Microstructure and Mechanical Properties of Poly(L-lactide) Scaffolds Fabricated by Gelatin Particle Leaching Method

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ABSTRACT: Biodegradable poly(L-lactide) (PLLA) scaffolds with well-controlled interconnected irregular pores were fabricated by a porogen leaching technique using gelatin particles as the porogen. The gelatin particles (280–450 μm) were bonded together through a treatment in a saturated water vapor condition at 70°C to form a 3-dimensional assembly in a mold. PLLA was dissolved in dioxane and was cast onto the gelatin assembly. The mixtures were then freeze-dried or dried at room temperature, followed by removal of the gelatin particles to yield the porous scaffolds. The microstructure of the scaffolds was characterized by scanning electron microscopy with respect to the pore shape, interpore connectivity, and pore wall morphology. Compression measurements revealed that scaffolds fabricated by freeze-drying exhibited better mechanical performance than those by room temperature dying. Along with the increase of the polymer concentration, the porosity of the scaffolds decreased whereas the compressive modulus increased. When the scaffolds were in a hydrated state, the compressive modulus decreased dramatically. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 98: 1373–1379, 2005

Key words: biodegradable; biomaterials; scaffolds; mechanical properties; tissue engineering

INTRODUCTION

Cell transplantation has been investigated as an alternative treatment to whole organ transplantation for failing or malfunctioning organs such as the liver and pancreas.[1] Donor tissue is harvested and dissociated into individual cells, which are attached and cultured onto a proper substrate that is ultimately implanted at the desired site of the functioning tissue.[2] Because many isolated cell populations can be expanded *in vitro* using cell culture techniques, only a very small number of donor cells may be needed to prepare an implant.[3] Consequently, the living donor need not sacrifice an entire organ, thus significantly expanding the donor pool. However, isolated cells cannot form new tissues on their own.[4] Thus, a temporary scaffold is needed to serve as an adhesive substrate for implanted cells and as a physical support to guide the formation of the new organ. In the tissue engineering approach, the scaffold plays a pivotal role in cell seeding, proliferation, and new tissue formation in 3 dimensions.[5–8]

Biodegradable polymers have been attractive candidates for temporary scaffolding materials because they degrade as the new tissues are formed, eventually leaving nothing foreign to the body.[9] Aliphatic polyesters such as poly(glycolic acid), poly(lactic acid) (PLA), and their copolymer of poly(DL-lactic-*co*-glycolic acid) have been fabricated into porous 3-dimensional (3-D) biodegradable scaffolds. These scaffolds have been used in tissue engineering of cartilage,[10,11] bone,[12] skin,[13] ligament,[14] bladder,[15] and liver.[16] Although these porous 3-D biodegradable scaffolds have shown great promise in the research of engineering a variety of tissues, the engineering of clinically useful tissues and organs is still a challenge. The research of the "ideal" scaffolds for tissue engineering has yet to be carried out.

The following are the physical and chemical requirements of scaffolds for tissue engineering:

1. biocompatibility and biodegradability;
2. the ability to modulate hydrophilicity for promotion of cell adhesion;
3. enhancement of cell growth;
4. retention of differentiated cell function;
5. high porosity and large surface area (surface/volume ratio) to provide adequate space for cell seeding, growth, and extracellular matrix production as well as modulate pore size;
6. a uniformly distributed and interconnected pore structure (this is important so that cells are easily distributed throughout the scaffold and an organized network of tissue constituents can be formed); and

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7. adequate mechanical stability and strength to keep the original shape of tissues and organs.

A number of techniques such as phase separation,[17–22] emulsion freeze-drying,[23] gas foaming,[24,25] fiber bonding,[26] porogen leaching,[27–29] and 3-D printing[30] have been developed to produce highly porous polymer scaffolds for tissue engineering. Among these, porogen leaching is frequently employed because of its easy fabricating process, well-controlled microstructure of the resultant scaffolds, and no requirement for special machines.

The feasibility of the porogen leaching method has been demonstrated by utilizing different kinds of porogens such as inorganic crystals, organic crystals, and paraffin spheres. Yet, the often-argued question is the potential side effect of some porogens such as sodium chloride, because the porogens are not removed completely because of entrapment in the polymer matrix and insufficient leaching. Herein, we introduce gelatin particles as a new kind of porogen material to fabricate porous PLA scaffolds for tissue engineering. As well demonstrated previously, introduction of gelatin, the degradation product of collagen, improves the positive interaction between materials and cells, so that side effects can be completely avoided. A notable feature of the process is that the gelatin particles are bonded together to form a 3-D assembly through a water vapor treatment, followed by polymer solution casting. The porous PLA scaffolds with interconnected pore structures can then be obtained after removal of the solvent and the gelatin particles.

EXPERIMENTAL

Materials

Poly(L-lactide) (PLLA, number-average molecular weight = 99,000, weight-average molecular weight = 212,000) was synthesized using the method described previously.[31] Gelatin and 1,4-dioxane were obtained from Shanghai Chemical Industries Co. Ltd.

Preparation of polymer scaffolds

The gelatin particles (280–450 μm) were sieved from raw gelatin particles and used without further treatment. The gelatin particles (ca. 4.0 g) were added into a glass mold, which is 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. It was taken out 3.5 h later, and the protuberant top surface caused by the swelling of the gelatin particles was immediately pressed flat. After cooling to room temperature, 3.2 mL of the PLLA/1,4-dioxane solution was cast dropwise onto the gelatin particles assembly. The mold was maintained under a low pressure of 0.07–0.08 MPa to evolve the trapped air bubbles, and 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 and then freeze-dried to remove the 1,4-dioxane. As a comparison, the solvent was also removed by evaporation at room temperature to obtain the gelatin/PLLA blend.

The PLLA porous scaffolds were obtained by leaching the gelatin assembly in 400 mL of deionized water at 70°C for 2 days, changing the water every 16 h. To compare the remaining gelatin content, the scaffold was extensively washed under the same conditions with a larger amount of water. The scaffolds were finally dried at 37°C for 3 days and preserved in a desiccator.

Measurement of apparent density and porosity

The apparent density (ρ) and the porosity (ε) were determined by measuring the volume (v) and the mass (m) of the resultant scaffolds.[8] The ρ is defined as m/v whereas the ε is calculated according to

$$\varepsilon = 1 - \rho/\rho_p \tag{1}$$

where ρ_p represents the bulk density of PLLA. For a porous scaffold, ρ_p was given by

$$\rho_p = 1/[(1 - X_c)/\rho_a + X_c/\rho_c] \tag{2}$$

where ρ_a and ρ_c are the densities of the amorphous and crystalline regions, respectively. For PLLA, ρ_a = 1.248 g/mL, ρ_c = 1.290 g/mL,[32] and X_c is the crystallinity degree measured by differential scanning calorimetry:

$$X_c = \Delta H_m/\Delta H_m^o \tag{3}$$

where ΔH_m is the melting enthalpy of the measured PLLA and ΔH_m^o is the melting enthalpy of the 100% crystalline polymer (203.4 J/g).[8]

Scanning electron microscopy (SEM) observations

The scaffolds were cut into pieces with a razor blade. These pieces were coated with gold under a pressure of 50 mTorr for 180 s. The microstructure of the scaffolds was then observed under SEM with a Jeol Ltd. JSM-5510LV.

Mechanical property

Cylindrical samples (~20-mm diameter and ~16-mm height) were compressed by a mechanical tester (Zwick Roell). The cross-head speed was set at 1 mm/min. The compressive modulus was determined from

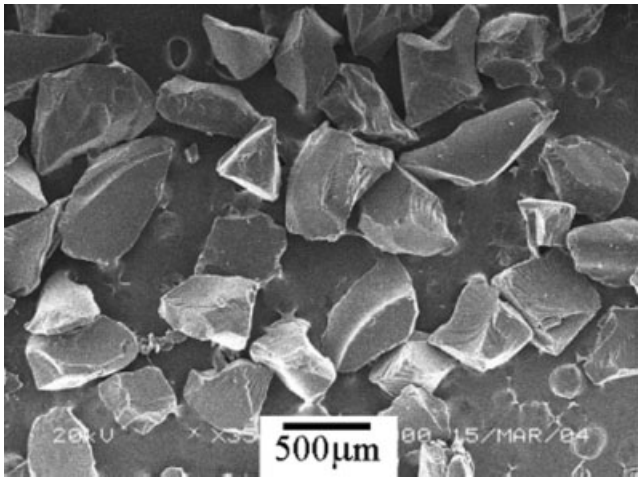

Figure 1 An SEM photomicrograph of 280–450 μm gelatin particles (original magnification ×35).

the compressive curve in the initial strain range of 2–6%. Hydrated samples were measured immediately after they were incubated in phosphate buffered saline (PBS, pH 7.4) for 12 h at 37°C.

Determination of residual gelatin content in scaffolds

The scaffolds were completely hydrolyzed with 6*M* HCl for 12 h at 120°C. The remaining gelatin in the scaffolds was determined by measuring the hydroxyproline content spectrophotometrically according to the method of Cheung et al.[33]

RESULTS AND DISCUSSION

The pore size of scaffolds is widely recognized as an important parameter for guiding the regeneration of a specific tissue or organ. In the present work we chose 280–450 μm gelatin particles as the porogen for cartilage tissue engineering (Fig. 1). To ensure the interconnected pore structure in the resultant scaffolds, the gelatin particles were treated in saturated water vapor at 70°C for 3.5 h prior to PLLA solution casting. This treatment produces firm bonding between the gelatin particles, forming a 3-D assembly. Another benefit of the vapor treatment was the good mechanical stability of the gelatin assembly compared to the loose gelatin particles. The loose gelatin particles were easily disturbed by a small droplet of polymer solution in the casting step, whereas the spatial relationships among the bonded gelatin particles were maintained in the casting process.

To balance processing feasibility and the mechanical property of the resultant scaffolds, the polymer concentrations were varied from 5 to 12%. Because the polymer solutions were rather sticky, especially at higher concentration, the prime problem during the casting process is how to evolve the entrapped air bubbles in the gelatin particle assembly, so that the polymers can distribute homogeneously in the whole matrix. Although low pressure benefits the infiltration of the polymer solution, a PLLA solution with a concentration of 12% could hardly fill into the gelatin particle assembly because of the evaporation of the solvent. Therefore, in the following experiments we chose three concentrations (0.05, 0.08, and 0.1 g/mL) to compare their influence on the properties of the scaffolds.

Two methods (room temperature drying and freeze-drying) were adopted to remove the organic solvent before particle leaching. For this purpose, a PLLA concentration of 0.1 g/mL was selected. Although the apparent density or porosity is rather similar between the room temperature dried and the freeze-dried scaffolds (Table I), their microstructure and mechanical performance had substantial differences (Figs. 2, 3). The huge pores in the room temperature dried scaffold only partially resembled the gelatin particles, because of the 3-D shrinkage of the scaffold that had been observed in the particle leaching procedure. Moreover, there were no small pores in the skeletons in between the huge pores; instead, ~50–120 μm irregular pores existed that were formed by the "polymer skins" enwrapped on the gelatin particles. In spite of the same polymer concentration, the room temperature dried scaffold had a much poorer compressive modulus than that of the freeze-dried one, the values of which were 0.24 ± 0.06 versus 1.59 ± 0.21 MPa, respectively. (These values were calculated from their stress–strain curves, as shown later.) This difference is apparently caused by their distinct microstructure. Hence, we can conclude that

TABLE I
Parameters (n = 3) of PLLA Scaffolds Fabricated under Different Conditions

Concentration (g/mL)	Particle size (μm)	Solvent drying method	Apparent density (g/cm^3)	Porosity (%)
0.1	280–450	Room temperature drying	0.070 ± 0.001	94.5 ± 0.1
0.1		Freeze-drying	0.065 ± 0.001	94.8 ± 0.1
0.08		Freeze-drying	0.052 ± 0.001	95.9 ± 0.1
0.05		Freeze-drying	0.030 ± 0.001	97.6 ± 0.1

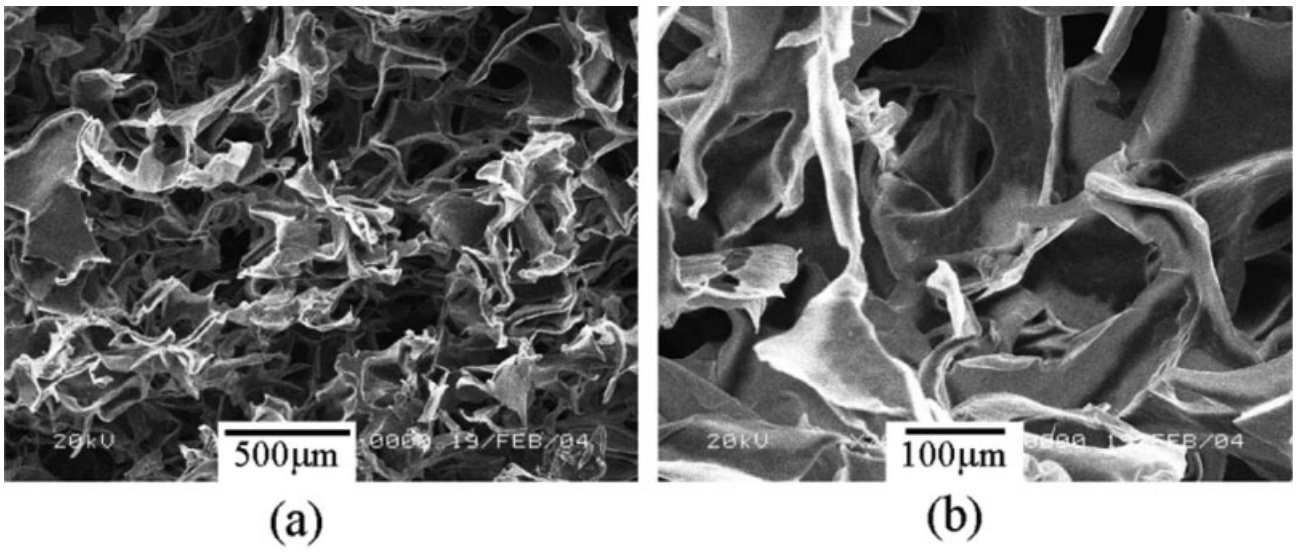

Figure 2 SEM photomicrographs of cross sections of the PLLA scaffold with solvent removal by the room temperature drying method (PLLA concentration = 0.1 g/mL) at original magnification (a) ×50 and (b) ×200.

freeze-drying is more promising than room temperature drying with respect to mechanical performance.

Figure 3 presents the inner morphologies of the scaffolds. Two kinds of pores exist in the scaffolds: one kind of 100s of microns that was the replica of the gelatin particle, and the other kind of several 10s of microns that distributed in between the larger pores. The larger pores resembled the macroscopic contours of the porogen and were well interconnected with each other. The large number of smaller pores (10–30 μm) located in the skeletons was produced by the mechanism of thermally induced phase separation (TIPS).[29] During the freezing procedure at −25°C, the phase separation occurred simultaneously in the PLLA/1,4-dioxane solution system, forming polymer-rich and polymer-poor phases. After removal of the solvent by freeze-drying, smaller pores were generated that resembled the shapes of the polymer-poor phases. It is worth noting that a smaller pore size in the skeletons was found when the polymer concentration was up to 10%. In contrast, a lower polymer concentration resulted in a more porous skeleton structure. This conclusion could also be drawn from the comparison of the apparent density or the porosity of the scaffolds fabricated at different polymer concentrations (Table I). Although the porosity of all of the scaffolds was above 94%, a lower polymer concentration always yielded higher porosity. As a tissue regeneration scaffold, the existence of these pores, especially the interconnected macropores, could be beneficial to both cell infiltration and mass transportation.

Mechanical properties are particularly important for scaffolds used in tissue engineering fields, because they are closely associated with the shape-retaining ability in practical operations and applications. For this purpose, the compressing performance of the scaffolds was investigated. Figure 4(a) shows the stress–strain curves of the scaffolds as a function of the polymer concentration. The curves display three apparent regions. At lower strains the stress increased almost in a linear manner. When the strains were larger than ~10%, platform regions appeared. When the strains were even higher, for example, 40–60%, depending on the polymer concentrations, the platform disappeared and the stress sharply increased.

These three regions reveal the alteration process of the scaffolds' microstructure during compression. In the first region, the deformation extent is small and might be considered as elastic; hence, the compressive modulus was calculated as shown in Figure 4(b). It is reasonable that the modulus increases when the polymer concentration is higher. This is a result of the higher polymer content per unit volume and smaller pores existing in the skeletons in between the huge pores, because the smaller pores could toughen the materials compared to the larger ones, providing the skeletons' thicknesses are similar. Note that during this stage the predominant parts to resist deformation should be the thinnest areas in the scaffolds. In the platform region, the skeletons in between the huge pores were continuously destroyed, leading to the increase of strain but an approximately constant stress. The gradual collapse of the scaffold resulted in close contact of the huge pores, accompanying by the disappearance of the interconnected channels [Fig. 5(a)]. Finally, most of the huge pores were also highly compressed [Fig. 5(b)], leading to the close contact of skeletons and a sharp increase of the stress in a short alteration range of strain.

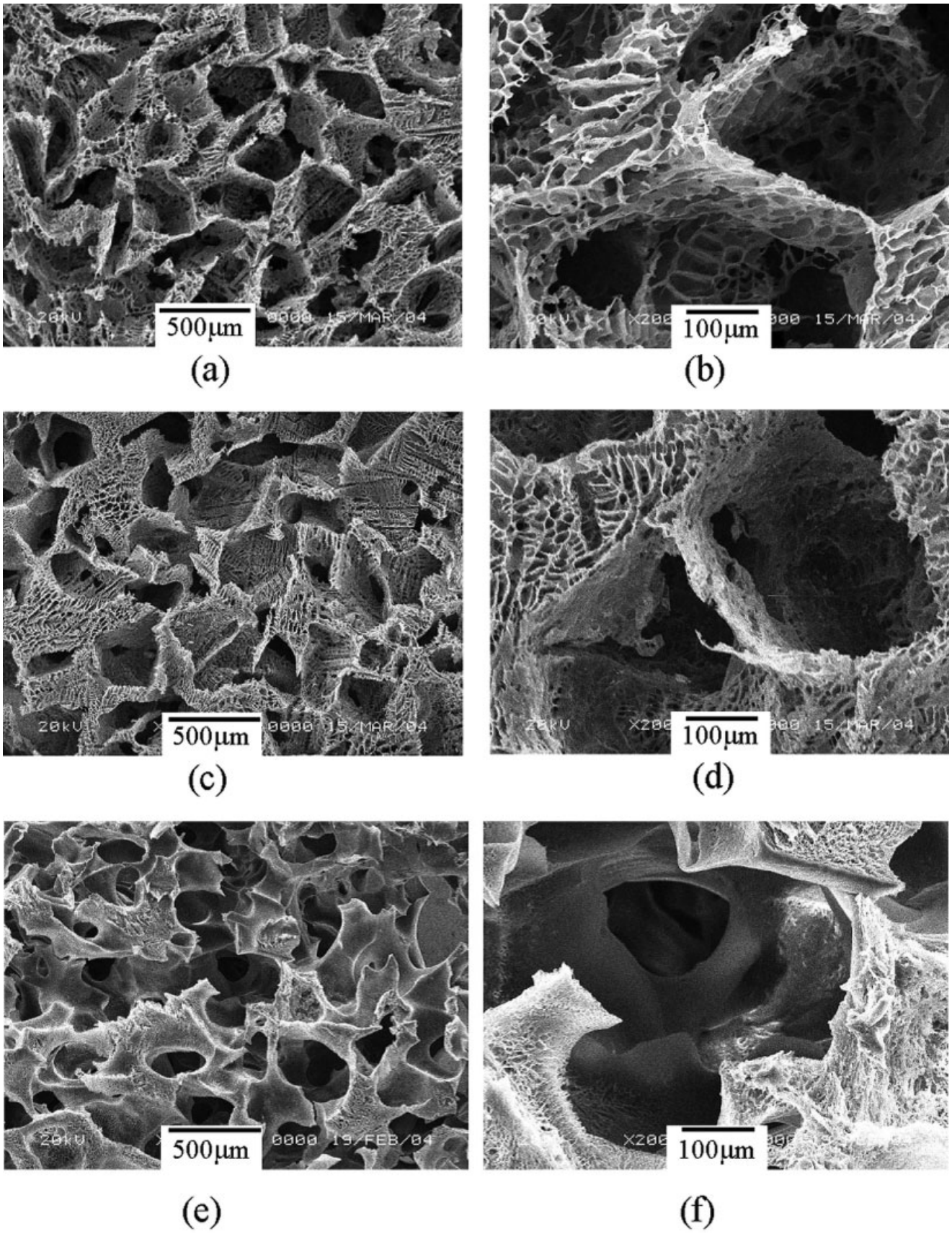

Figure 3 SEM photomicrographs of cross sections of the PLLA scaffolds with solvent removal by the freeze-drying method. The PLLA concentrations are (a) 0.05, (c) 0.08, and (e) 0.1 g/mL at original magnification ×50 and (b) 0.05, (d) 0.08, and (f) 0.1 g/mL at original magnification ×200.

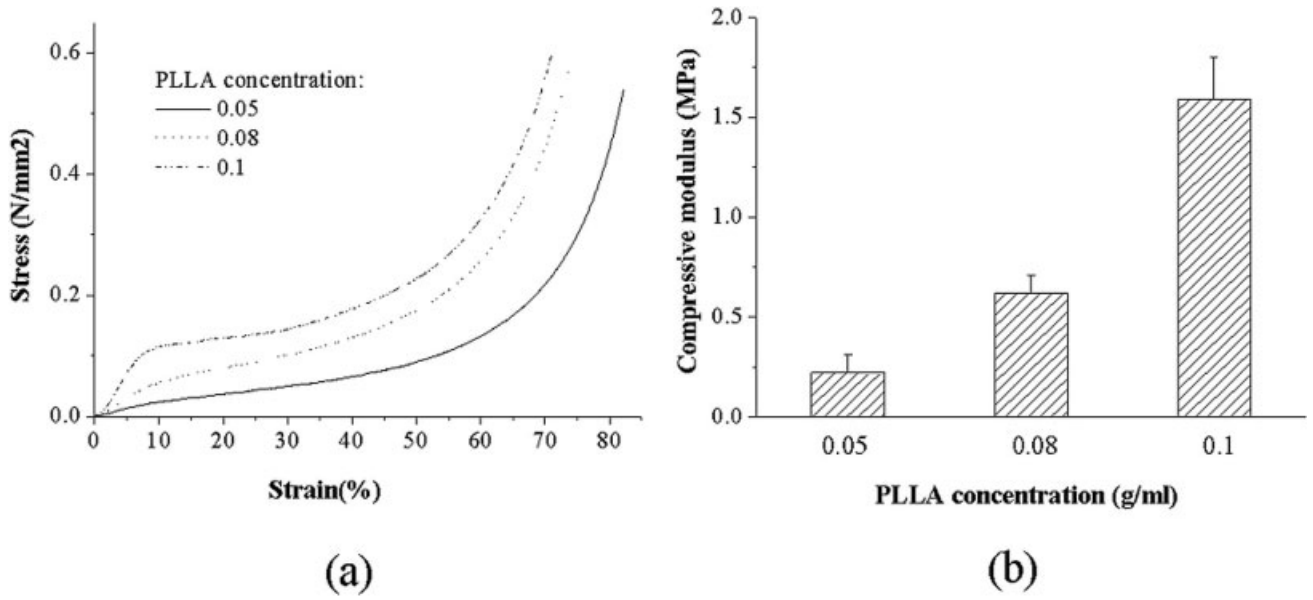

Figure 4 (a) Stress–strain curves of the PLLA scaffolds fabricated at different concentrations. (b) The compressive moduli (n = 3) of the scaffolds obtained from (a) at the initial strain range.

The mechanical performance in the hydrated state is extremely important because the scaffolds are used exclusively in contact with culture medium *in vitro* or body fluid *in vivo*. Thus, the scaffold fabricated with a PLLA concentration of 0.1 g/mL was again incubated in PBS at 37°C for 12 h. The measured compressive modulus in the wet state, however, was surprisingly decreased from 1.59 ± 0.21 to 0.75 ± 0.18 MPa (Table II, A and Aw). Although it is well understood that the water molecule may function as a plasticizer for most polymers, this should not be the main reason in the present case because we observed a reverse effect of water for the same scaffolds fabricated by TIPS. The most possible reason should be the remaining gelatin, which may adhere on the skeleton surface and toughen the scaffold in the dry state. While in the wet state, the gelatin can be dissolved, or at least swollen, thus losing its toughening effect. To verify this postulation, the remaining gelatin contents were varied as shown in Table II. The values indeed demonstrate that the moduli decreased along with the reduction of gelatin content in the dry state. At an extremely low

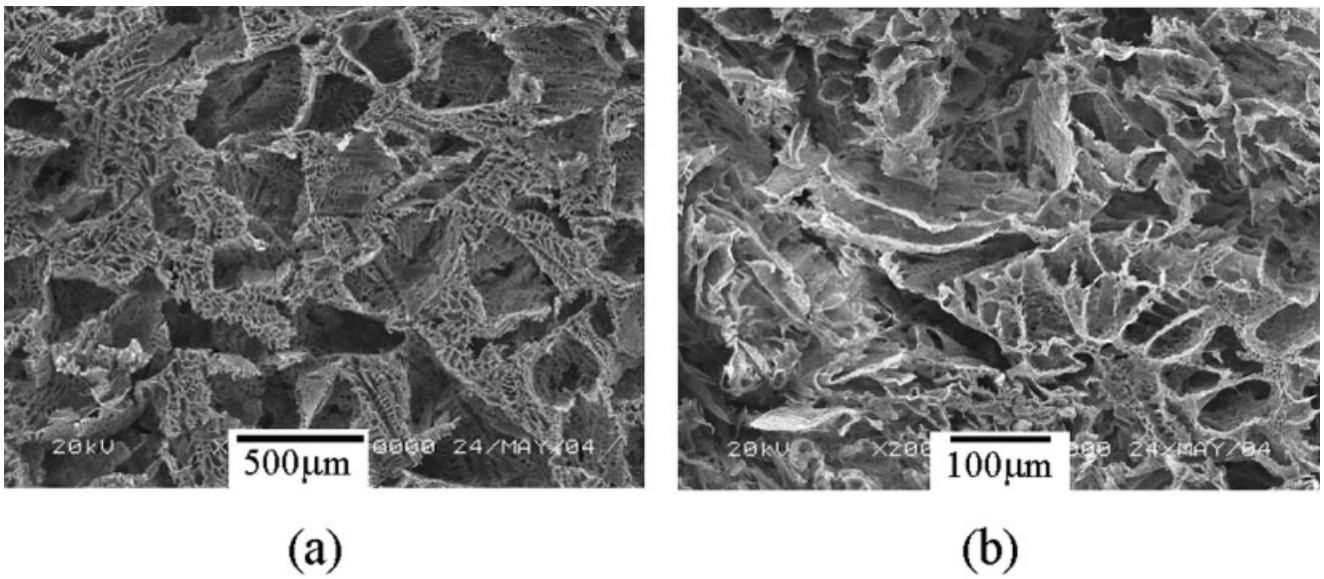

Figure 5 SEM photomicrographs of cross sections of the PLLA scaffolds after compressing at (a) 25% (original magnification ×50) and (b) 80% (original magnification ×200). PLLA concentration = 0.1 g/mL.

TABLE II
Compressive Moduli (n = 3) of PLLA Scaffolds as a Function of Remaining Gelatin Content and Drying State

Samples	State	Content of gelatin (mg/g) scaffold	Compressive modulus (MPa)
A	Dry	31.7 ± 2.23	1.59 ± 0.21
B	Dry	12.48 ± 2.80	1.22 ± 0.08
C	Dry	3.89 ± 1.13	0.76 ± 0.35
Aw	Wet	—	0.75 ± 0.18
Cw	Wet	—	0.61 ± 0.21

Aw and Cw, A and C that were incubated in PBS at 37°C for 12 h, respectively.

gelatin content, there was no significant difference between the wet and dry state (Table II, C, Aw, and Cw). In addition to improvement of the mechanical performance, the remaining gelatin is also advantageous to enhance cell–scaffold interaction that is basically required in tissue regeneration.[34–38] Research is underway to investigate the cell growth behaviors *in vitro* and tissue response *in vivo* in these porous scaffolds.

CONCLUSIONS

We demonstrated here that porous PLLA scaffolds were successfully fabricated by using porogen leaching and solvent removal techniques. A notable feature was that the porogen (gelatin particles) were bonded together to form a 3-D assembly through a water vapor treatment in prior polymer solution casting. Solvent removal by freeze-drying is more promising than by room temperature drying because the former produced scaffolds with higher mechanical performance. SEM observations revealed that smaller pores emerged in the scaffolds fabricated by freeze-drying, in addition to the huge pores replicating the porogen contours. Along with the increase of the polymer concentration, the compressive modulus also increased. Hydrated scaffolds exhibited lower mechanical performance that was mainly caused by the dissolution or swelling of the adhered gelatin on the skeleton surfaces in between the pores. We predict that the remaining gelatin is also advantageous to enhance cell–scaffold interaction that is basically required in tissue regeneration.

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