FABRICATION OF INTERCONNECTED POROUS POLY(LACTIC ACID)
SCAFFOLDS BASED ON DYNAMIC ELONGATIONAL FLOW PROCEDURE,
BATCH FOAMING AND PARTICULATE LEACHING

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Abstract

This study reports a highly porous interconnected poly(lactic acid) (PLA) scaffold fabrication method, which utilized self-developed vane extruder melt blending followed by supercritical carbon dioxide (Sc-CO2) foaming and particulate leaching. PLA as polymer matrix was blended with polyvinyl alcohol (PVA) and NaCl particles via vane extruder, which generates global dynamic elongational flow. Then, the prepared blends were foamed by Sc-CO2 followed by leaching the porogens. It was found that most NaCl particles could preserve their original size after vane extruder blending and the macropore density and size can be tuned by modifying the salt concentration and particle size. The scaffolds prepared using this approach could achieve a porosity up to 93% with well interconnected porous structure.

Introduction

Tissue engineering scaffold could provide temporarily three-dimensional supports and a template for the tissue generation [1]. An ideal scaffold should be highly porous with an interconnected, open porous structure to provide a three-dimensional (3D) substrate for cell growth and nutrition supply. Various techniques including fiber bonding [2], solvent casting/particulate leaching [3], phase separation technology [4], gas foaming [5], solid freeform fabrication [6] have been introduced and developed to produce scaffolds.

Each fabrication method has its own advantages and disadvantages. The melt-molding/particulate leaching was proposed to fabricate scaffolds without using of organic solvent [7, 8]. The deficiency of this technique is the lack of interconnectivity between the pores at low volume fraction of porogen particles. To improve the interconnectivity between the pores, conventional immiscible polymer blending technique was adapted to produce interconnected, porous, three-dimensional scaffolds [9, 10]. The strategy consists of melt blending two immiscible polymers to create the co-continuous blend morphology. After formation of the co-continuous blend morphology, one of the polymer phase is sacrificed by selective removing from the blend to form the porous structure with fully interconnected pores. The melt blending technique by utilizing extrusion process provides an economical and controllable method of producing scaffolds. However, the drawback of the technique is that the maximum porosity of 50-60% is achievable and it is not suitable to be used in tissue engineering without further modification. Recently, Reignier et al. have improved the above method by combing the polymer leaching and particle leaching to obtain highly porous interconnected networks [11]. By removing the sacrificial polymer phase and porogens, the porosity of the scaffolds can be achieved to 88%.

To further improve the porous structure of the scaffolds, Tung group employed polymer/particle leaching combined with gas foaming to prepare porous PLA, PCL and thermoplastic polyurethane (TPU) scaffolds. After foaming and leaching processes, porous scaffolds with multimodal porous structure and interconnected channels were obtained [12-15].

However, polymers are faced with shear deformation field in the screw extruder. High shear stresses especially in the melting zone can cause great attrition of particulate, which reduces the particle size of the porogen [15]. Elongational flow field in polymer processing has many advantages over the shear deformation field [16]. Recently, our lab designed a novel polymer processing equipment known as vane extruder of which continuous dynamical converging channels can generate strong elongational deformation field in the whole process [17]. Experimental study demonstrates that the vane extruder has a much higher dispersive mixing efficiency than twin-screw extruder.

The purpose of this study is to prepare porous scaffolds using vane extruder combined with Sc-CO2 foaming and particulate leaching techniques. PLA was chosen as the scaffold matrix material. NaCl and PVA were used as water soluble porogens. The effect of NaCl concentration on the scaffold morphology and the porosity was investigated.
Materials and Experiments

Materials

Biodegradable PLA (2002D, Mw= 208,000 g/mol, 4.25% D-lactic acid) was supplied by Nature Works LLC. PVA (GM-14S) with density of 1.27 g/cm3 was provided by Japanese Synthetic Chemistry Corp. Edible NaCl crystals were purchased from Guangdong Province Salt Industry Group Corp., China and to desired size ranging from 75 to 150 μm. Materials were pre-dried for 8 h at 80 °C under vacuum prior to using. Commercial purity grade CO₂ (purity grade, 99%) purchased from Shengtong Corp., China was used as physical blowing agent.

Vane Extruder Device

A vane extruder was employed in this study to prepare PLA/PVA/NaCl blends. As illustrated in Figure 1, instead of screw in the conventional extruder, the vane extruder is comprised of several vane plasticizing and conveying units (VPCUs) along the axial direction. The stator, vanes, baffles and the rotor of VPCUs compose a closed chamber. The volume of the closed chamber changes periodically as rotor rotates during processing because of the eccentric distance from the stator to the rotor. A dynamical converging channel is obtained along the circumferentially direction. Thus, higher stress and dynamic elongational flow field are generated.

The vane plasticizing units feed materials as the volume increases, and discharge materials as the volume decreases. The vane extruder used in this study consists of 12 VPCUs. The inner diameter of the stator are 46 mm, the outer diameter of the rotor is 40 mm, and eccentric distance between the rotor and stator is 3 mm.

Figure 1. Schematic structure of vane extruder

2.3 Preparation of Porous PLA Scaffolds

Pre-dried PLA, PVA and NaCl were mixed at certain proportions and compounded via the vane extruder at a rotation speed of 100 rpm. The temperature profiles were 160 °C, 170 °C, 170 °C, 160 °C from hopper to die. For melt blending, the PLA/PVA was kept constant at 50/50 by volume to ensure a co-continuous morphology of the polymer matrix, while the NaCl content was varied from 10% to 40% of the polymer matrix by volume. The prepared PLA/PVA/NaCl blends were foamed in a high-pressure vessel. The samples were heated to 150 °C and saturated with SC-CO₂ at 10 MPa for 1 h. They were subsequently cooled to the foaming temperature of 100 °C and foamed by instantaneously depressurizing the pressure to the atmosphere level to induce cell nucleation and growth. Finally, the water soluble phase PVA and NaCl were leached with circulating water at 40 °C for at least 72 h (water was changed every 6 h) until the sample weight was constant. The porous scaffolds were vacuum-dried before use. Figure 2 illustrates the schematic of the fabrication process of porous PLA scaffolds.

Figure 2. Schematic illustration of preparing porous PLA scaffolds.

Characterization

The morphology of the blends and the foams was observed under a scanning electron microscope (Quanta 200, FEI, USA) at 10 kV. The samples were immersed into liquid nitrogen and fractured in cross section. The fractured surfaces were coated with a thin layer of gold prior to observation.

The porosity of the scaffolds was calculated using the following Equation (1) [18]:

\[ \text{Porosity} = \frac{W_{d} \rho - W_{m}}{W_{d} \rho} \times 100\% \]  

(1)

where, \( \rho \) is the density of PLA; \( W_{d} \) is the volume of the foamed samples, which was measured with a density balance (DH-600 DahoMeter, Dongguan Hongtuo Instrument Co. Ltd); \( W_{m} \) is the weight of the sample determined by a electronic scale with high accuracy.

The salt content of each blends was analyzed using a thermogravimetric analyzer (NETZCH TG 209, Germany). The samples were heated from 30 °C to 700 °C at a rate of 20 °C/min. To evaluate the influence of leaching time on the NaCl residue, porous scaffolds at different leaching time were burned in a muffle furnace (KSW-4-12, Shanghai Chongming Experimental Instrument Factory) at 700 °C to burn polymer matrix. The remaining NaCl particles were measured by the aforementioned electronic scale and the content of NaCl was calculated according to the following Equation (2).

\[ \Phi_{\text{NaCl}} = \frac{W_{\text{NaCl}}}{W_{\text{sca}}} \times 100\% \]  

(2)

where, \( W_{\text{NaCl}} \) was the weight of residue after burning in the muffle furnace and \( W_{\text{sca}} \) was the weight of the scaffolds before leaching. Moreover, Laser diffraction particle size analyzer (LS 13320, USA) was employed to determine the size of NaCl particles before and after blending.

SPE ANTEC™ Indianapolis 2016 / 221
Results and Discussion

Scaffold Morphology

DSC was conducted on neat PLA and PVA to determine the processing temperature of PLA/PVA blends. As shown in Figure 3(a), it can be observed that the melting temperature of PLA and PVA are 152 °C and 192 °C, respectively. Based on our preliminary work, it was found that when the highest processing temperature was 200 °C, the viscosity of PLA became very low and hard to solidify. Moreover, the color of PVA became yellow demonstrating the decomposition of PVA during processing. Therefore, the highest processing temperature profile was in between the two melting points of the PLA and PVA. Due to the good flow ability of PLA phase at the chosen temperature and the shear heat during processing, PVA phase was also easily processed.

After leaching for different time periods, the morphology of PLA/PVA blends was explored, and the results are shown in Figure 3. Figure 3(b) shows the microstructure of the blend after extracting the PVA phase for 24 h. It can be found that most PVA phase was leached out except some solid cores, which was formed by the entrapment of PVA in the PLA matrix. However, after leaching for 72 h, the solid pores disappeared with the appearance of interconnected channels (as shown in Figure 3(c)). Pores with the diameter range of 5-30 μm were also observed in the blends after leaching for 72 h. These were attributed to the removal of unmelted PVA phase under the used processing temperature profile (which was slightly lower than the melting point of PVA as shown in the Figure 3(a)). The larger pores created by the unmelted PVA phase might be beneficial for the nutrition and waste transportation during cell culture.

Figure 3. (a) DSC curves of neat PLA and PVA, respectively; SEM results of (b) PLA/PVA blend after leaching for 24 h, (c) PLA/PVA blend after leaching for 72 h, (d) higher magnifications of the dashed red square part in image (c).

The Effect of NaCl Content on the Scaffold Morphology

The scaffold morphology can be tuned by adjusting the salt concentration in the scaffolds. It can be seen that higher content of NaCl particulates in the blends introduced more large pores of the scaffolds along with the sacrifice of the PLA/PVA/NaCl melt fluidity during processing. The viscosity of the suspension is known to increase with the increase of solid particulate concentration. Therefore, the maximum content of NaCl that can be attained was 40%, beyond which the extrude mixture lost their integrity. The morphology of the scaffolds prepared by varying the NaCl concentrations is shown in Figure 4. All scaffolds showed a multimodal porous structure with distinct pore size and pore architecture. The macro pores ascribed to continuous PVA phase and salt particles were homogeneously distributed in the PLA matrix. The size of the macro pores varied throughout the whole scaffold from tens to hundreds of micrometers. The micrometer network created by the extraction of the PVA porogen polymer shown as cylindrical pores distributed uniformly with the pore size ranging from nanoscale to tens of micrometers.

However, although the PVA polymer and NaCl particles as porogens introduced interconnected channels and macropores for the scaffolds, which played a major role for the interconnectivity of the scaffolds, there were still thick skin layers among the macropores to affect the interconnectivity of the scaffolds. These are not beneficial for cell interactions and nutrition transportation in tissue engineering. On the other hand, the porosity of scaffolds was increased with the increase of NaCl contents. The porosity of the scaffold was 70% with the highest NaCl content. This was not optimal for nutrient transportation during cell culture. Therefore, further technique need to be used to enhance the scaffold porosity.

Figure 4. Morphology of PLA porous scaffolds as a function of NaCl content: (a) 10 vol%; (b) 20 vol%; (c) 30 vol%; (d) 40 vol%. The PLA/PVA composition ratio is kept at 50/50 (v/v) for all the blends (the scale bars in the

SPE ANTEC™ Indianapolis 2016 / 222
images and insert images are 100 μm and 20 μm, respectively.

**The Effect of Gas Foaming on Scaffold Morphology**

To further enhance the porosity and interconnectivity of the scaffolds, supercritical CO₂ foaming was used to combine with the particulate leaching. The morphology of the scaffolds is shown in Figure 5. Multimodal pore structure was observed in the foamed scaffolds. After extraction of NaCl particles, macropores with the size of hundreds of micrometers were left in the scaffolds. Besides, there were dense microscale pores after foaming distributed on the walls of the macropores, which were ascribed to the foaming of PLA matrix. However, as shown in Figure 5(a), those created macropores were not interconnected due to the limited content of NaCl in the foamed PLA/PVA/NaCl blend at the composition ratio of 50/50/10.

For the blends of PLA/PVA/NaCl with the composition ratio of 50/50/20, after foaming, the macropores were connected (shown in Figure 5(c)). The number of the macropores was also increased, and it can be seen that the wall of macropores was composed of many small pores and micropores. Those small pores were fully interconnected. The porosity and interconnectivity of the scaffold were achieved to 93% and 96%, respectively. On the other hand, it was noticeable that the macropores created by NaCl particles after foaming were larger than their original size. The possible explanations were as follows: during foaming, debonding occurred between the NaCl particles and PLA matrix, therefore, NaCl particles slipped in the polymer matrix under the encouragement of the foaming process, which caused larger macropores than the real size of NaCl particles after leaching. When the NaCl content was further enhanced, the PLA/PVA/NaCl blends was difficult to foam and the porosity of the scaffolds was not increased (The results were not shown here). These was because the increment of non-melt polyvinyl alcohol and higher loading of NaCl particles make the CO₂ adsorption of the PLA matrix reduce, thus the foaming capacity of the blend was weakened.

![Figure 5. Morphology of PLA scaffolds prepared by combination of particulate leaching and supercritical CO₂ foaming, (a) the PLA/PVA/NaCl volume composition ratio is 50/50/10 and (b) the PLA/PVA/NaCl volume composition ratio is 50/50/20.](image)

**NaCl residues in the Scaffold**

TGA was used to quantitatively determine the residual NaCl content of the blends. The results are showed in Figure 6(a), the NaCl content of the different components are found to be 17.3%, 29.8%, 42.4%, 53.2%, respectively, which are closed to the theoretical values, demonstrate that vane extruder has good mixing effect. To evaluate the effect of leaching time on the residual NaCl content, both unfoamed and foamed PLA scaffold were selected and immersed in the 40 °C thermostatic water to extract the water soluble components, and then burned in muffle furnace at 700 °C for 6 h to obtain the residual NaCl components. Figure 6(b) shows the weight of the residual NaCl of unfoamed and foamed PLA scaffolds at different leaching time. As expected, the weight of NaCl decreased as the leaching time increased. It is noted that foamed PLA scaffold needs less than 24 h to fully extract the water soluble NaCl particles in the scaffolds, in comparison, the unfoamed scaffolds takes 72 h to fully remove the NaCl particles. These was result from the higher porosity and better interconnectivity of the foamed scaffolds, which was consistent with the SEM results.

![Figure 6. (a) TGA curves of PLA/PVA/NaCl blends with different NaCl contents; (b) The NaCl residues of unfoamed and foamed PLA/PVA/NaCl (50/50/20) components at different leaching time.](image)

**Morphology of NaCl Particles after Processing**

It has been reported that high shear stress especially in the melting zone of the extruder can cause sharp attrition of the salt particles during blending in the previous studies. While in this study, the vane extruder was used to prepare the blends, which generates strong elongational stress during compounding. Figure 7 depicts the morphology and particle size distribution of NaCl before and after blending by vane extruder, twin extruder and triple extruder. It can be seen that before mixing, the NaCl powder is mainly composed of uniformly distributed large particles. However, after going through the extruder, the particle size distribution showed a bimodal peak, especially for the twin and triple extruder. In contrast, for the NaCl particles processed by...
vane extruder, most NaCl particles were still larger than 90 μm. This indicated that the shape and particle size of NaCl particles could be preserved by the elongational stress.

The possible dispersing mechanism of NaCl particles dispersing in the polymer matrix under steady shear or volume elongational flow field is shown in Figure 7. For the steady shear case, the velocity gradient is perpendicular to the flow direction. The NaCl particles were inclined to move in a strong shear layer and rotate by itself, faced with strong shear force. While, for the volume elongation flow field, the polymer molecular chains were much easier to move than shear flow[19], therefore, the NaCl particles were faced with a stretched force result in a easier movement without broken during processing. Therefore, the NaCl particles could preserve their cubic shape under volume elongational flow field, which was consistent with the SEM micrographs.

![Figure 7](image)

**Figure 7.** The morphology of NaCl particle before and after processing by different flow field. (a) original NaCl particles before processing, (b) NaCl particles processed by vane extruder, (c) NaCl particles processed by extruder and (d) NaCl particles processed by triple screw extruder.

Conclusions

A novel highly porous interconnected polylactic acid (PLA) scaffold fabrication method was proposed in this study, which is based on self-developed vane extruder melt blending followed by supercritical carbon dioxide (Sc-CO2) foaming and particulate leaching. The maximum porosity and interconnectivity of the prepared scaffolds could be achieved to 93% and 96%, respectively. Moreover, the obtained scaffolds showed multimodal porous structure with interconnected channels. The polymer blending process was conducted on vane extruder which is a novel polymer processing equipment dominated by elongational flow field. Compared with twin and triple screw extruder, most NaCl particles preserved their original size after processing by vane extruder, indicates the vane extruder was more suitable for precisely controlling the size of macropores in the scaffolds.

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