Formation of Porous PLGA Scaffolds by a Combining Method of Thermally Induced Phase Separation and Porogen Leaching

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ABSTRACT: A novel method for the fabrication of porous poly(L-lactide-*co*-glycolide) (PLGA) scaffolds by combining thermally induced phase separation and porogen leaching is presented in this article. Big pores with about 75–400 μm diameters in the obtained scaffolds were generated by the porogen, sucrose particles, while small pores with diameters less than 20 μm induced via phase separation. Extraction of the solvent, chloroform by ethanol at cool temperatures could reduce the scaffold toxicity. Effects of PLGA concentration, freezing temperature, volume fraction of porogen, and introduction of β-tricalcium phosphate (β-TCP) on morphology, porosity, and compressive properties of the scaffolds were systematically discussed. Results showed that the size of small pores decreased by decreasing the polymer concentration and reducing the freezing temperature, whereas the interconnectivity of the scaffolds was improved by increasing the porogen fraction. The compressive modulus and strength were significantly lowered by increasing the scaffold porosity, that is, by increasing porogen fraction, or decreasing the polymer concentration, or reducing the freezing temperature. Addition of β-TCP into the scaffolds did not influence the compressive modulus significantly but tended to decrease the compressive strength. The obtained scaffolds with diverse pore sizes would be potentially used in bone tissue engineering. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 109: 1232–1241, 2008

Key words: scaffold; PLGA; phase separation; morphology; mechanical properties

INTRODUCTION

Because of wounds and diseases, the need to repair bone defects has become more and more prevalent recently. There are many bone graft options including autografts, allografts, or xenografts, but the bone grafts are still deficient to the requirement.[1,2] Tissue engineering, as a technique to create new tissues from cultured cells in a scaffold directed by signals, has now been considered as a potential alternative for organ or tissue reconstruction. Meanwhile, biodegradable porous scaffolds, serving as transplant vehicles for cultured cells or templates to guide tissue regeneration, play an important role.[3–5]

Three-dimensional (3D) porous scaffolds based on biodegradable aliphatic polyesters, such as poly(L-lactide-*co*-glycolide) (PLGA), have been widely used in tissue engineering.[6] Highly porous scaffolds with an interconnected pore structure are needed in many cases to facilitate cell seeding, adhesion, proliferation, and eventual tissue regeneration.[7] In addition, an ideal scaffold should have suitable pore size and reliable biomechanical properties as well as biocompatibility to match the repaired tissue.[8]

A number of methodologies and technologies have been reported for preparing porous scaffolds, such as fiber bonded nonwoven,[9] phase separation,[10–12] freeze drying,[13] gas foaming,[14,15] porogen leaching,[16–18] 3D printing,[19] fused deposition modeling,[20] and electrospinning.[21] Among these methods, a commonly used one was thermally induced phase separation (TIPS)[10,12,22] by which a homogeneous biodegradable polymer solution could induce phase separation via reducing the temperature. The phase separation might occur in the process of liquid–liquid or solid–liquid demixing. In the first case, a uniform polymer solution was separated into a polymer-lean liquid phase and a polymer-rich liquid phase, whereas the polymer-lean phase would subsequently grow and coalesce to form pores in scaffolds. On the other hand, when the temperature was low enough, solid–liquid demixing would happen to form frozen solvent and the concentrated polymer phase. Then, the pores would be developed by removal of the frozen solvent.[11]

The approach of porogen leaching to prepare porous scaffold has been widely used before. The

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porosity, pore size, and pore morphology of the prepared scaffolds could be easily controlled by adjusting the fraction, size, and shape of the porogen. Additionally, there have been several methods by combining the porogen leaching with others to prepare the porous scaffolds. Ma et al. have made good achievements in 3D nanofibrous poly(L-lactide) scaffolds by sugar or gelatin particles leaching accompanied with freeze-drying.[23,24] Ding et al. reported an injection molding/porogen leaching method to fabricate biodegradable 3D porous scaffolds with external anatomical shape accorded with the repaired tissue.[25] Scaffolds prepared by combining methods could have large as well as small pores. Interconnected pores larger than the cells for the repaired tissues were essential for the infiltration of the cells into the scaffold, whereas smaller pores could help the exchange of cellular nutrients and waste products.[26]

In this article, a novel method of TIPS/porogen leaching was developed to prepare PLGA scaffolds with interconnected bigger and smaller pores. Effects of polymer concentration, freezing temperature, volume fraction of porogen, and introduction of β-tricalcium phosphate (β-TCP) on the morphology, porosity and compressive properties of the scaffolds were discussed systematically.

EXPERIMENTAL

Materials

PLGA (70/30) was kindly supplied by Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, China, and purified by dissolving in chloroform and precipitating in ethanol. The weight-average relative molecular mass ($\overline{M}_w = 2.55 \times 10^5$) and polydispersity ($\overline{M}_w/\overline{M}_n = 1.62$) of PLGA were determined using gel permeation chromatography (Agilent 1100) in tetrahydrofuran. β-TCP (particle size in the micrometer scale, $d_{50} = 3.22$ μm) was purchased from National Engineering Research Center for Biomaterials, Sichuan University, China. Sucrose particles sieved into 200–400 μm were used as porogen. All the chemicals used were of analytical quality throughout the study.

Scaffold preparation

PLGA was dissolved in chloroform under a magnetic stir at room temperature with desired concentrations (0.12 g/mL, 0.15 g/mL, or 0.20 g/mL). A certain amount of the sieved sucrose particles (volume fraction percentage of the total volume of PLGA and porogen) were added into the polymer solution, and the mixture was stirred with a glass-bar to form a pastelike dope of PLGA/porogen/solvent. A given amount of the dope was put into a customized cylindrical Teflon mold and pressed with a clamp. Five minutes later, the mold with dope was transferred into a refrigerator at preset freezing temperatures (4°C, −18°C or −60°C) for 12 h to induce phase separation. Then, the frozen cylindrical specimen was removed from the mold and put into ethanol, which was precooled at −18°C (4°C for the specimen was frozen at 4°C) to extract chloroform. After 12 h, the specimen was taken out of ethanol and left in the air for about 4–6 h for stabilization and ethanol evaporation. The cylindrical specimen was again put into gently stirred distilled water for 24 h to leach out the sucrose. Finally, the obtained porous PLGA scaffold in a diameter of about 6 mm and a height of about 12 mm was dried in the air for 24 h and in vacuum for another 24 h to further eliminate the residual water and ethanol. For preparation of PLGA/β-TCP scaffolds, a certain amount of β-TCP (mass percentage of PLGA and β-TCP) was added into the clear PLGA solution and dispersed under ultrasonic stir for 45 min before the porogen was added. Both PLGA and PLGA/β-TCP scaffolds were stored in a desiccator for further uses.

Determination of porosity of the scaffolds

A modified liquid replacement method based on the reported ref. 27 was employed to determine the porosity of the prepared scaffold. A scaffold with the mass of m_s was put into a weighing bottle, which contained a certain amount of ethanol and weighed as m_a. After the interconnected pores were imbued with ethanol under vacuum, the scaffold was removed and then put into a density bottle which had been full of ethanol. After adding the scaffold, a certain amount of ethanol equaled to the scaffold overflowed from the density bottle. The masses of the density bottle before and after holding the scaffold were designated as m_1 and m_2, respectively. After the scaffold being removed, the weighing bottle with the residual ethanol was weighed as m_b. Then, the scaffold porosity could be determined according to the following equations:

$$\text{Porosity} = a/b$$

$$a = m_a - m_b - m_s$$

$$b = m_1 - [m_2 - (m_a - m_b)]$$

SEM analysis

The specimens were fractured after frozen in liquid nitrogen, and then the fresh-fractured section was

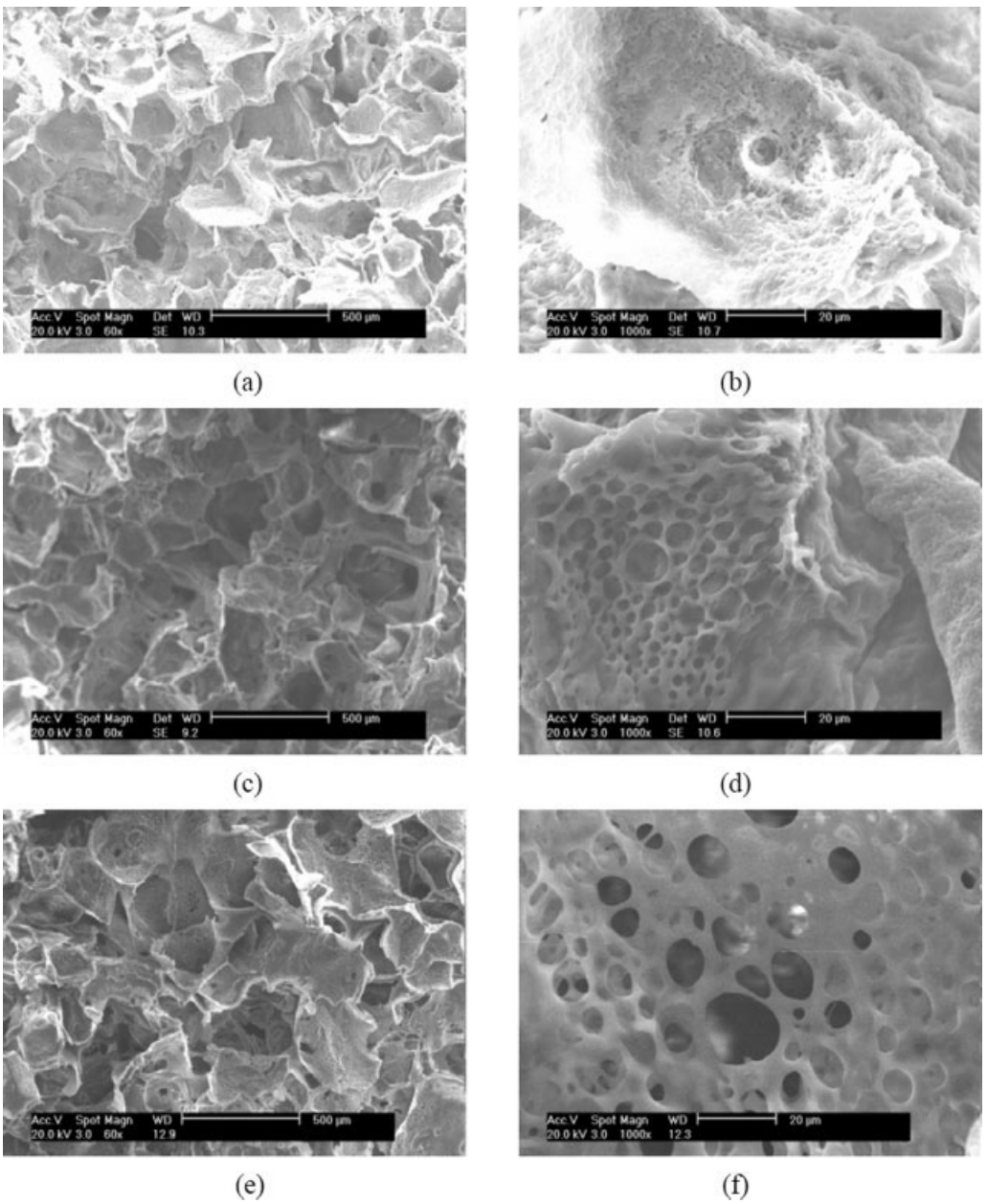

Figure 1 SEM micrographs (left 60×, right 1000×) of PLGA scaffolds prepared from 0.12 g/mL (a,b), 0.15 g/mL (c,d), and 0.20 g/mL (e,f) polymer solutions, respectively, with 90% porogen at −60°C for freezing.

observed under a Philips XL30 scanning electron microscope after gold coating.

Mechanical properties

Compressive properties of the porous scaffolds were measured at ambient temperature using M350-20KN tensile testing machine. The cylindrical porous scaffolds in ϕ6 mm × 12 mm were used as the testing specimens. The crosshead was run at 1 mm/min. The compressive modulus was determined by the linear slope of 0–4% strain in the compressive stress–strain curves, and the compressive strength was referred to as the compressive stress at 10% of strain according to the refs. 2, 28. All results were the averages of at least three to five measurements.

RESULTS AND DISCUSSION

It is ideal for a tissue-engineering scaffold possessing interconnected pores and suitable pore size to satisfy the ingrowth of cells. In this study, porogen leaching was used to form big pores with diameters in about several hundred microns in the PLGA scaffolds, in which the big pore structure and size could be adjusted by the porogen, sucrose. The TIPS method

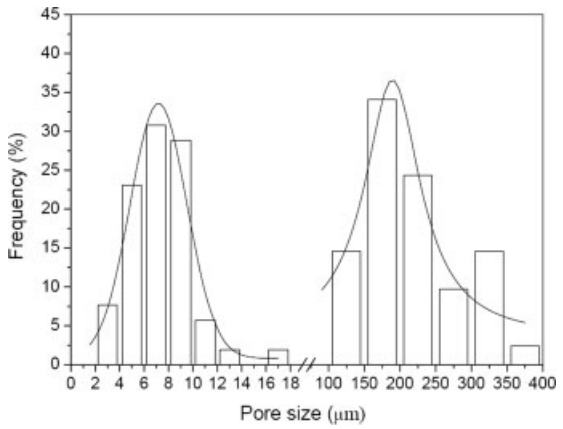

Figure 2 Pore size distribution in the PLGA scaffold prepared from a 0.20 g/mL polymer solution with 90% porogen at −60°C for freezing.

was also used to develop small pores in the range of several ten microns on the wall of the big pores, which would conduce to the exchange of nutrition and cellular waste products. It was assumed that the liquid–liquid phase separation generated by temperature reducing was mainly influenced by polymer concentration, freezing temperature, and volume fraction of the porogen. These factors might also have effects on morphology, porosity, and mechanical properties of the scaffolds and are systematically discussed in the following paragraphs.

Morphology

Polymer concentration

Figure 1 indicates the effect of the polymer concentration on the morphology of the PLGA scaffolds

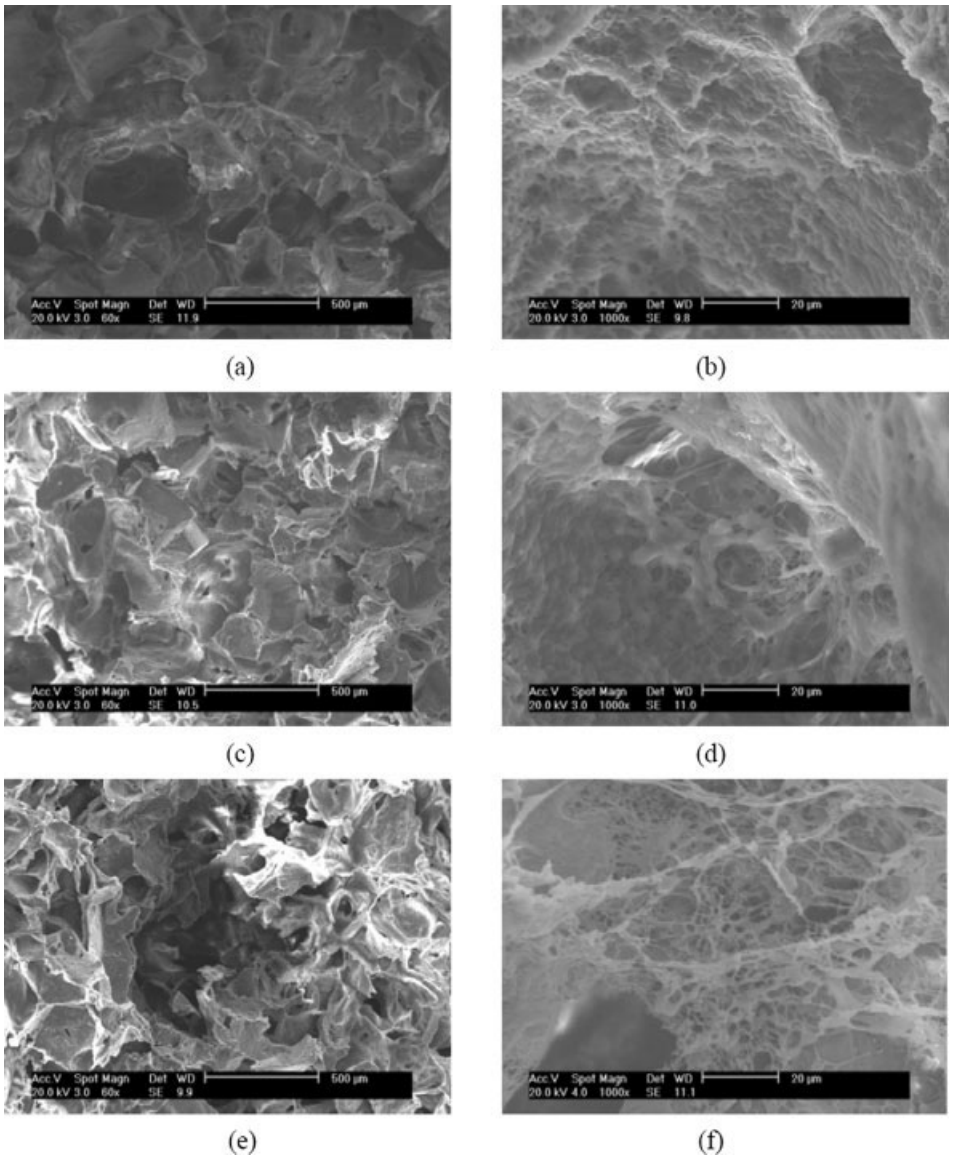

Figure 3 SEM micrographs (left 60×, right 1000×) of PLGA scaffolds prepared from a 0.20 g/mL polymer solution with 85% porogen at different freezing temperatures: (a,b) 4°C; (c,d) −18°C; (e,f) −60°C.

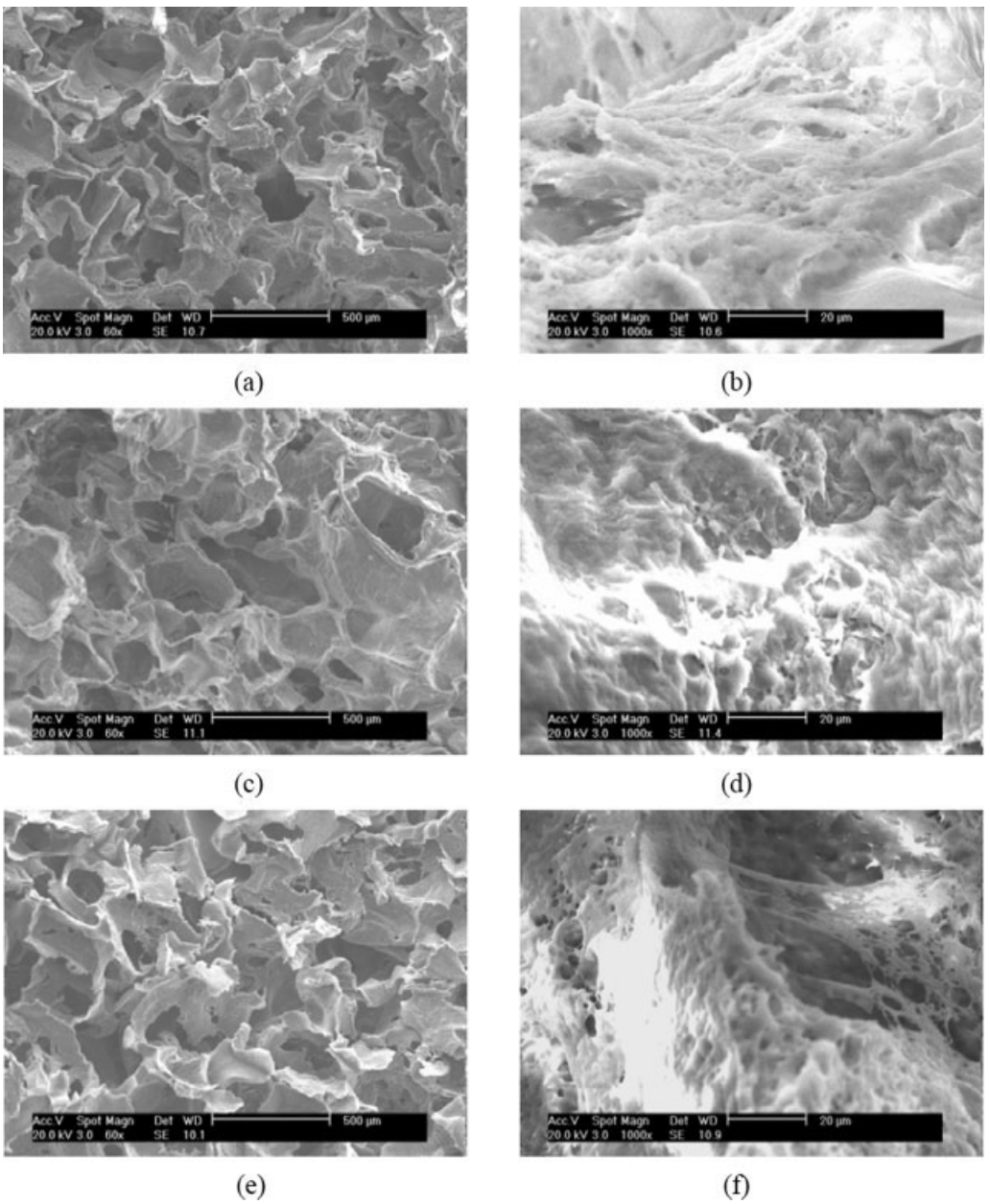

Figure 4 SEM micrographs (left 60×, right 1000×) of PLGA scaffolds prepared from a 0.15 g/mL polymer solution with 80% (a,b), 85% (c,d), and 90% (e,f) porogen at −18°C for freezing.

with 90% porogen at −60°C for freezing. The typical pore size distribution in the scaffolds from the 0.20 g/mL solution is shown in Figure 2. It could be clearly seen that the two distribution peaks of the smaller and bigger pores appeared at around 2–18 μm and 75–400 μm, respectively. It was assumed that the TIPS process by annealing the samples to the freezing temperature led to the formation of small pores with a distribution less than 20 μm [Fig. 1(b,d,f)], while the removal of porogen gave rise to big pores showing no clear differences in morphology [Fig. 1(a,c,e)]. As the concentration of the PLGA solution increased from 0.12 g/mL to 0.20 g/mL, the interconnected small pore size showed an increasing tendency with a broader distribution [Fig. 1(b,d,f)]. When the polymer concentration was 0.15 g/mL, honeycomb-like pores with average size around 3–4 μm were generated [Fig. 1(d)]. It suggested that more small pores with smaller sizes were obtained from the lower polymer concentration solution. It was seemingly evident that when the phase separation occurred in a lower polymer concentration, the volume ratio of polymer-lean phase to polymer-rich phase would be higher, providing more spaces and opportunities for the formation of small pores. The kinetics of phase separation was also faster than that

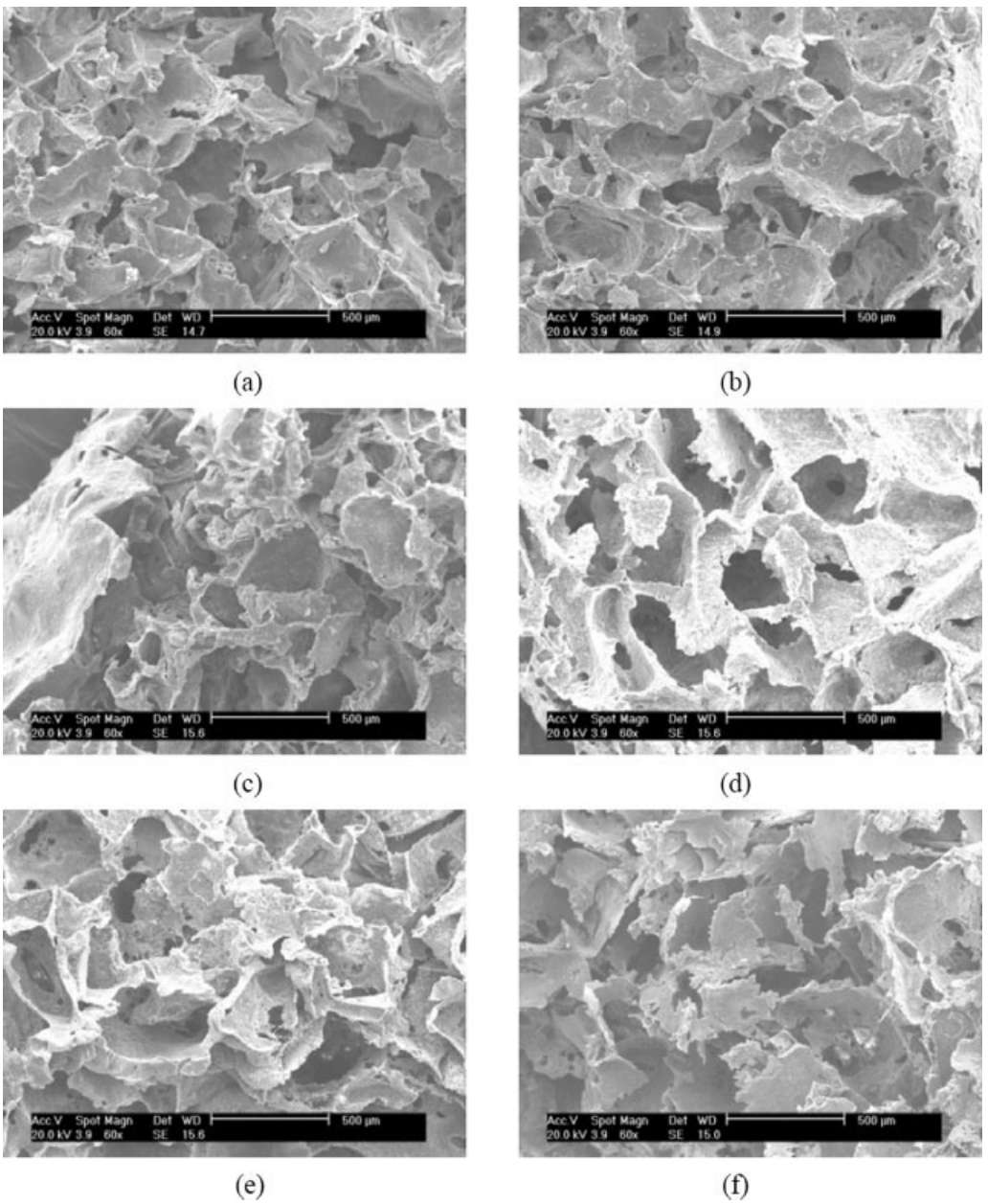

Figure 5 SEM micrographs (60×) of PLGA scaffolds after introduction of β-TCP in mass percentages of 10% (a), 20% (b), 30% (c), 40% (d), 50% (e), and 60% (f), respectively, prepared from a 0.15 g/mL polymer solution with 80% porogen at −18°C for freezing.

of the coalescence in the polymer-lean phase, hence the small pores with a larger amount and smaller sizes were produced in the scaffolds.

Freezing temperature

Figure 3 shows that the SEM micrographs of PLGA scaffolds prepared from a 0.20 g/mL polymer solution with 85% porogen at different freezing temperatures. Small pores in several microns clearly appeared on the wall of the big pores [Fig. 3(b,d,f)]. The small pore size in the scaffolds prepared at different freezing temperatures mainly distributed from 1 to 10 μm and became smaller as the freezing temperature decreased. Kim et al.[29] reported that the rate of pore size development decreased dramatically at the lower quenching temperature during fabrication of porous PLLA scaffolds via TIPS, which was especially evident in the high-polymer solution concentration. When the freezing temperature decreased, the PLGA solubility in chloroform dropped, which would result in faster phase separation of the homogeneous polymer solution, that is, the kinetics

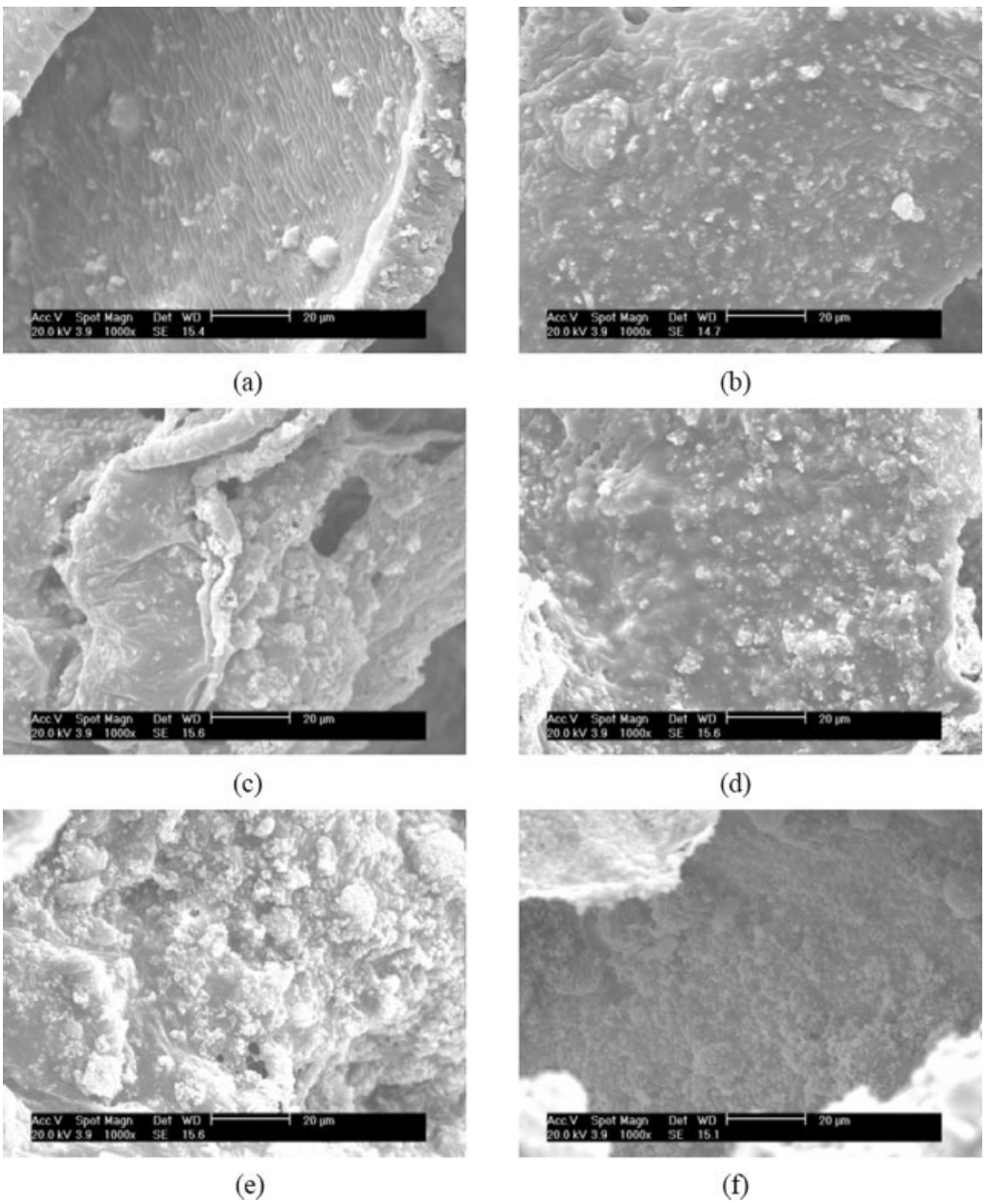

Figure 6 SEM micrographs (1000×) of PLGA scaffolds after introduction of β-TCP in mass percentages of 10% (a), 20% (b), 30% (c), 40% (d), 50% (e), and 60% (f), respectively, prepared from a 0.15 g/mL polymer solution with 80% porogen at −18°C for freezing.

of phase separation became faster with a lower freezing temperature. The subsequent growth and coalescence of the polymer-lean phase would develop to form the smaller pores in scaffolds.

After the phase separation, the scaffolds were then subjected in an extra amount of precooled nonsolvent, ethanol. Chloroform could be extracted by ethanol. The space occupied by chloroform was filled with ethanol. Extracting chloroform by ethanol would possibly reduce the toxicity of scaffolds for further uses. This work will be carried out in our further studies.

Porogen fraction

SEM micrographs of the PLGA scaffolds produced from a 0.15 g/mL polymer solution containing 80, 85, and 90% porogen, respectively, at −18°C for freezing are shown in Figure 4. Results of the lower magnification micrographs [Fig. 4(a,c,e)] exhibited that the interconnectivity of the scaffold was improved as the volume fraction of porogen increased. The distribution of the big pore size in the scaffolds was broad from 75 to 400 μm and mainly in the range from 150 to 300 μm, which means that the pro-

TABLE I
Porosities and Compressive Properties of the PLGA and PLGA/β-TCP Scaffolds

No.	Polymer concentration (g/mL)	Freezing temperature (°C)	Porogen fraction (%)	β-TCP percentage (%)	Porosity (%)	Compressive modulus (MPa)	Compressive strength (MPa)
1	0.15	−18	80	0	83.5	4.26 ± 0.81	0.310 ± 0.022
2	0.15	−18	85	0	90.3	2.06 ± 0.77	0.246 ± 0.086
3	0.15	−18	90	0	90.6	1.97 ± 0.69	0.178 ± 0.034
4	0.15	−60	80	0	84.8	3.37 ± 1.09	0.336 ± 0.038
5	0.15	−60	85	0	90.0	1.70 ± 0.11	0.198 ± 0.054
6	0.15	−60	90	0	92.0	1.49 ± 0.14	0.176 ± 0.018
7	0.12	−18	85	0	89.1	1.71 ± 0.41	0.243 ± 0.056
8	0.20	−18	85	0	91.3	3.30 ± 0.52	0.291 ± 0.030
9	0.20	4	85	0	90.9	2.01 ± 0.88	0.179 ± 0.067
10	0.20	−60	85	0	91.3	1.49 ± 0.43	0.217 ± 0.04
11	0.15	−18	80	10	90.0	5.14 ± 3.17	0.296 ± 0.056
12	0.15	−18	80	20	91.4	5.21 ± 3.04	0.177 ± 0.025
13	0.15	−18	80	30	91.2	6.64 ± 1.03	0.131 ± 0.041
14	0.15	−18	80	40	90.3	4.78 ± 0.83	0.264 ± 0.062
15	0.15	−18	80	50	90.8	4.68 ± 2.28	0.201 ± 0.028
16	0.15	−18	80	60	89.5	2.61 ± 1.92	0.110 ± 0.058

cess of removing the porogen particles had an important effect on the pore size distribution. The small pore size distribution in the scaffolds [Fig. 4(b,d,f)] was estimated about 1–12 μm, which was consisted with the reported reference that liquid–liquid phase separation could give rise to isotropic pores of 1–30 μm depending on the process parameters and the thermodynamics of the polymer/solvent system.[30]

Introduction of β-TCP

To improve biocompatibility and compressive properties of the PLGA scaffolds for bone tissue engineering, β-TCP, was introduced. The scaffold preparation conditions were carefully designed as 0.15 g/mL polymer concentration, 80% porogen fraction, and −18°C freezing temperature. Mass percentages of β-TCP with respect to the total mass of PLGA and β-TCP were selected as 10, 20, 30, 40, 50, and 60%. SEM micrographs of the PLGA/β-TCP scaffolds are shown in Figures 5 and 6. These figures suggested that addition of β-TCP into the PLGA scaffolds influenced slightly the size of big pores and interconnectivity of the scaffolds. The big pores with pore size distribution in 75–400 μm were connected with each other. As the mass percentage of β-TCP increased, the amount of β-TCP on the walls of scaffolds also increased, but the amount of small pores decreased. The introduction of the fourth component made the mechanism of the phase separation complex. It was assumed that the polymer-rich phase during the liquid–liquid phase separation when freezing contained a large amount of β-TCP. The β-TCP-containing polymer-rich phase would be transferred into the big pore wall and the large amount of β-TCP might limit the small pore formation on it.

Porosity

Porosities of the PLGA and PLGA/β-TCP scaffolds are listed in Table I. Results indicated that the porosity of the scaffold increased with increasing porogen fraction at a freezing temperature and a given PLGA concentration (Nos. 1–3 and Nos. 4–6). For a porogen volume fraction of 80%, the scaffold porosity was around 80–85% (Nos. 1 and 4). If the porogen fraction was set at 85% or 90%, the porosity increased to about 89–92%. The scaffold porosity increased slightly with increasing the polymer concentration (Nos. 2,7,8) and lowering the freezing temperature (Nos. 8–10).

As an ideal scaffold, it should possess high porosity (60–90%) to benefit the cell ingrowth.[31] The porosity of the scaffolds was generally determined by volume fraction of the porogen, and the maximum amount of porogen was controlled by the processing feasibility and the required mechanical properties of the scaffold.[2] In our study, the maximum volume fraction of the porogen was 95% in the case of a lower polymer concentration, that is, 0.12 g/mL, whereas it had to be decreased to 90% when the polymer concentration was higher to 0.15 g/mL or 0.20 g/mL. Once the porogen amounts were more than the maximum fraction, the polymer/sucrose/solvent dope would be difficultly stirred, and the porogen particles could not be dispersed homogeneously. The method we used to determine the porosity of the scaffolds seemed not to be perfect because of the deviation by ethanol used.

Porosities of the PLGA/β-TCP scaffolds are also listed in Table I (Nos. 11–16). It suggested that the scaffold porosities were nearly 90% after the addition of β-TCP, and the values changed slightly with the β-TCP mass percentage increased from 10 to

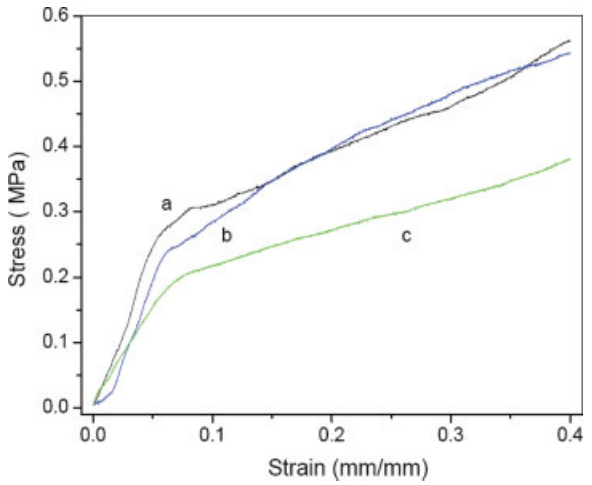

Figure 7 Typical stress–strain curves of PLGA scaffolds prepared from a 0.15 g/mL polymer solution with 80% (a), 85% (b), and 90% (c) porogen at −18°C for freezing. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

60%. The porosities of the PLGA/β-TCP scaffolds were significantly higher than that (83.5%) of PLGA prepared at the same conditions (No. 1). The inorganic β-TCP could interfere in the phase separation when the polymer/porogen/solvent/β-TCP dope was frozen. The interconnectivity and porosity of the PLGA/β-TCP scaffolds could be caused by the tight contact between porogen and β-TCP particles.

Mechanical properties

High mechanical strength is the guarantee for an ideal scaffold to meet the requirement of the repaired tissues, especially for those used for bone-tissue engineering. Compressive tests were often performed to evaluate the mechanical properties of porous scaffolds.[28] In this study, the typical stress–strain curves of the PLGA scaffolds prepared from a polymer solution of 0.15 g/mL with different volume fractions of porogen at −18°C for freezing are shown in Figure 7. Other stress–strain curves of the PLGA scaffolds prepared at different freezing temperatures were similar like these.

According to the ref. 28, the compression process of a porous PLGA scaffolds generally composed of a linear elastic deformation at small strain and a yielding deformation at large strain, followed by a plateau region and solidifying region at very large strain and extremely large strain. Porosity of the PLGA scaffolds prepared in this work was around 83–92%. Therefore, yielding peak was not observed while only flexure deformation was found from the stress–strain curves shown in Figure 7(a,b), and the flexure deformation was further lowered when the porogen fraction was larger at 90% [Fig. 7(c)].

In this work, the compressive stress at 10% strain was defined as the compressive strength according to ISO 844-2004 for determination of compressive properties of rigid cellular plastics. The compressive modulus was calculated as the initial slope of linear elastic region at 0–4% strain. Mechanical properties of the scaffolds prepared from a 0.15 g/mL polymer solution with different porogen fractions at −18°C or −60°C for freezing are listed in Table I (Nos. 1–3 and 4–6). As expected, the compressive modulus and strength decreased with the porogen volume fraction increasing at a given freezing temperature, similar to the result in the ref. 28. It could also be seen from Table I (Nos. 1–6 and Nos. 8–10) that the compressive modulus and strength of the PLGA scaffolds prepared from the 0.15 g/mL or 0.20 g/mL polymer solutions containing a given amount porogen tended to decrease at a lower freezing temperature. In addition, when the porogen volume fraction and the freezing temperature kept unchanged, the compressive modulus and strength of the PLGA scaffold also exhibited a decrease tendency with decreasing the solution concentration (Nos. 2,7,8).

Decreasing the freezing temperature would increase the possibility of phase separation, which led to generation of more small pores, while diluting the polymer solution would also result in more small pores as it was explained in above section. The existing small pores became the defects of the scaffold and result in the reduction of their compressive properties.

Typical compressive stress–strain curves and their compressive properties of PLGA/β-TCP scaffolds are shown in Figure 8 and Table I (Nos. 11–16),

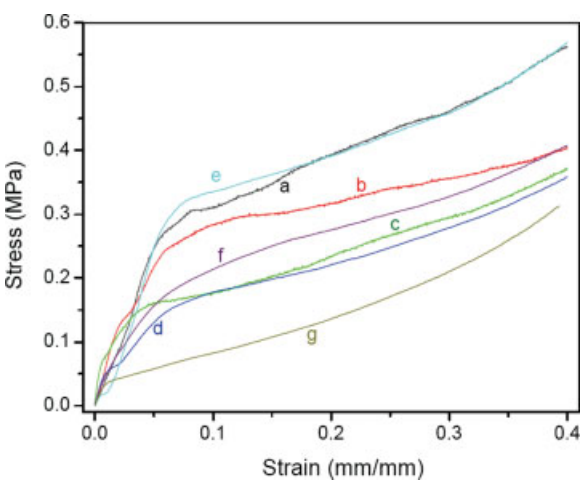

Figure 8 Typical stress–strain curves of PLGA scaffolds after introduction of β-TCP in different mass percentages prepared from a 0.15 g/mL polymer solution with 80% porogen at −18°C for freezing. (a) 0% β-TCP; (b) 10% β-TCP; (c) 20% β-TCP; (d) 30% β-TCP; (e) 40% β-TCP; (f) 50% β-TCP; (g) 60% β-TCP. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

respectively. Results suggested that the compressive properties tended to increase after addition of β-TCP, but not in the case of 60% β-TCP. When the β-TCP mass percentage was 10–50%, the compressive modulus of the composite PLGA/β-TCP scaffolds showed slightly higher values when compared with that of the PLGA scaffold prepared at the same conditions (No. 1), whereas the compressive strength showed a little decrease. The drop in the compressive strength might be caused by the higher porosity (Table I, No. 11–16) of the PLGA/β-TCP scaffolds than that of PLGA (Table I, No. 1). When the mass percentage was 60%, the compressive modulus and strength decreased significantly, both of which were obviously lower than the PLGA scaffold without β-TCP. It could be seen from Figure 6 that when the β-TCP mass percentage was 10–40%, the materials consist of a continuous polymer-mineral matrix and the interfacial connection in these scaffolds were better, so that the compressive properties of these composite PLGA/β-TCP scaffolds were improved. But the composite scaffolds with a larger amount of β-TCP consisted of polymer-linked β-TCP, and there was almost no continuous polymer matrix between the inorganic particles. Besides, lack of interconnection between the particles in the material also resulted in the poor compressive properties of these PLGA/β-TCP scaffolds.

CONCLUSIONS

Porous PLGA and PLGA/β-TCP scaffolds have been fabricated successfully by TIPS/porogen leaching method. The scaffolds were highly porous with about 90% porosity. There were interconnected big pores ranged from 75 to 400 μm and small pores in microns distributing on the walls of the big pores. Compressive tests indicated that diluting the polymer concentration, decreasing freezing temperature, or increasing volume fraction of the porogen would result in the decrease of the compressive modulus and strength of the PLGA scaffolds. Addition of β-TCP showed a slight effect on the compressive modulus but tended to lower the compressive strength. The PLGA and PLGA/β-TCP scaffolds obtained would be potentially applied in bone-tissue engineering.

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