Available online at www.sciencedirect.com
ScienceDirect
Acta Biomaterialia 3 (2007) 531–540
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

In vitro and in vivo degradability and cytocompatibility of poly(L-lactic acid) scaffold fabricated by a gelatin particle leaching method

Yihong Gong, Qingliang Zhou, Changyou Gao *, Jiacong Shen

Department of Polymer Science and Engineering, Zhejiang University, Key Laboratory of Macromolecule Synthesis and Functionalization, Ministry of Education, Hangzhou 310027, China

Received 4 June 2006; received in revised form 7 October 2006; accepted 14 December 2006

Abstract

Porous poly(L-lactic acid) (PLLA) scaffolds fabricated by a gelatin particle-leaching technique have good mechanical property and cytocompatibility, as demonstrated by a previous in vitro study. Here we investigate further the in vitro degradation of the scaffolds in terms of weight loss, water uptake, weight-average molecular weight, thermal behavior and morphology during a 39 week period in phosphate-buffered saline. The water uptake decreased dramatically during the initial stage due to release of the remaining gelatin, and then increased slightly with degradation time. The weight-average molecular weight decreased linearly as a function of time, while the crystallinity steadily increased with slightly decreased melting temperature. After degradation, many defects and big holes were seen in the scaffolds by scanning electron microscopy. Cartilage regeneration and scaffold disappearance in vivo were compared by implanting the construct into nude mice for 30–120 days. While the scaffolds maintained their intact pore structure after 23 weeks of degradation in vitro, they almost disappeared in vivo at the same time, implying a faster degradation rate in vivo. By 120 days after implantation, the scaffolds were hardly seen in the newly formed cartilage-like tissue. The regenerated cartilages could not maintain their predesigned shape after a long period of in vivo culture due to the weakening of the mechanical strength of the constructs as a result of PLLA degradation. The regions occupied initially by PLLA scaffold were filled later by collagen type II secreted by the chondrocytes, but with no evident basophilic proteoglycan

Keywords: Poly(L-lactic acid) (PLLA); Porous scaffolds; Degradation; Tissue engineering

1. Introduction

In many tissue engineering applications, scaffolds with a porous structure are often desirable for the purpose of cell infiltration, extracellular matrix (ECM) production, vascularization and tissue ingrowth [1–4]. Porous poly(L-lactic acid) (PLLA) scaffolds have been widely used to guide the regeneration of cartilage [5–7], bone [8], skin [9], ligament [10], bladder [11] and liver [12] in a tissue engineering manner. It has been processed into porous scaffolds by a variety of methods, such as porogen leaching [13–17], phase separation [18–20] and electrospinning [21,22].

The success of aliphatic polyesters in tissue engineering relies largely on their degradability and biocompatibility, as well as their good processibility and mechanical properties. This is very promising since the foreign materials will eventually be removed from the body as the new tissues are formed [1–3]. In principle, the degradation rate of the scaffold should match the rate of tissue formation. Therefore, the degradation behavior of a scaffold has crucial impact on the long-term performance of a tissue-engineered cell/scaffold construct [23]. PLLA has been known to degrade by simple hydrolysis of the ester bonds into lactic acid, which is eventually removed from the body by the normal

* Corresponding author. Fax: +86 571 87951948.
E-mail address: cygao@mail.hz.zj.cn (C. Gao).

1742-7061/$ - see front matter
doi:10.1016/j.actbio.2006.12.008

metabolic pathways [24]. Several factors, such as polymer molecular weight, polydispersity [25], crystallinity [26], shape and morphology [27], are known to affect the rate of hydrolytic degradation of PLA. Other factors, such as pH, ionic strength, temperature and buffering capacity of the medium in which the degradation occurs, also influence the degradation kinetics [28].

In a previous study we have reported that porous PLLA scaffolds with controllable microstructure and mechanical performance can be fabricated by a gelatin particle leaching method [16,29]. A notable feature of the process is that the gelatin particles are bonded together to form a three-dimensional assembly through a water vapor treatment before the polymer solution is cast. In this paper, we shall focus on the degradation behavior of this porous scaffold in vitro in PBS at 37 °C. Moreover, in vivo implantation of the chondrocytes/scaffold constructs into nude mice are performed to assess the cytocompatibility and degradability of the scaffolds. These properties are essential to the evaluation of the applicability of the scaffolds in guiding the regeneration of cells and tissues.

2. Materials and methods

2.1. Materials

The PLLA ($M_n = 99{,}000$, $M_w = 212{,}000$) was synthesized using the method described previously [30]. Gelatin and 1,4-dioxane were obtained from Shanghai Chemical Industries Co. Ltd. A 10% (w/v) PLLA/1,4-dioxane solution was prepared for further use.

2.2. PLLA scaffold fabrication

PLLA was fabricated into porous scaffolds by a porogen-leaching technique with gelatin particles as the porogen [16]. Briefly, the gelatin particles (280–450 μm) sieved from raw gelatin were added into a glass mold, a cylindrical vial with a diameter of 22 mm. The vial was tapped gently to make a flat surface of the gelatin particles, followed by a slight pressing. The vial was then carefully moved into a vessel filled with saturated water vapor at 70 °C. After 1.5 h, the vial was removed, and the protuberant top surface caused by the swelling of the gelatin particles was pressed to be flat immediately. After cooled to room temperature, 0.8 ml of PLLA/1,4-dioxane solution with a concentration of 0.1 g ml^{-1} was cast dropwise onto the gelatin particles assembly. The mold was then maintained under a low pressure of 0.07–0.08 MPa to evolve the trapped air bubbles, then the pressure was released to make the polymer solution fill the cavities in-between the gelatin particles. The mixture was frozen at −25 °C for 3 h, then freeze-dried to remove the 1,4-dioxane. The PLLA porous scaffolds were obtained by leaching the gelatin assembly in 100 ml of deionized water at 70 °C for 10 h.

2.3. In vitro degradation of the PLLA scaffolds

The scaffolds were cut into a $2 \times 4 \times (8–10)$ mm shape with a razor blade. Each sample, of weight (W_0) ~ 15 mg, was immerged into 5 ml of phosphate-buffered saline (PBS) (pH 7.4; 0.8 g NaCl; 0.2 g KCl; 2.9 g $Na_2HPO_4 \cdot 12H_2O$ and 0.2 g KH_2PO_4 were dissolved in 1 l of distilled water). The samples in glass tubes were placed in a water bath at 37 °C for up to 39 weeks. Every 2 days the buffer solution was replaced with 5 ml of fresh PBS. At a given time point, three samples were taken out and rinsed with distilled water three times. The surface water was adsorbed by a filter paper and the wet weight ($W_{t,w}$) was measured immediately. These samples were subsequently air-dried at 30 °C to a constant weight (W_t) before being subjected to characterizations of molecular weight, thermal properties and morphology.

The weight loss and water uptake were defined as $(W_0 - W_t)/W_0 \times 100\%$ and $(W_{t,w} - W_t)/W_t \times 100\%$, respectively, according to Refs. [46,47]. Each result was the average of three parallel measurements, expressed as mean ± standard deviation.

2.4. Instrumental characterizations

The PLLA molecular weight was determined by gel permeation chromatography (GPC) (Waters 515). The samples were dissolved in tetrahydrofuran (THF) at a concentration of ~0.5%. THF was used as a flow phase at a flow rate of 1 ml min^{-1}. The temperature was set at 40 °C. The molecular weight and polydispersity index were obtained by referring to a calibration curve recorded from polystyrene standards (polysciences).

The thermal properties of the PLLA scaffolds, including melting temperatures and corresponding enthalpy changes, were measured by a Perkin–Elmer DSC 7 calorimeter. The samples (between 2 and 4 mg) were heated at a rate of 10 °C min^{-1}. The peak temperatures of melting endotherm were recorded as T_m. The intrinsic degree of crystallinity (X_c) was calculated by $X_c = \Delta H_m/\Delta H_m^o$, where ΔH_m is the melting enthalpy of the measured PLLA and ΔH_m^o is the melting enthalpy of 100% crystalline polymer (203.4 J g^{-1}) [31].

To observe the morphology change under a scanning electron microscope (SEM), samples with different degradation time were coated with a gold layer under a pressure of 50 mtorr for 180 s. The microstructure of the scaffolds was then observed by SEM (JSM-5510LV, Japan).

2.5. In vitro chondrocyte seeding and culture

Chondrocytes were isolated from New Zealand rabbit ears (the rabbits were sacrificed under the institutional ethical guidelines) by digesting the cartilage chips with 0.2% collagenase II (Sigma) and cultured in Ham's F-12 medium supplemented with 20% fetal calf serum (FBS), 300 mg l^{-1} glutamine, 50 mg l^{-1} vitamin C, 100 U ml^{-1} penicillin and 100 U ml^{-1} streptomycin. The cell suspension was then