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The effects of molecular weight and supercritical CO_2 on the phase morphology of organic solvent free porous scaffolds

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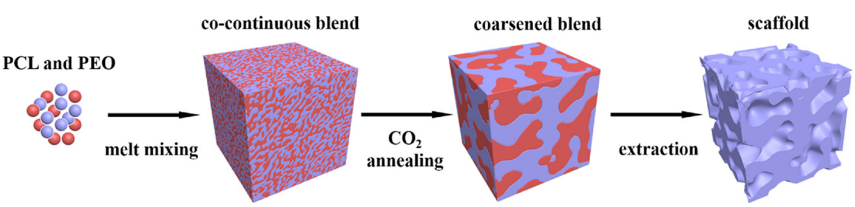
GRAPHICAL ABSTRACT

The porous scaffolds were prepared by extracting the PEO phase from co-continuous PCL/PEO blends which were annealed in CO_2. Due to the plasticization of CO_2, the temperature in CO_2 annealing is lower than the temperature in atmosphere to obtain the same pore size. Low annealing temperature is beneficial to maintain the mechanical properties of scaffold. The preparation of scaffold completely avoids the use of organic solvents.



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ABSTRACT

Porous poly(ε-caprolactone) (PCL) scaffolds were prepared by extracting the poly(ethylene oxide) (PEO) phase from co-continuous PCL/PEO blends, which were annealed in supercritical carbon dioxide ($scCO_2$). It was found that the coarsening temperature of PCL/PEO blend in $scCO_2$ is lower than that in atmosphere, which indicated that the $scCO_2$ could be used as an effective tool for morphology control. The effects of molecular weight of PEO, annealing time, temperature and pressure on the phase morphology of polymer blends have been systematically studied. The average pore size of PCL porous scaffold increases with the increase of annealing time, temperature and pressure. The coarsening effect of the PCL/PEO blends decreases with increasing molecular weight of PEO. The average pore size is in the range of 10–130 μm with high interconnectivity. In addition, the preparation of scaffold is organic solvent free preparation for the reason that PEO and PEG are water-soluble polymers.

1. Introduction

A variety of polymers have been proposed to the preparation of three-dimensional porous scaffolds for tissue engineering. Among them, poly(ε-caprolactone) (PCL) has been widely used in tissue engineering and drug delivery owing to its favorable mechanical and biodegradable properties [1–4]. Degradation of PCL occurs due to the bulk and surface hydrolysis of ester linkages which results in a slow biodegradation. Degradation of PCL can also be altered according to specific needs for each application [5]. In addition, PCL is biocompatible, easily processable and does not elicit immune responses. These excellent properties make PCL have potential for application in tissue engineering applications. The application of PCL in tissue engineering has been studied and a variety of methods to prepare PCL porous scaffold have been investigated, including fused deposition modeling (FDM) [6], particulate leaching [7], supercritical CO_2 ($scCO_2$) foaming [8–10], and so on. However, there are some unavoidable problems with these methods, such as poor interconnectivity. The morphology in immiscible binary blends depends on the interfacial properties and the composition of the components. Co-continuous polymer blends have been examined

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in the past and show the potential for the production of tissue engineering scaffolds with high pore interconnectivity [11–13]. The polymer blend with co-continuous phase structure is subjected to static annealing process and selective extraction. Since the phase structure of the polymer blends is co-continuous structure, the polymer blend possesses an interconnected network, which contributes to the formation of porous materials in which the pores are interconnected. In a recent study, Mehr et al. [14] prepared PCL scaffolds with highly controlled porous structure and a fully interconnected internal network via melt blending of PCL and poly(ethylene oxide) (PEO). The result shown that the scaffolds have close to 100% pore interconnectivities, sharp unimodal pore size distribution. However, the PCL/PEO blends need to be processed at 160 °C in order to achieve the coarsening of the phase structure. The temperature of 160 °C is so high for PCL that may result in degradation, and a decrease in mechanical properties. Therefore, the low temperature processing method is with great significance.

Supercritical carbon dioxide ($scCO_2$) possesses excellent properties, such as moderate supercritical condition, environmentally and combination of gas-like diffusivity and liquid-like density. There is a relatively strong affinity between the $scCO_2$ and polymer with carbonyl group, such as PCL and polylactic acid (PLA). On account of these excellent properties, $scCO_2$ has become a unique medium for polymer processing [15–20]. There are several studies on tissue engineering scaffolds prepared by supercritical foaming [10,21,22]. However, the pore of the scaffold material prepared by supercritical foaming is normally closed-cell structure. The closed-cell of scaffold couldn't provide a timely material exchange for the metabolism products of the cell. Some particles, such as salt or sugar, are selected as porogenic agents to increase the connectivity of pores. However, the porogenic agents couldn't be removed completely, which is very detrimental to tissue engineering. Our group [22] firstly presented a novel to prepare porous scaffold based on the phase morphology control in $scCO_2$-assisted annealing process. The porous scaffolds were prepared by quiescent annealing the co-continuous poly(ε-caprolactone) (PCL)/polylactide (PLA) blends in $scCO_2$. It was found that the size of phase was larger than samples processed under atmosphere at the same temperature. In other words, the processing temperature in $scCO_2$ was lower than that in atmosphere to obtain the same pore size. The application of $scCO_2$, acting as tool for assisting static annealing is feasible in the preparation for tissue engineering scaffold.

Both Polyethylene glycol (PEG) and poly(ethylene oxide) (PEO) are polymers of glycol. The difference between PEG and PEO is molecular weight. The PEG possesses a molecular weight lower than 2.0×10^4, while that of PEO is higher than 1.0×10^5. It is important that both of PEG and PEO are water-soluble polymers. Due to its nontoxicity nature, PEG and PEO are widely used in medicine [23–26]. There are several studies on PCL/PEO blend for preparing scaffold. Mehr et al. [4,14] prepared a porous PCL material with 100% pore interconnectivities, sharp unimodal pore size distribution. The results showed that the static annealing of the blends can yield a sharp unimodal pore size distribution, as opposed to non-annealed structures that demonstrated a polymodal and irregular size dispersity. Yin et al. [27] fabricated the highly interconnected 3D porous scaffolds with aligned pore structure by combination of solid phase extrusion of PCL/PEO co-continuous blends with phase removal. Allaf et al. [28] prepared three-dimensional interconnected porous PCL/PEO scaffolds by combining cryomilling and compression molding/polymer leaching techniques. The results showed that the resultant porous scaffolds exhibited co-continuous morphologies with 50% porosity and mean pore sizes of 24 and 56 μm were achieved by varying milling time.

In this study, the porous PCL scaffolds were prepared by combining supercritical CO_2 techniques with static annealing. Due to the introduction of $scCO_2$, the treatment temperature is much lower than that in the literature. In addition, the preparation of scaffold is organic solvent free preparation due to the water soluble nature of PEO and PEG. The effects of molecular weight of PEO and supercritical CO_2 condition on the phase morphology of PCL/PEG or PCL/PEO blends have been systematically studied.

2. Experimental section

2.1. Materials

PCL was purchased from Sigma Aldrich ($M_W = 8.0 \times 10^4$) with a density of 1.146 g ml^{-1}. PEG ($M_n = 2.0 \times 10^4$) was supplied by Chengdu Kelong Chemical Reagent Co. (Chengdu, China) with a density of 1.2 g ml^{-1}. PEO was supplied by Ryoji ($M_n = 1.0 \times 10^5$, 3.0×10^5 and 5.0×10^5) with a density of 1.2 g ml^{-1}. Before using, the PEG, PEO and PCL were dried in vacuum ovens at 40 °C for 24 h to reduce moisture content. CO_2 with a purity of 99.5% was obtained from Qiaoyuan Gas Co. (Chengdu, China).

2.2. Blend preparation and quiescent annealing procedure

PCL/PEG and PCL/PEO blends with different content (calculate by volume fraction) were prepared by melt mixing with Haake MiniLab II at 80 °C and 100 rpm for 8 min. After mixing, the resulting blends were rapidly quenched in liquid nitrogen to freeze-in the phase morphology in order to investigate the morphological evolution. The pure PCL samples were prepared under the same processing conditions. Then, PCL/PEG, PCL/PEO blends and pure PCL were dried in vacuum oven at 40 °C for 24 h. The experimental samples with a thickness of 2 mm were prepared by compression molding at 80 °C for 5 min. PCL blending with PEO ($M_n = 1.0 \times 10^5$, 3.0×10^5 and 5.0×10^5) were marked as PCL/PEO10, PCL/PEO30 and PCL/PEO50, respectively. The PCL/PEO10 blends were annealed at various temperatures and CO_2 pressures for different annealing time using a house-made vessel. The annealing conditions of PCL/PEO10 were showed in Table 1. The PCL/PEG, PCL/PEO10, PCL/PEO30 and PCL/PEO50 were annealing in 50 °C and 10 MPa CO_2 for 60 min. After annealing, the vessel was quickly cooled to 25 °C by pouring the cooling water into the interlayer before the CO_2 was released. Once the temperature was reduced to 25 °C, the CO_2 pressure was released in a slow rate to prevent foaming. In contrast, the experiment was carried out at the same temperature for the same time in atmosphere.

2.3. Preparation of the porous scaffold

After annealing, the specimens were etched in order to acquire the porous scaffold. The etching solution was distilled water. As a water-soluble polymer, PEG or PEO could be completely dissolved by distilled water. The solvent was changed every eight hours to ensure the PEG or PEO effectively extracted. After extraction, the samples were dried under vacuum oven until the weight of sample was stable. It is crucial that no organic solvents were used in the preparation process.

2.4. Continuity measurement

As porogen phase, PEO was completely etched to obtain the porous scaffold. The continuity of PEO in porous scaffolds was researched. The continuity of PEO is defined as the ratio of the weight of PEO etched to

Table 1
The annealing conditions of PCL/PEO10.

	1	2	3	4
Pressure	50 °C, 60 min, **5 MPa**	50 °C, 60 min, **10 MPa**	50 °C, 60 min, **15 MPa**	50 °C, 60 min, **20 MPa**
Temperature	10 MPa, 60 min, **45 °C**	10 MPa, 60 min, **50 °C**	10 MPa, 60 min, **55 °C**	10 MPa, 60 min, **60 °C**
Time	50 °C, 10 MPa, **30 min**	50 °C, 10 MPa, **60 min**	50 °C, 10 MPa, **120 min**	50 °C, 10 MPa, **240 min**