L phase separation. In particular, the Xc of BC/PLLA100 was significantly high despite the presence of the BC fiber in the PLLA framework. This was probably because the BC fiber acted as a nucleating agent for PLLA during phase separation. For this reason, the crystallization of PLLA could start at the surface of the BC fiber, resulting in an ivy-like morphology in which leaf-like PLLA crystals are entangled within the BC network. It is interesting to note that the size of leaf units was minimized when the content of PLLA was increased (see Figure 1). This was because of the higher viscosity induced by both dense concentration and fast crystallization of PLLA, which limited the initial L–L phase separation and induced minimized crystal morphology. This suggests that the pore size and surface morphology can be manipulated by the starting PLLA concentration. To evaluate how easily the morphology can be controlled, a Brunauer–Emmet–Teller (BET) adsorption test was performed (Figure S3, Table S1), which revealed that the specific surface area of the BC/PLLA monolith decreased with increasing amounts of PLLA. This was possibly due to the relatively flat surface of the PLLA unit (Table S1) and the change in the PLLA surface structure caused by phase separation in the presence of BC. Therefore, we demonstrated that the ivy-like BC/PLLA monoliths with highly porous morphology and good controllability of pore size could be effectively tailored using a simple TIPS technique.

Next, a compressive test was performed to examine the change in the mechanical strength of BC/PLLA monoliths (Figure 3, Table S1). The sample was compressed perpendicularly or parallelly to the BC layers to measure the mechanical anisotropy of the monoliths. It was surprising that the BC/PLLA100 exhibited four times higher compressive stress than the PLLA100 at 70% strain (Figure 3A, Table S1) despite the fact that a dried neat BC gel has critical fragility. Moreover, BC/PLLA showed considerable improvement in terms of brittleness, whereas PLLA100 was cracked and collapsed under 30% strain during compression (the test was continuously conducted after 30% strain as a matter of convenience) (Figure 3B). This may be because the 3D BC network acted as a reinforcement agent, like a steel frame, for the brittle PLLA monoliths. In addition, the mechanical anisotropy of BC/PLLA was observed according to the direction of compression (Table S1). It was revealed that a higher stress was required for the compression parallel to the BC layer than that for the perpendicular. This phenomenon was reported previously, namely, the mechanical strength of a BC gel changed dramatically because of its anisotropic structure. In this study, the difference between these orientations was limited to
Within a small range probably because the PLLA unit was filled throughout the BC structure. Thus, it was revealed that the interpenetration of the BC fiber efficiently enhanced the brittle PLLA monoliths consisting of leaf-like crystals.

Finally, the change in wettability was investigated through a static water angle experiment (Figure 4, Table S1). The BC/PLLA100 monolith exhibited a notably lower contact angle (102.1 ± 1.7°) than did the pristine PLLA (128.8 ± 1.1°) (Figure 4A). Furthermore, the BC/PLLA100 monolith spontaneously absorbed water droplets, whereas PLLA100 retained the drops on its surface (Figure 4B). Regarding BC/PLLA50 and BC/PLLA75, the hydrophilicity dramatically improved, as water droplets were rapidly absorbed in the monoliths (Table S1) and the contact angle was not available. This fact suggests that incorporating hydrophilic BC enables the PLLA monoliths to manipulate its wettability. In previous studies, the hydrophilicity of PLLA monoliths was improved using BCNWs. However, the water contact angle remained at 57° in the BCNW/PLLA monolith. In our research, it was demonstrated that a large BC structure can be introduced throughout the PLLA monoliths using a TIPS technique. In addition, we found that the hydrophilicity of PLLA monoliths could be easily controlled by the ratio of a BC gel (4.4–8.6 wt %).

**CONCLUSION**

In this study, we developed a novel monolithic composite composed of PLLA and BC gel using a simple TIPS technology and elucidated the changes in morphology, mechanical properties, and wettability. BC/PLLA monoliths exhibited a unique ivy-like double network wherein a leaf-like PLLA unit and the BC fiber co-existed. It was clearly observed that this ivy-like structure contributed to the reinforcement of the PLLA monoliths and exhibited four times higher compressive strength than that of neat PLLA. Furthermore, it was revealed that the interpenetration of a BC structure changes the hydrophobic PLLA monoliths to hydrophilic materials and can thus considerably expand the use of PLLA monoliths. Thus, we demonstrated for the first time that the hydrophilic PLLA monoliths with both high mechanical properties and the ability to control morphology can be prepared by incorporating BC gel and by using a simple TIPS technique.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b01968.

- SEM image of anisotropic BC/PLLA, DSC thermograms of PLLA100 and BC/PLLA100, N2 adsorption isotherms of the monoliths, table of physical properties, and analysis conditions (PDF)

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The paper was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

**Notes**

The authors declare no competing financial interest.

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