



Porous poly(L-lactic acid) sheet prepared by stretching with starch particles as filler for tissue engineering



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ABSTRACT

Porous poly(L-lactic acid) (PLLA) sheets were prepared by uniaxial stretching PLLA sheets containing starch filler. Here, the starch filler content, stretching ratio, stretching rate and stretching temperature are important factors to influence the structure of the porous PLLA sheets, therefore, they have been investigated in detail. The pore size distribution and tortuosity were characterized by Mercury Intrusion Porosimetry. The results revealed that the porosity and pore size enlarged with the increase of the starch filler content and stretching ratio, while shrank with the rise of stretching temperature. On the other hand, the pore structure almost had no changes with the stretching rate ranging between 5 and 40 mm/min. In order to test and verify that the porous PLLA sheet was suitable for the tissue engineering, the starch particles were removed by selective enzymatic degradation and its in vitro biocompatibility to osteoblast-like MC3T3-E1 cells was investigated.

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1. Introduction

Poly(L-lactic acid) (PLLA), as a synthetic resorbable polymer, has been approved by the US Food and Drug Administration (FDA) for direct contact with biological fluids due to its biocompatibility (Rasal, Janorkar, & Hirt, 2010). Therefore, the PLLA products have been tailored for many applications in the biomedical field due to its biocompatibility, such as sutures, pins, screws, drug delivery systems, and scaffolds for bone tissue engineering. In the development of scaffolds for tissue engineering, it is recognized that scaffolds should be porous, biocompatible, bioactive, easy to manufacture, and with appropriate mechanical properties (Hutmacher, 2000). A lot of methods have been used to fabricate the porous PLLA as scaffolds, such as salt leaching (Kothapalli, Shaw, & Wei, 2005), phase separation (Chen, Tu, & Tsay, 2010), gas foaming (Nofar & Park, 2014), emulsion freeze-drying (Haaparanta et al., 2010), emulsion solvent evaporation (Shi et al., 2009; Shi, Sun, & Gan, 2011), selective enzymatic degradation (Liu, Li, Garreau, & Vert, 2000; Wang, Fan, & Hsiue, 2005) and rapid prototyping (Tanodekaew, Channasanon, Kaewkong, & Uppanan, 2013), or a

combination of some of them. However, PLLA scaffolds fabricated by above methods are difficult to engineer clinically useful tissues and organs, which are attributed to the fatal drawbacks of uncontrollable porous structure or the introduction of a toxic solvent, except for the rapid prototyping. Therefore, exploring new methods for the fabrication of porous materials remains a challenge.

Gore-Tex, made from polytetrafluoroethylene (PTFE) by W.L. Gore, is the first porous membrane made by stretching method in the year of 1976 (Gore & Newark, 1976, 1980). In recent years, the most developed porous polymers formed with membrane stretching are PTFE, polypropylene (PP), polyethylene (PE) and the inorganic fillers have been added to the polymers to form microporous sheets easily. Mizutani et al. prepared microporous PP sheets by biaxial stretching PP sheets containing CaCO₃ filler (Nago, Nakamura, & Mizutani, 1992), and the dependency of the sheet properties on the particle size of filler (Nago et al., 1992), filler content (Mizutani, Nakamura, Kaneko, & Okamura, 1993), and stretching ratio (Nago & Mizutani, 1996, 1998; Nakamura, Kaneko, & Mizutani, 1993) have been studied. The results revealed that the size of the CaCO₃ particles did not so much affect the porosity but evidently affected the maximum pore size (D_{max}) and pore size distribution (Mizutani et al., 1993; Nago et al., 1992). While the D_{max} also enlarged with the increase of filler content due to that the PP phase could be stretched much more with decreasing PP

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content at the same stretching ratio (Mizutani et al., 1993; Nago & Mizutani, 1994). The pore size and porosity reasonably increased while the sheet thickness decreased with increasing the stretching ratio. After removing the filler particles, the porosity increased when the stretching ratio was smaller, then slightly decreased when the stretching ratio was bigger, it was due to compaction of the fibrous PP texture caused by the removal of the filler particles as a spacer (Nago & Mizutani, 1998). At the same time, the mechanical properties also could be controlled by adjusting stretching ratios in machine and transverse directions. Also, the PP porous sheets have been prepared by combining a specific crystalline morphology followed by annealing and stretching (Saffar, Carreau, Aji, & Kamal, 2014). From the above mentioned, the features of the stretching method can be concluded as follows: (1) easy controllability of pore size and porosity by adjusting stretching ratios, filler type and content; (2) adjustable mechanical properties by balance the stretching ratios in machine and transverse directions in biaxial stretching; (3) uniform stretchability is very easy to realize for polymeric materials (Mizutani et al., 1993).

While, for the PLLA stretching behavior, there have been several studies mostly focused on the changes of crystalline morphology (Xie et al., 2012), phase behavior (Mulligan & Cakmak, 2005; Stoclet, Seguela, Lefebvre, & Rochas, 2010), dimensional stability (Aou, Kang, & Hsu, 2005), mechanical properties (Yu, Liu, Xie, Chen, & Li, 2008), and enzymatic degradation (Rangari & Vasanthan, 2012) of the PLLA films during the uniaxial or biaxial stretching process under different conditions. Up to now, there has been little research implemented on the preparation of porous PLLA sheets by stretching method. In this study, the starch was used as filler, and the porous PLLA was prepared by stretching method. The effects of filler content, stretching ratio, stretching rate, stretching temperature, and the removal of starch filler on the porous structure of PLLA were investigated in detail. Meanwhile, the *in vitro* biocompatibility of the porous PLLA sheet was also estimated.

2. Experimental

2.1. Materials

The PLLA (Grade 4032D, the melting temperature $T_m = 169^\circ\text{C}$, the glass transition temperature $T_g = 62^\circ\text{C}$) used in this work was a commercially available product from Natureworks LLC (USA). It exhibited a weight-average molecular weight of $2.07 \times 10^5 \text{ g mol}^{-1}$ and polydispersity of 1.74 as determined by gel permeation chromatography (GPC). Normal corn starch was provided by Changchun Jincheng Corn Development Co., Ltd., Da Cheng Group (China), the mean particle size of the starch was $12.5 \pm 3.0 \mu\text{m}$. The thermostable α -amylase and glucoamylase was purchased from the Jiangsu Ruiyang Biotech Co., Ltd (China).

2.2. Preparation of the porous PLLA sheets

Before processing, PLLA and starch were dried at 80°C in a vacuum oven for 24 h. The drying was necessary to ensure that the starch could not be plasticized by water; therefore, the starch could keep the original particles shape during the processing. PLLA/starch composites with a series of weight ratios (80/20, 70/30, 60/40, 50/50, 40/60; wt/wt; marked as LS-20, LS-30, LS-40, LS-50, LS-60) were prepared by using a torque rheometer (XSS-300, Shanghai Kechuang Rubber Plastic Mechanical Equipment Co., Ltd., China). The melt compounding was performed at 175°C and a screw speed of 50 rpm during a total mixing time of 8 min, until the viscosity had reached a nearly constant value. After mixing, all the samples were cut into small pieces and then were hot-pressed at 180°C for 3 min using a frame with thickness of 1 mm, then followed by

cold-press at room temperature to form the base sheets for stretching. The compression molding steps were carried out carefully in order to obtain the same treatment for every sample.

The sheets were stretched with the aid of a tensile-testing machine (Instron-1121, USA) at a crosshead speed of 5 mm/min. The tensile-testing machine was equipped with a high-low temperature environmental chamber. Sheet stretching was carried out at 60°C , which was closed to the glass transition temperature of PLLA. The samples with gauge dimensions of $4 \text{ mm} \times 20 \text{ mm} \times 1 \text{ mm}$ (dumbbell-shaped) and $20 \text{ mm} \times 50 \text{ mm} \times 1 \text{ mm}$ (rectangle) were used. The sheets were kept in the environmental chamber for 3 min under the preset temperature for equilibrium. Various stretching ratios were performed for different PLLA/starch ratio, thus, various porous PLLA sheets were acquired. Meanwhile, the stretching rate of 5–40 mm/min and the stretching temperature of 60 – 85°C were also carried out to test the influence of stretching rate and stretching temperature on the porous structure of PLLA. Due to the specificity of the thermostable α -amylase and glucoamylase, the starch filler could be removed from the composites while the PLLA matrix was retained. Thus different kinds of porous morphologies before and after removing the starch filler were acquired. The enzymatic degradation of the starch filler was carried out in phosphate buffer solution (pH=5.5) containing 2 mg/mL of thermostable α -amylase and 2 mg/mL of glucoamylase at 50°C with shaking at 130 rpm. Here, the mixed enzyme solution of the thermostable α -amylase and glucoamylase was used to shorten the degradation time. The phosphate buffer solution was periodically updated, and the starch filler could be removed completely within a week.

2.3. Characterization and measurements

2.3.1. Scanning electron microscopy (SEM)

The morphology of the porous PLLA was observed using a field emission scanning electron microscopy (SEM; XL30 ESEM FEG, FEI Co., Eindhoven, The Netherlands) at an accelerating voltage of 10 kV. The samples after tensile testing were immersed in liquid nitrogen for about 3 min, and then broke off. The cryo-fractured surfaces of the samples before and after removing the starch filler were coated with a thin layer of gold to acquire the SEM micrographs, therefore, the dispersion of the starch particles in the PLLA matrix and the porous morphology of the PLLA sheets before and after removing the starch filler were recorded.

2.3.2. Porosity

The porosity of the sheets was calculated by using the apparent density of the base sheets and the porous sheets obtained. Apparent density of the base sheet (ρ_0) and the porous PLLA sheet (ρ) formed by stretching before removing the starch were estimated by buoyancy method in water. Apparent density of the porous PLLA sheet (ρ) formed by stretching and selective enzymatic degradation was estimated by the basic definition of density. The samples with gauge dimensions of $20 \text{ mm} \times 10 \text{ mm} \times 1 \text{ mm}$ were used. At least three specimens with the same size were tested for each sheet. The porosity of the porous sheet was calculated by the following equation:

$$\text{Porosity}(\%) = (1 - \rho/\rho_0) \times 100\% \quad (1)$$

where, for the porous PLLA sheet before removing the starch filler, the ρ_0 is the density of the PLLA/starch composites, after removing the starch filler, the ρ_0 is the density of the PLLA.

2.3.3. Mercury Intrusion Porosimetry (MIP)

The average pore size and pore size distribution of the porous sheets were measured by MIP (AutoPore IV 9500, Micromeritics,

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