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Materials Letters 62 (2008) 2029–2032
materials letters
www.elsevier.com/locate/matlet

Preparation of poly(L-lactic acid)/β-tricalcium phosphate scaffold for bone tissue engineering without organic solvent

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Received 6 September 2007; accepted 5 November 2007
Available online 17 November 2007

Abstract

A new method was developed to prepare porous composite poly(L-lactic acid)/β-tricalcium phosphate (PLLA/β-TCP) scaffold. The method was composed of pressing the preheated mixtures of PLLA, β-TCP and salt particles in a stainless steel mold, followed by the second heating and molding, and leaching pore-forming agent. The fabricated scaffold has a homogeneously interconnected porous structure with high porosity and compressive strength. The immersion of PLLA/β-TCP in simulated body fluid (SBF) and *in vitro* osteoblasts culture indicate that the porous PLLA/β-TCP scaffold possesses good abilities of bone-like apatite formation and cell adhesion. As the new method does not use any organic solvent, it offers an advantage to avoid problems associated with organic solvent residue. It has a promising potentiality in preparing porous scaffold for bone tissue engineering.

Keywords: Poly(L-lactic acid); β-Tricalcium phosphate; Composite materials; Bone tissue engineering; Porosity

1. Introduction

Calcium phosphate ceramics are confined to non-load or low-load bone repair fields due to their brittleness. An approach to overcome this limitation is the combination with polymers to yield composite materials [1–3]. Several preparation methods of fabricating porous composite scaffolds have been reported, including porogen leaching [4], thermally induced phase separation [5], freeze-extraction and freeze-gelation methods [6] and so on. However, the scaffold produced by these methods has low mechanical strength. The interfacial property between calcium phosphate and polymer is one of the major influencing factors on the mechanical properties of composite. To improve the interfacial integrity of composite and to obtain high mechanical property, surface modification of ceramics has been employed [1]. However, thus new surfactants would be introduced, besides a large number of organic solvent used to dissolve polymer. These residual organic solvent and surfactants have the potential harm to cell and tissue.

Therefore, to solve the problems of the organic solvent residual and interfacial compatibility, we developed powder mixing/compression molding/low temperature treating/particulate leaching technique (PCLP) to prepare PLLA/β-TCP composite scaffolds without organic solvent employed. Process conditions of preparing composite scaffold were investigated. To investigate the bioactivity of this scaffold, *in vitro* immersion and cell culture were also studied.

2. Materials and methods

2.1. Preparation of PLLA/β-TCP scaffold

PLLA powder (10 μm ≤ particle size ≤ 20 μm, M_W: 600 kDa) was purchased from Institute of Organic Chemistry, Chinese Academy of Sciences (Chengdu). β-TCP powder with the mean

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doi:10.1016/j.matlet.2007.11.014

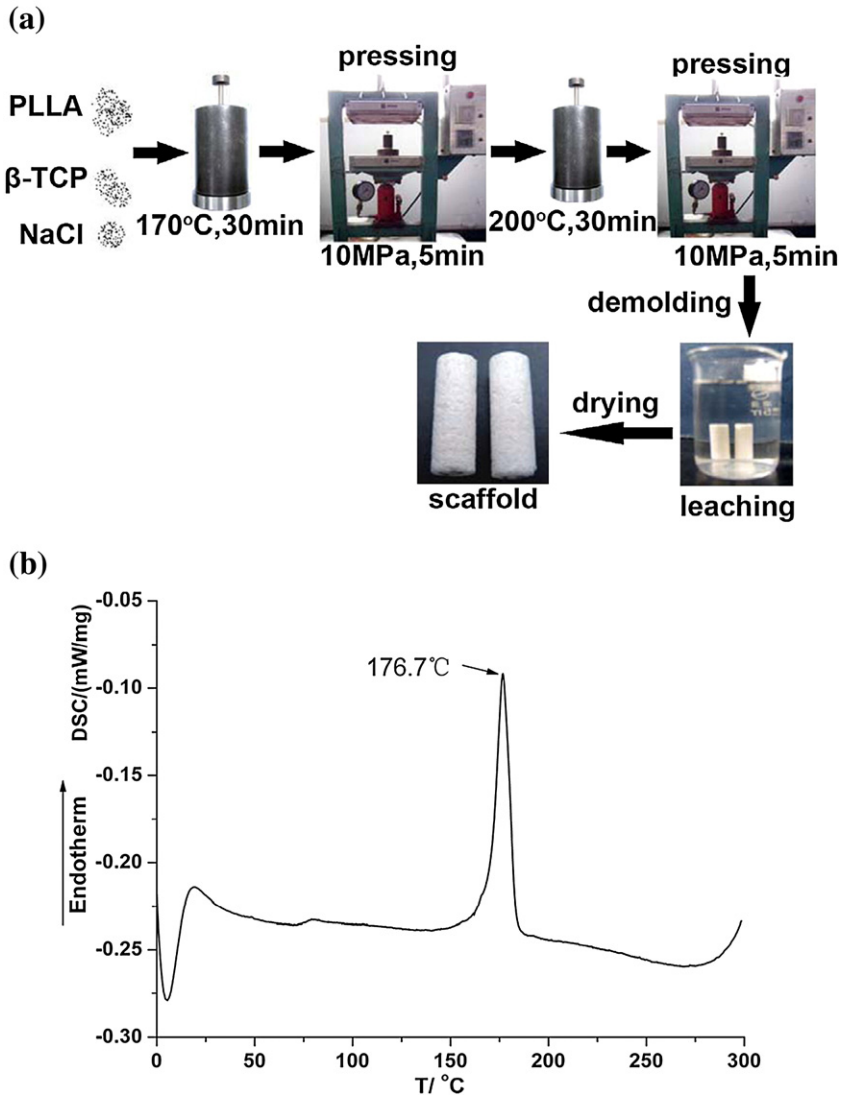

Fig. 1. Schematic graph of the whole procedure of novel PCLP method (a) and the DSC curve of PLLA (b).

particle size of 2.41 μm was prepared in our laboratory. PLLA, β-TCP fine particles and sieved sodium chloride particles (200 ≤ particle size ≤ 400 μm) were mixed by magnetic force stirrer. The schematic graph of fabricating procedure is illustrated in Fig. 1a. The mass ratios of salt particulates to compounds (PLLA and β-TCP) were 0.8:1, 1.6:1, and 2.4:1, respectively, namely the percentage of salt in the whole weight of mixture is 44.44%, 61.54%, 70.59%, respectively. Table 1 details the experiments conditions used in the preparation of PLLA/β-TCP scaffold. PLLA, β-TCP, and salt were homogeneously mixed by magnetic force stirring for 30 min and loaded into a stainless steel mold (inner diameter: 5 mm). The mold loading mixtures was heated at 170 °C (slightly lower than the melting temperature of PLLA, which was determined by differential scanning calorimetry curve shown in Fig. 1b) for 30 min, and the mixture in mold was quickly pressed at a pressure of 10 MPa for 5 min to yield solid cylinder (the ratio of height to diameter was 2:1). Next, the mold was secondly heated at 200 °C (slightly higher than the melting temperature of PLLA) for 30 min. Then the mixture in mold was quickly further pressed for the second time at a pressure of 10 MPa for 5 min to obtain high mechanical properties. The pore-forming agents were subsequently leached from the composites by soaking the composites in de-ioned water with shaking. In order to leach thoroughly the pore-forming agents, the water was replaced by fresh de-ioned water every 6 h till no precipitation was observed when silver nitrate was added into the replaced water. And then the composites were dried in a vacuum oven.

Table 1
Experiment conditions of preparing porous composite scaffold

Sample no.	NaCl: (PLLA+ β-TCP) (w/w)	PLLA: β-TCP	Compressive strength (MPa) (±S.D., $n=3$)	Porosity (%) (±S.D., $n=3$)
1	0.80	65:35	9.51 (4.04)	42.37 (3.15)
2	1.60	65:35	4.42 (0.29)	60.23 (3.01)
3	2.40	65:35	2.56 (0.22)	64.56 (0.99)
4	0.80	35:65	6.01 (0.34)	45.40 (1.28)
5	1.60	35:65	1.42 (0.01)	70.70 (2.83)
6	2.40	35:65	1.03 (0.24)	71.67 (1.87)

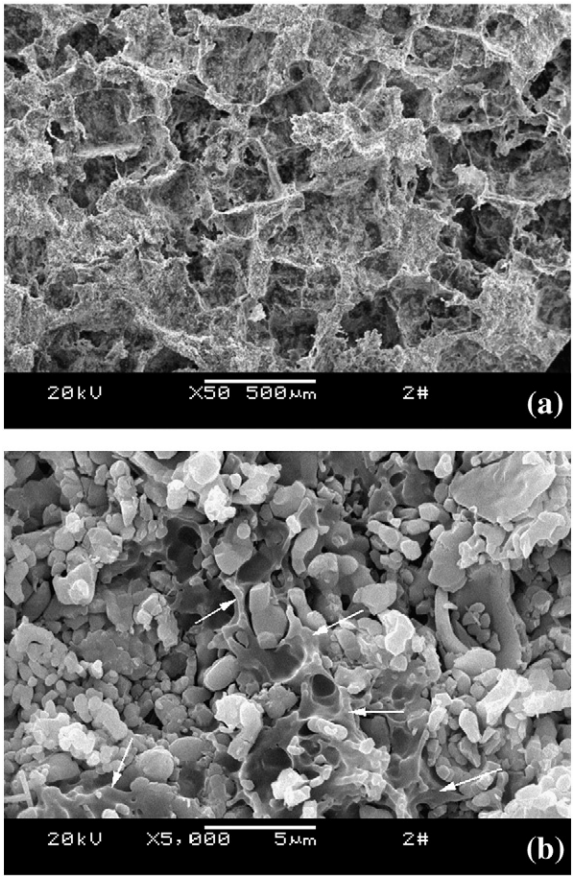

Fig. 2. SEM images of pore of composites scaffolds: section morphology (a) and the high magnification of inner pore (b). The white arrow on the photograph represents PLLA.

2.2. Characterization

The porosity of scaffold was measured by using Archimedes immersion technique. The method was described in detail in our previous literature [7]. The pore diameter and morphology of scaffold were observed by scanning electron microscope (SEM) (JSM-5900LV, Japan) after gold coating. The compressive strength of the scaffold was evaluated on an AG-10TA electronic apparatus (Shimadzu, Japan).

2.3. Bioactive and cell adhesion study in vitro

Scaffolds were immersed in 150 ml SBF prepared according to the procedure of Tadashi Kokubo [8] at 37 °C in the water bath. At the designed interval time, scaffolds were taken out and the formation of apatite was observed by SEM. Osteoblasts were cultivated in culture flasks for 2 weeks, and cell suspension (4×10^6 cells/ml) was pipetted onto the surface of scaffolds in 24-well culture plates (Falcon Co.) Cell-scaffolds were cultured in a humidified incubator at 37 °C with 5% CO_2 for 72 h. After 72 h, cell-scaffolds were taken out and observed by SEM.

3. Results and discussion

The porosity and pore size of the scaffold are important features to evaluate biomaterial properties for tissue engineering. Sufficient pore size allows maximum osteoconduction with sufficient nutrient transfer for optimal tissue growth, although there are conflicting reports on pore size in favor of new bone formation and growth [9–11]. The porosity and pore size can be controlled by the salt weight and particulate size. The porosity in Table 1 indicates that the porosity of scaffold increases with more sodium chloride particulates, which coincides with the fact that the same percentage of pore-forming agents was used. The value of the pore size determined from the SEM is similar with the particle size of the used sodium chloride. The polymer fraction also had some effects on the porosity and the pore wall structure. Lower polymer fraction resulted in more pore structures and higher porosity. The reasons may be that lower PLLA content is hard to form continuous phase to combine with β-TCP, resulting in more pore left. Therefore, the porosity and pore structure of the scaffold could be manipulated by varying the shape, weight fraction, size of the particulates and the polymer fraction.

The compressive strength of PLLA/β-TCP scaffold decreases with porosity increasing as shown in Table 1. The results further indicate that the compressive strength of scaffold with high fraction of β-TCP is lower than that with low fraction β-TCP group at the same porogen fraction. This reason is similar with the explanation for the change of porosity.

Fig. 2a exhibits interconnected pore structure of PLLA/β-TCP scaffold. Fig. 2b shows PLLA became continuous phase and reticulation, which made the β-TCP particles disperse homogeneously in the PLLA continuous phase. The interconnectivity of pores and the degree of melting of polymers were controlled depending on the treating temperature and time. To determine the appropriate temperature

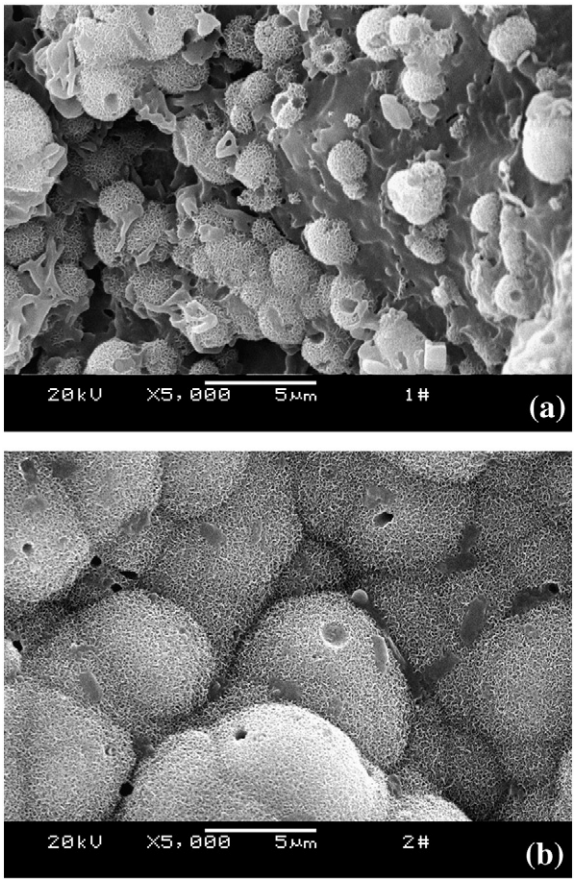

Fig. 3. SEM images of spherical crystals formed on scaffold after 1 week (a) and 2 weeks (b).

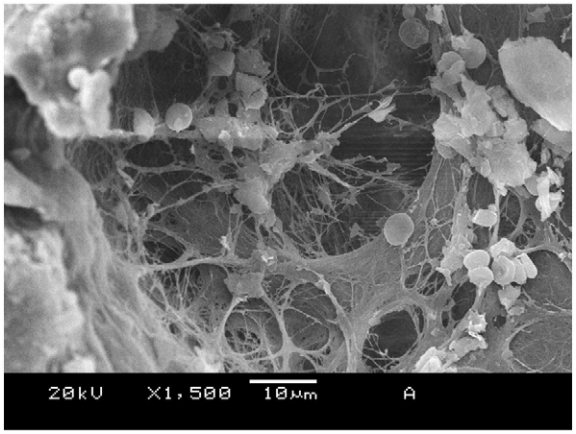

Fig. 4. SEM of cell adhered on the scaffold.

and time of heat treatment, a number of experiments according to the proprieties of PLLA (DSC was shown in Fig. 1b) were performed. From Fig. 1b, we chose 170 °C as the preheating temperature and 200 °C as the followed heating temperature. In addition to the temperature, it is important to choose the heat treating time. We chose 30 min as twice heat treating time. For the second time of heat treatment, too short would result in a lack of consolidation and too long resulted in scaffolds with closed pore structures.

Fig. 3a shows the SEM images of bone-like apatite formed on the wall of inner pore immersed after 1 week. The crystal spheres were regarded as early stage of Ca–P precipitation. Precipitation started at individual granules and the granules gradually developed on the specimen surface and inner pore after 2 weeks as shown in Fig. 3b.

Fig. 4 shows the morphological features of osteoblasts cultured on the scaffold for 3 days. The result shows that the osteoblasts attached and spread well. SEM indicates that a confluent layer of cells was present together with collagen bundles and calcified globular accretions. The cells extend filopodia to across the valley of inner and form multiple points' attachment from the surface, indicating the good cell adhesion property of scaffold.

4. Conclusions

A new method has been successfully developed to prepare PLLA/β-TCP scaffold. The scaffold has high porosity with interconnected porous structure and high compressive strength. The porous PLLA/β-TCP scaffold indicates good ability of bone-like apatite formation *in vitro* and good cell adhesion property. Such a superior characteristic of scaffold prepared by the novel method should result from the fact that the β-TCP particles could disperse homogeneously in PLLA continuous phase matrix. The melting PLLA consolidate the β-TCP particles. As the new method does not use any solvent, it offers an advantage to avoid problems associated with solvent residue.

Acknowledgements

This project was financially sponsored by the *National High Technology Research and Development Program of China* (863 Program: 2002AA326080). We thank the analytical and testing center of Sichuan University for performing the SEM observation and mechanical properties tests.

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