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A novel route to the generation of porous scaffold based on the phase morphology control of co-continuous poly(ε-caprolactone)/polylactide blend in supercritical CO_2

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ABSTRACT

Three-dimensional porous polylactide (PLA) scaffolds of almost fully interconnected pores were successfully prepared by extracting the poly(ε-caprolactone) (PCL) phase from co-continuous PCL/PLA blends. Quiescent annealing of the blends in supercritical CO_2 ($scCO_2$) prior to the PCL extraction, reported for the first time in this paper, allowed control over the average pore size from 50 to 150 μm of the PLA scaffolds. It was found that the PCL/PLA blend underwent a significant coarsening process in $scCO_2$ under proper condition, which indicated that the CO_2 pressure adjustment could be used as an additional effective tool for morphology control and the scaffold structure could be tailored by careful control of annealing time, temperature and CO_2 pressure. Moreover, the structure of the blend could be changed at a relative low temperature in the presence of $scCO_2$, this fact added the advantage over the conventional melt-processed treatment, as minimizing the degradation of PLA to maintain the mechanical properties of the scaffolds. A porous PLA scaffold was used to study the NIH 3T3 fibroblast cell culture and the cells successfully proliferated in the scaffold, which suggested biocompatibility and high potential of this scaffold being used in tissue engineering application.

1. Introduction

Tissue engineering scaffold works as a support for cell proliferation, playing a key role in the regeneration of tissue and organs. Several techniques have been proposed for the fabrication of porous scaffolds such as fiber bonding [1], solvent casting and particulate leaching [2], phase separation [3], gas foaming [4] and freeze-drying technique [5], etc. However, preparing a scaffold with highly controlled pore sizes and interconnectivity has been and is still currently an experimental challenge. To improve the extent of control on pore structure and interconnectivity of the porous scaffold, a preparation method based on immiscible polymer blending has been developed [6,7], in which the melt blending of immiscible polymers at co-continuous compositions followed by quiescent annealing and selective extraction, could be an effective approach for the production of porous scaffolds. Since co-continuous morphology is essentially a vast network of interpenetrating fiber-like structure, extracting the sacrificial phase can result in a fully interconnected three-dimensional porous material. Besides, it has been found that a co-continuous blend can undergo coarsening effect and the phase structure vary significantly under quiescent annealing [8–10], this acts as an effective tool for the control of scaffold structure. Sarazin et al. [11] have prepared a porous polylactide (PLA) material with fully interconnected pores and highly controlled pore size using this method. This fabrication process, however, is a melt-based technique, the phase structure can be controlled only in molten state of a polymer system, which presents an inherent disadvantage, the use of high temperature [6,7,11–14]. Consequently, quiescent annealing in molten state could inevitably lead to the degradation of polymers and this would deteriorate the mechanical property of the final scaffold.

Supercritical CO_2 ($scCO_2$), has the combination of gas-like diffusivity and liquid-like density, which makes $scCO_2$ a unique medium for polymer synthesis and processing [15–18]. It is well known that $scCO_2$ is soluble in many polymers and leads to substantial and drastic changes in the viscosity, melting temperature and interfacial tension, etc. [19–23], while they are the crucial

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parameters in determining the final resulting morphology of the immiscible binary polymer blends [24–29]. Previous studies [30–34] focused on the assistance of $scCO_2$ in dynamic extrusion process and the results show that the size of dispersed phase was decreased and the miscibility of polymers were improved. Moreover, Jenkins et al. [35] found that $scCO_2$ enables the melt blending processing to take place at a lower temperature and thus the poly(3-hydroxybutyrate-co-hydroxyvalerate) (PHBV)/poly(ε-caprolactone) (PCL) blend prepared by $scCO_2$-assisted blending showed negligible levels of thermal degradation. Therefore, $scCO_2$ tends to be a promising solvent for morphology control of polymer blends. On the one hand, it is reasonable to assume that the phase structure of polymer blends could be modified in the presence of $scCO_2$, though there are quite few published reports in which the $scCO_2$ is applied in the annealing process of polymer blends in a static way. On the other hand, the $scCO_2$-assisted annealing process may be carried out at a relative low temperature due to its unique property of reducing the melting temperature, viscosity and interfacial tension of a polymer blend system, which is in favor of minimizing the occurrence of degradation of polymers and thus maintaining the mechanical property of the final scaffold product. In addition, the solubility of $scCO_2$ in some polyesters is relatively high resulted from the high affinity for CO_2 due to the specific Lewis acid-base type interactions between the carbonyl groups and CO_2 molecules [21,36–38]. For example, the CO_2-induced melting point decreasing rate of PLA (−2.1 °C/MPa) is greater than that of non-polar iPP (−0.83 °C/MPa) [39]. Therefore, two biodegradable and biocompatible polyesters PCL and PLA are used for investigation in this paper.

Inspired by the conjecture, a novel PLA scaffold fabrication strategy is proposed in this study, by applying the polymer blending in combination with $scCO_2$ fluid technique. With the assistance of $scCO_2$, the processing temperature in fabrication process is expected to be significantly lowered and the degradation degree of a polymer would thereby be decreased. Therefore, the $scCO_2$ assisted annealing process appears to be a feasible alternative to the conventional high-temperature treatment. For the purpose of increasing the pore sizes, the quiescent annealing of a polymer blend in $scCO_2$ atmosphere is conducted in our investigation. In this paper, the effect of annealing temperature, time, CO_2 pressure and viscosity on the morphology of PCL/PLA blends are systematically studied. PLA scaffolds are prepared by this fabrication method based on the detailed morphological analysis of PCL/PLA blends. The biocompatibility of the PLA scaffold fabricated are preliminarily evaluated with the NIH 3T3 fibroblast cells.

2. Experimental section

2.1. Materials

PLA pellets were supplied by NatureWorks LLC (Grade 2002D; $M_n = 8.8 \times 10^4$, $M_w/M_n < 1.5$) with a density of 1.24 g ml^{-1} (20 °C), and its reported ₙ-isomer content is 4.3 mol%. PCL pellets were purchased from Sigma-Aldrich ($M_n = 7–9 \times 10^4$, $M_w/M_n < 2$) with a density of 1.145 g ml^{-1} (20 °C). To decrease their moisture level, the PLA and PCL pellets were dried in vacuum ovens at 65 °C and 30 °C, respectively, for 24 h before use. CO_2 (>99.5% purity) was obtained from Qiaoyuan Industry Co. (Chengdu, China).

2.2. Blend preparation and quiescent annealing procedure

The PCL/PLA blends were prepared by melt mixing with a twin-screw extruder (Haake MiniLab II) at 180 °C and 50 rpm for 5 min. After mixing, the blends were rapidly quenched in liquid nitrogen to freeze-in the morphology. All these concentrations were reported as volume fraction. The neat PCL and PLA samples were also prepared under the same processing conditions for the properties comparison. In order to determine the composition of co-continuous blends, PCL/PLA of various mixing ratios were prepared for SEM observation and rheological measurements. The binary blends PCL/PLA were annealed at various temperatures and CO_2 pressures for different annealing time. After annealing, the samples were cooled immediately and the pressure was relieved at a slow rate in case of forming. For comparison, quiescent annealing was conducted for the samples in atmosphere at the same temperature and for the same annealing time.

2.3. Preparation of the porous scaffold

A schematic of the scaffold fabrication process is shown in Fig. 1. Quiescent annealing of the blends PCL/PLA with co-continuous structure was carried out in a house-made autoclave. The PCL phase was extracted with acetic acid. Acetic acid was a solvent for PCL and a non-solvent for PLA, the solvent was changed regularly to ensure an efficient extraction. After extraction, the specimens were then rinsed with distilled water to remove any trace of acid and were subsequently dried under vacuum until contrast weight was obtained.

2.4. Sample characterization

2.4.1. Measurement of the interfacial tension

Deformed drop retraction method developed by Carriere et al. [40] allowed the measurement of the interfacial tension over a shorter time scale, was hence used to estimate the interfacial tension between PCL and PLA in atmosphere. This method consisted of studying the kinetics of relaxation of a deformed droplet, in this case, a short PLA fiber between two PCL thin films. The relaxation kinetics was based on a theoretical equation describing the shape evolution of an ellipsoidal liquid drop suspended in an infinite fluid domain. Upon cessation of the flow,

$$D = D_0 exp\left[-\frac{40(p+1)}{(2p+3)(19p+6)} \left(\frac{\sigma}{\eta_m R_0}\right) t \right] \quad (1)$$

where D is the drop deformation parameter defined as $D = (L-B)/(L+B)$, where L and B are the major and minor axis of the ellipsoidal drop, respectively; D_0 is an initial deformation parameter; p is the viscosity ratio between the viscosity of the drop and the matrix phase; σ is the interfacial tension; t is the time; η_m is the viscosity of the matrix phase; and R_0 is the radius of the drop at equilibrium. The retraction process of a PCL thread embedded in the PLA matrix was recorded at several temperatures using an optical microscope equipped with a hot stage.

2.4.2. Dynamic mechanical thermal analysis

DMTA measurements were performed on a Q800 DMA from TA Instruments, were used to study the miscibility of the PCL/PLA blends. Rectangular samples from the blends in dimensions of 35 × 10 × 1 mm were tested in the single cantilever bending mode.

2.4.3. Continuity measurement

The continuity of the porogen phase PCL in scaffolds as a measure of pore interconnectivity was analyzed in polymer blend. The samples were weighted to measure the quantity of extracted PCL and the remaining amount of PLA and a gravimetric method was used to calculate the continuity of the porogen phase, using the simple equation: