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Technical Note

A method for solvent-free fabrication of porous polymer using solid-state foaming and ultrasound for tissue engineering applications

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Abstract

Most of the existing fabrication techniques for tissue engineering scaffolds require the use of organic solvents that may never be fully removed even after long leaching hours. The residues of these organic solvents reduce the ability of biological cells to form new tissue. This paper presents an approach toward solvent-free fabrication of tissue engineering scaffolds. Interconnected porous structures were created using solid-state foaming and ultrasound. The material used in this study was polylactic acid (PLA) and the blowing agent was CO_2. In order to determine suitable process conditions, saturation and foaming studies were first conducted. Selected foam samples were then processed using pulsed ultrasound. The microstructures before and after the ultrasound processing were compared. It was shown that the inter-pore connectivity of the solid-state foams was substantially enhanced. The combined solid-state foaming and ultrasound processing provide a way to fabricate porous polymer for potential tissue engineering applications.

Keywords: Solvent-free fabrication; Tissue engineering scaffold; Polylactic acid (PLA); Ultrasound; Solid-state foams; Porous polymer

1. Introduction

Over the past decade, tissue engineering has moved beyond the realm of transplantation and into the realm of fabrication [1]. The vision is that in the future doctors will be able to shape the scaffolds into intricate structures that mimic specific tissue and organs, load the scaffolds with living cells and nutrient, and implant them to replace diseased or damaged organs without the need of retrieving the scaffolds. For this to be successful, the fabrication of biodegradable tissue engineering scaffolds is crucial. Existing fabrication methods for tissue engineering scaffolds include the fiber bonding [2], solvent casting [3–5], phase separation [6–11], gas foaming with particulate leaching [12–16], and rapid prototyping techniques [17–19]. Almost all these methods require organic solvents, which may reduce the ability for biological cells to form new tissue if not fully removed. Other methods involve using salt particulates as porogen. The concerns for this are the lengthy leaching steps and the residual salt effects. To overcome these problems, other combinations of materials and pore-forming techniques must be explored [2].

The solid-state foaming process has been studied to generate microcellular foams for biomedical applications [20]. The process does not involve any organic solvents or chemical blowing agents. Instead, it uses gases such as CO_2 and N_2. The pore sizes that have been achieved range from sub-micrometers to a few hundred micrometers. However, the disadvantage of the process is that the foams it produces are mostly close-pored and not suitable for tissue engineering applications. In this study, we explore the possibility of using ultrasound to break the pore walls of the solid state foams. Biodegradable polymer samples were first foamed in the solid-state foaming process to achieve suitable pore sizes. Then the foamed samples were processed using ultrasound. The microstructures of the processed samples were compared with those of the original foams. It is shown that ultrasound can substantially enhance the inter-pore connectivity of the solid-state foams, which suggests that the combined solid-state foaming and ultrasound process could be used to fabricate biodegradable porous polymer for tissue engineering applications.

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2. Experimental

2.1. Materials

Biodegradable polylactic acid (PLA) was used in the study. The PLA samples were acquired in thin sheets (0.25 mm) (WMI, Taiwan) and compression molded into thicker samples (1.1–1.3 mm). The density of the samples was 1.25 g/cm^3. PLA is a semicrystalline polymer. The crystallitnity of the samples after the compression molding was around 5%. The glass transition temperature was 60 °C. Medical grade CO_2 was used as the physical blowing agent. The CO_2 was obtained from Airgas Nor Pac, Inc.

2.2. Experimental setup and procedure

The PLA samples were foamed in a typical solid-state foaming process [20]. Before foaming a PLA–CO_2 sorption study was conducted at room temperature with the gas pressures at 3–5 MPa. Following the sorption study at 5 MPa, a desorption study was conducted by retrieving a sample from the pressure vessel and measuring its weight periodically in the atmospheric environment. The results of these sorption and desorption studies were used to determine the saturation time and desorption time, both of which are important for the subsequent foaming process.

The foaming experiments were conducted in two groups. In the first group, the saturation time and desorption time were kept constant, while the major process parameters including saturation pressure, foaming temperature, and foaming time were varied according to Table 1. Desorption time was defined as the time elapse between when the samples were retrieved from the pressure vessel to when they were foamed. The purpose of this group of experiments was to identify the relationship between the pore size and the major foaming process parameters. In the second group of experiments, the effects of the saturation time and desorption time were explored. Table 2 shows the parameters used in the second group of the experiments.

After foaming, the relative density of the samples was measured according to ASTM standard [21]. Foamed samples with the lowest relative density were chosen to apply ultrasound (Model VC750 from Sonics Concept, Inc.). The ultrasonic processor had a frequency of 20 kHz and a maximum power of 750 W. The samples were held with a fixture in distilled water. The water container was located on a positioning table that was computer controlled (MAXNC 10 from MAXNC, Inc.). The sonotrode was placed 2 mm above the sample surface. Pulsed ultrasound was used with a 1:9 on and off ratio and the average electrical power was maintained at 100 w. The total ultrasound treatment time was 60 s. The water temperature was 21 °C.

2.3. Sample characterization

FEI Siron XL 30 EDAX EDS scanning electronic microscope (SEM) was used for microstructure characterization. Image processing software, *ImageJ*, from the NIH website was used to analyze the pore size distribution. The pore size was measured from the SEM images as the Feret's diameter, i.e., the greatest distance possible between any two points along the boundary of the pore.

Table 1
Parameters in the first group foaming experiments

Variable	Values
Saturation pressure (MPa)	3, 4, 5
Saturation time (h)	165
Desorption time (min)	60
Foaming temperature (°C)	100, 130, 140, 150
Foaming time (s)	5, 20, 60

Table 2
Parameters in the second group foaming experiments

Variable	Values
Saturation pressure (MPa)	5
Saturation time (h)	3.5, 30
Desorption time (min)	10–50
Foaming temperature (°C)	100
Foaming time (s)	10

3. Results and discussion

3.1. CO_2 saturation and desorption of PLA

The CO_2 saturation results of PLA samples are shown in Fig. 1. The equilibrium CO_2 concentration in the polymer samples was 11%, 15% and 21% for saturation pressures of 3, 4, and 5 MPa, respectively. Despite the difference in saturation pressures, all the samples reached the equilibrium in less than 20 h. The saturation results suggest that the solid-state foams can be produced in a relatively short time frame. Fig. 2 shows the results from the saturation and desorption studies with a sample saturated at 5 MPa. It is seen that the gas concentration started to decrease at a faster rate once it was taken out the pressure vessel. Within six hours, the gas concentration decreased from 20% to about 8%. This indicates that the desorption time could be a significant factor affecting the foaming process. Samples foamed at different desorption times could have different relative density since the gas concentration of the saturated samples could be dramatically different. Therefore, desorption time should be carefully controlled to obtain consistent foaming results.

3.2. The effects of foaming parameters

A statistical analysis was conducted on the main effects and the second order interaction effects of the three major foaming process parameters. Table 3 shows the results of an *F*-test using the standard least squares regression algorithm. Based on the *F*-test results, it can be determined that the main effects of the saturation pressure and foaming temperature have significant effects on the relative foam density. The main effect of the foaming time is insignificant. However, the interaction effect of the foaming temperature and foaming time is significant.

The main effects of these three parameters are plotted in Fig. 3. In general, foaming temperature has the most significant effect on the relative density. The higher the foaming temperature is, the lower the relative density will be. As the saturation pressure increases, the relative density decreases. When the foaming time increases, the relative density also increases. The interaction effects of these three parameters are plotted in Fig. 4. The interaction of the foaming temperature and time shows strong effect, which means they have to be considered jointly to achieve a low