

Fabrication of Graded Macroporous Poly(lactic acid) Scaffold by a Progressive Solvent Casting/Porogen Leaching Approach

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ABSTRACT: Biodegradable porous scaffolds play an important role as transplant vehicles for cultured cells or templates to guide tissue regeneration. Graded porous scaffolds can be utilized to study the interactions between cells or tissues and scaffolds with different pore sizes. In this study, a progressive solvent casting/porogen leaching (PSC/PL) approach was developed to fabricate a porous PLA scaffold with an axial pore size grade. The graded macroporous PLA scaffold was built up by solvent casting layer-by-layer progressively in a Buchner funnel with the assistance of a vacuum pump to facilitate the removal of solvent. The grade was achieved by gradually decreasing the size and content of salt at different layers. The result-

ing scaffold layers had an average thickness of 1 mm, a progressive pore size from $166 \pm 41 \mu\text{m}$ to $453 \pm 18 \mu\text{m}$, and compressive moduli between $2.12 \pm 0.08 \text{ MPa}$ and $2.56 \pm 0.18 \text{ MPa}$, which correspond to models of each layer (The compressive modulus of the whole graded macroporous PLA scaffold was $2.18 \pm 0.24 \text{ MPa}$). This method allows the fabrication of grade macroporous PLA scaffold in a simple, homogenous, and fast way. © 2011 Wiley Periodicals, Inc. *J Appl Polym Sci* 125: 571–577, 2012

Key words: macroporous polymers; microstructure; particle size distribution; layer growth; compression

INTRODUCTION

Biomaterials play important roles in the replacement and regeneration of human tissues. Porous biomaterials exhibit the potential of bone ingrowth which depends on porous parameters such as pore size, porosity, and pore interconnectivity. Most porous biomaterials developed and studied are homogenous in terms of pore size distribution, porosity distribution, composition, and mechanical properties. To optimize the structural, mechanical, and biological properties, porous biomaterials with graded/gradient porosity and pore size have been developed.¹ Graded porous scaffolds are useful to study the interactions between cells or tissues and scaffolds with different pore sizes.

Recently, the researches on polymer scaffolds with gradient pore size have drawn more attention. A few techniques have been reported on the fabrication of polymer scaffolds with graded pore size. Oh et al.² fabricated polycaprolactone cylindrical scaffolds with

gradually increasing the pore size along the longitudinal direction by a centrifugation method. Yao et al.³ developed a method for the growth of graded porous polystyrene/poly(lactic acid) blend structures utilizing variable coarsening rates in a graded temperature field in a special annealing setup. Woodfield et al.⁴ prepared porous poly(ethylene glycol)-terephthalate-poly(butylene terephthalate) copolymer scaffolds by a 3D fiber deposition technique. The scaffolds were constructed by successively layering a 0–90° pattern of molten copolymer through a nozzle onto a computer-controlled axes table. Harley et al.⁵ fabricated tubular scaffolds of collagen–glycosaminoglycan with a radial pore size graded by a spinning technique. Wu et al.⁶ reported a method for building porous chitosan-g-poly-caprolactone copolymer scaffolds with a graded porous microstructure along the longitudinal direction by layer-by-layer assembly followed by particulate-leaching process. A thin layer of mixed powders was spread and pressed on a circular stainless steel mould and then a motor-controlled syringe reciprocally moved above the polymer and deposited very small solvent droplets in a way so that the powders were bound into many parallel filaments in one layer. After one layer was completed, the bottom of the mould was lowered by around 1 mm and turned 36° and a new layer of polymer powders were added and the fabrication was repeated until the required constructs were built.

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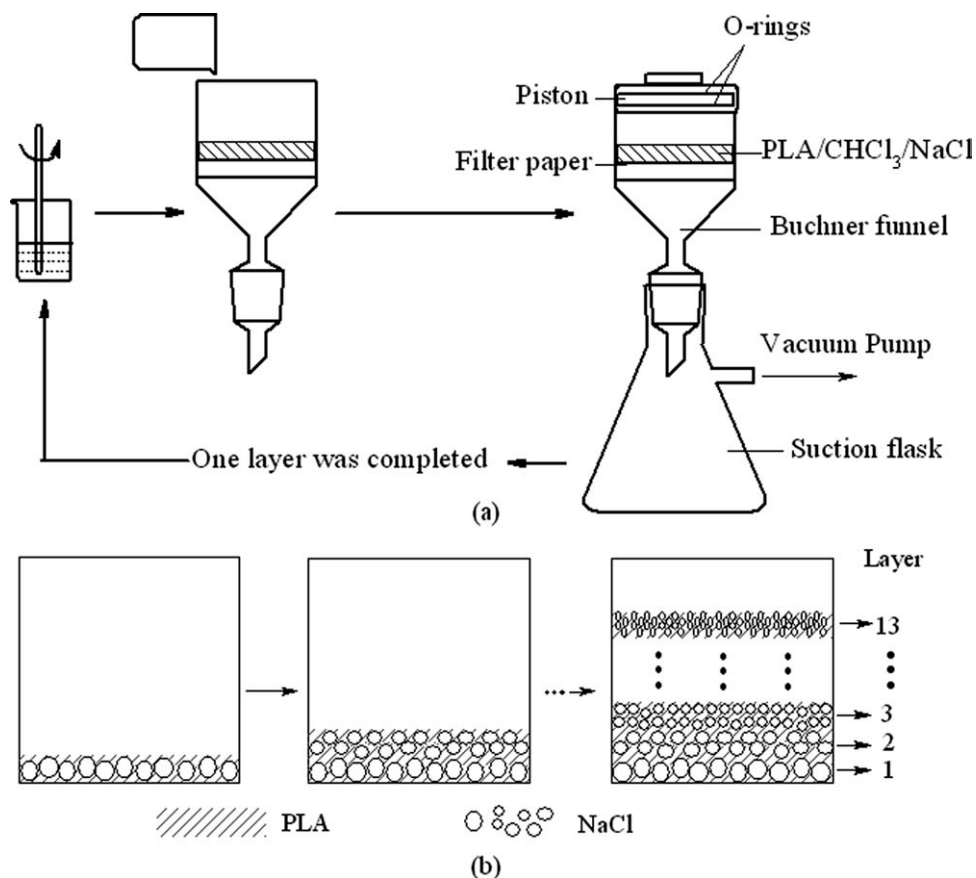


Figure 1 Schematic diagram of the fabrication process: (a) the apparatus and (b) the PSC/PL method.

In our previous study, a modified solvent casting/porogen leaching (SC/PL) method was developed to fabricate thick poly(lactic acid) (PLA) scaffolds with controlled pore size and porosity, and much shorter processing time.⁷ The advantage of this method resides in the capability of producing thick scaffolds efficiently with the assistance of vacuum volatilization without using any special and complicated apparatus. The possibility of using a progressive solvent casting/porogen leaching (PSC/PL) method to fabricate graded macroporous PLA scaffold was investigated in this study. In addition, scaffolds with porosity up to 90% had been reported to allow good cell penetration and proper vascularization of the ingrown tissue.⁸ To obtain a graded macroporous scaffold with high porosity, a large amount of porogen was used. The pore size, pore distribution, porosity, pore interconnectivity, and compressive properties correspond to models of each layer in the graded macroporous PLA scaffold were investigated in this study.

MATERIALS AND METHODS

Materials

PLA particles (Shenzhen Bright China Industrial Co., Ltd., China) used in this study have inherent viscos-

ity of 13.9 mL/g in chloroform at 25°C. Its density (ρ_{PLA}) is 1.270 g/mL as determined by m/V , its glass temperature and melting point are 55°C and 175°C, respectively. The granular sodium chloride (NaCl) was ground with a mortar to obtain sizes of 100–210, 210–310, 310–420, and 420–500 μm , respectively, after sieving with the standard testing sieves.

Scaffold fabrication

The graded macroporous PLA scaffold was fabricated by solvent casting layer-by-layer progressively as shown in Figure 1. To obtain 90% porosity with a graded pore size, the ratio of PLA to NaCl was kept at 1 : 9 by weight in different casting layers. The casting solution was prepared by dissolving 0.33 g of PLA in 2 mL of chloroform (CHCl_3) and then NaCl with desired size was dispersed evenly in the solution. A total of 13 casting solutions by reducing the size of NaCl were used to fabricate the graded macroporous PLA scaffold; their compositions are summarized in Table I. The casting solution was stirred for 3 min and volatilized until it became a pasty mixture. The pasty mixture was then poured into a Buchner funnel (50 mm inner diameter) with a suction flask connected to a vacuum pump. A piston was then sealed on top of the Buchner funnel to

TABLE I
Size and Content of NaCl in Different Casting Solutions which Formed the 13
Layers in the Graded Macroporous PLA Scaffold

Layer No.	PLA (g)	NaCl (100–210 μm) (g)	NaCl (210–310 μm) (g)	NaCl (310–420 μm) (g)	NaCl (420–500 μm) (g)
1	0.33	0	0	0	3.00
2	0.33	0	0	0.75	2.25
3	0.33	0	0	1.50	1.50
4	0.33	0	0	2.25	0.75
5	0.33	0	0	3.00	0
6	0.33	0	0.75	2.25	0
7	0.33	0	1.50	1.50	0
8	0.33	0	2.25	0.75	0
9	0.33	0	3.00	0	0
10	0.33	0.75	2.25	0	0
11	0.33	1.50	1.50	0	0
12	0.33	2.25	0.75	0	0
13	0.33	3.00	0	0	0

prevent the volatilization of the solvent. The whole setup was allowed to stand for 20 min so that a uniform surface was formed on the bottom of the mixture. A vacuum pressure of 0.08 ± 0.01 MPa was then applied to volatilize the remaining solvent in the mixture. When the color of the mixture turned from yellow to white, the vacuum was stopped and the pressure was released. Then, a new casting solution was poured into the Buchner funnel and the above procedures were repeated until the required constructs were built. After complete removal of the solvent by vacuum, the scaffold was obtained by salt leaching and then air-dried for 24 h at 30°C and vacuum-dried (<1 mmHg) for another 12 h at 30°C. All samples were kept in a vacuum desiccator to avoid degradation before the characterization.

To determine the porosity, layer thickness, pore size, and mechanical property of each layer formed from different casting solution precisely, samples with homogeneous structure identical to each of the 13 layers were fabricated by accumulating the same casting solution 10 times following the procedure described above.

Scaffold characterization

The thickness of the sample with homogeneous structure was measured by a vernier caliper and the corresponding layer thickness in the graded macroporous PLA scaffold was 1/10 of the measured thickness. The porosity, pore size, and mechanical property of each layer formed from different casting solution were represented by the properties of the corresponding homogeneous structure.

A simple method was applied to test the interconnectivity of pores between layers in the graded macroporous PLA scaffold. Eight drops of black ink were added to 50 mL of water and well stirred.

After the graded scaffold was soaked by alcohol, the prescribed black ink water mixture was dropped onto the top surface of the graded scaffold drop-by-drop. Then, the sample was cut open to inspect the ink marks. The image was captured by a digital camera.

Porosity of the scaffold was determined by the modified liquid replacement method reported previously.^{7,9} The scaffold was permeated with ethanol completely through repeated cycles of vacuum and air charge. The weights of the dry (w_{dry}) and wet (w_{wet}) samples were measured. The porosity of the scaffold, Φ , was calculated from the weight difference between dry and wet samples according to eq. (1).

$$\Phi = \frac{(w_{\text{wet}} - w_{\text{dry}})/\rho_{\text{ethanol}}}{(w_{\text{wet}} - w_{\text{dry}})/\rho_{\text{ethanol}} + w_{\text{dry}}/\rho_{\text{PLA}}} \times 100\% \quad (1)$$

where ρ_{ethanol} and ρ_{PLA} are the density of ethanol and PLA, respectively. It was assumed that the volume of pores equaled the volume occupied by the absorbed ethanol, and the amount of ethanol absorbed by the PLA was negligible. The porosity data were analyzed by Origin 7.0 software.

The quantities of salts and solvent remained inside the graded macroporous PLA scaffold were determined by a Perkin-Elmer TGA-7 instrument. A temperature range from 20 to 400°C was scanned at a heating rate of 10 K/min. Nitrogen was used as a flushing gas with a flow rate of 20 mL/min. The specimen in each thermogravimetric analysis weighed about 12 mg. Perkin-Elmer proprietary software was used to calculate the derivative thermogram.

The top surface and vertical section of the graded macroporous PLA scaffold were observed by the

scanning electron microscopy (SEM, Jeol JSM-6490). Samples (2–3 mm in thickness) were cut using a razor blade and sputter-coated with gold. The SEM measurement was performed at 20 kV. The average pore size of the scaffolds was measured using an image analysis program (Nano Measurer 1.2.5).

The axial compressive moduli of the porous scaffolds (whole scaffolds and scaffolds correspond to models of each layer) were measured by the universal mechanical testing machine [MTS, CMT-4204, MTS Industrial Systems (China) Co., Ltd.]. Cylindrical specimens of 10 mm height (for the graded scaffold, the height of the specimens is the total height of the graded scaffold) and 10 mm diameter were compressed at an upper load limit of 1 kN with a crosshead speed of 1 mm/min at room temperature. The compressive modulus was estimated from the stress–strain curve where the slope was fitted to the curve at 2 and 10% of elongation. At least five specimens were tested for each sample, and the averages and standard deviations were determined.

RESULTS AND DISCUSSION

Graded macroporous PLA scaffold was successfully fabricated using the PSC/PL approach. The graded macroporous PLA scaffold was constructed by vacuum-assisted filtration of 13 casting solutions layer-by-layer. According to our previous work, the rate of volatilization was accelerated and the amount of residual solvent remained in the scaffold was greatly reduced by applying the vacuum.⁷ At room temperature, longer than an hour was required to complete volatilizing the solvent to produce a scaffold with 1 mm thickness under atmospheric pressure. However, the volatilization time was shortened to about half an hour in the case of applying vacuum.

Table II shows the detailed layer thicknesses of each layer in the graded macroporous PLA scaffold. The density of PLA and NaCl are 1.270⁷ and 2.165 g/cm³. The volume of PLA and NaCl were calculated by m/ρ . Based on the results of calculation, the volume of 0.33 g PLA and 3.00 g NaCl are 0.26 mL and 1.39 mL, respectively. If the gaps between the NaCl particles were exactly filled by PLA, the layer thickness should be 0.84 mm inside a 50 mm inner diameter Buchner funnel. However, all the actual layer thicknesses were larger than this value, which indicates that the PLA could not fill up all the gaps between the NaCl particles and the volume of PLA/NaCl depends mainly on the accumulation volume of NaCl particles. According to Table II, the average thickness of the graded macroporous PLA scaffold was 1 mm. The total height of the graded macroporous PLA scaffold by actual measurement coincides with the sum of the values in Table II.

TABLE II
The Detailed Layer Thicknesses, Porosities, and Pore Sizes of Each Corresponding Layer in the Graded Macroporous PLA Scaffold

Layer no.	Layer thickness (mm)	Porosity (%)	Pore size (μm)
1	1.08	91.3 \pm 0.8	453 \pm 18
2	1.05	91.1 \pm 0.7	425 \pm 21
3	0.98	90.9 \pm 0.4	396 \pm 17
4	1.02	90.4 \pm 0.8	373 \pm 22
5	1.06	89.8 \pm 0.6	332 \pm 24
6	1.04	89.8 \pm 0.8	307 \pm 23
7	0.99	88.9 \pm 0.3	284 \pm 22
8	0.98	88.1 \pm 0.7	261 \pm 25
9	1.02	88.2 \pm 0.2	237 \pm 25
10	0.98	87.6 \pm 0.8	214 \pm 27
11	0.97	87.7 \pm 0.3	192 \pm 38
12	0.95	87.3 \pm 0.4	178 \pm 41
13	0.94	87.4 \pm 0.9	166 \pm 47

The SEM micrographs of the top surface and parts of the vertical section of the graded macroporous PLA scaffold are shown in Figure 2. Based on the layer thickness and different particle sizes, the layer boundaries were marked by white line in Figure 2(b–d). From the SEM micrograph of the top surface [Fig. 2(a)], many pores are observed on the top surface. It can be deduced that some NaCl particles were projecting on the surface when the layer was formed. This phenomenon corresponds with the result that the PLA cannot fill the gaps between the NaCl particles. In fact, no distinct boundary was directly observed between different layers from the SEM micrographs [Fig. 2(b–d)] because the NaCl particles were projecting on the surface of the layer. Moreover, it can be observed that a graded structure was developed with decreasing pore sizes from the bottom to the top. No trace of remaining porogen can be observed. And, the pores were distributed homogeneously.

Figure 3 shows the digital photo of ink seepage created by 0.45 mL ink and 0.65 mL ink. The results of ink seepage showed that the graded macroporous PLA scaffold was well interconnected in each layer and their boundaries. Before leaching process, the PLA/NaCl was a dual-phase continuity structure. After leaching process, the well interconnected pores were formed in the PLA scaffold. Moreover, most pore walls were broken for the irregular contraction of the PLA during volatilizing, which also contributes to the interconnectivity. These imply that the PSC/PL method is suitable for accumulating layers of different particles to build a graded structure.

Figures 4(a and b) show the thermogravimetric analysis (TGA) and derivative thermogram curves derived from the pyrolysis of the raw PLA and the graded macroporous PLA scaffold. The raw PLA

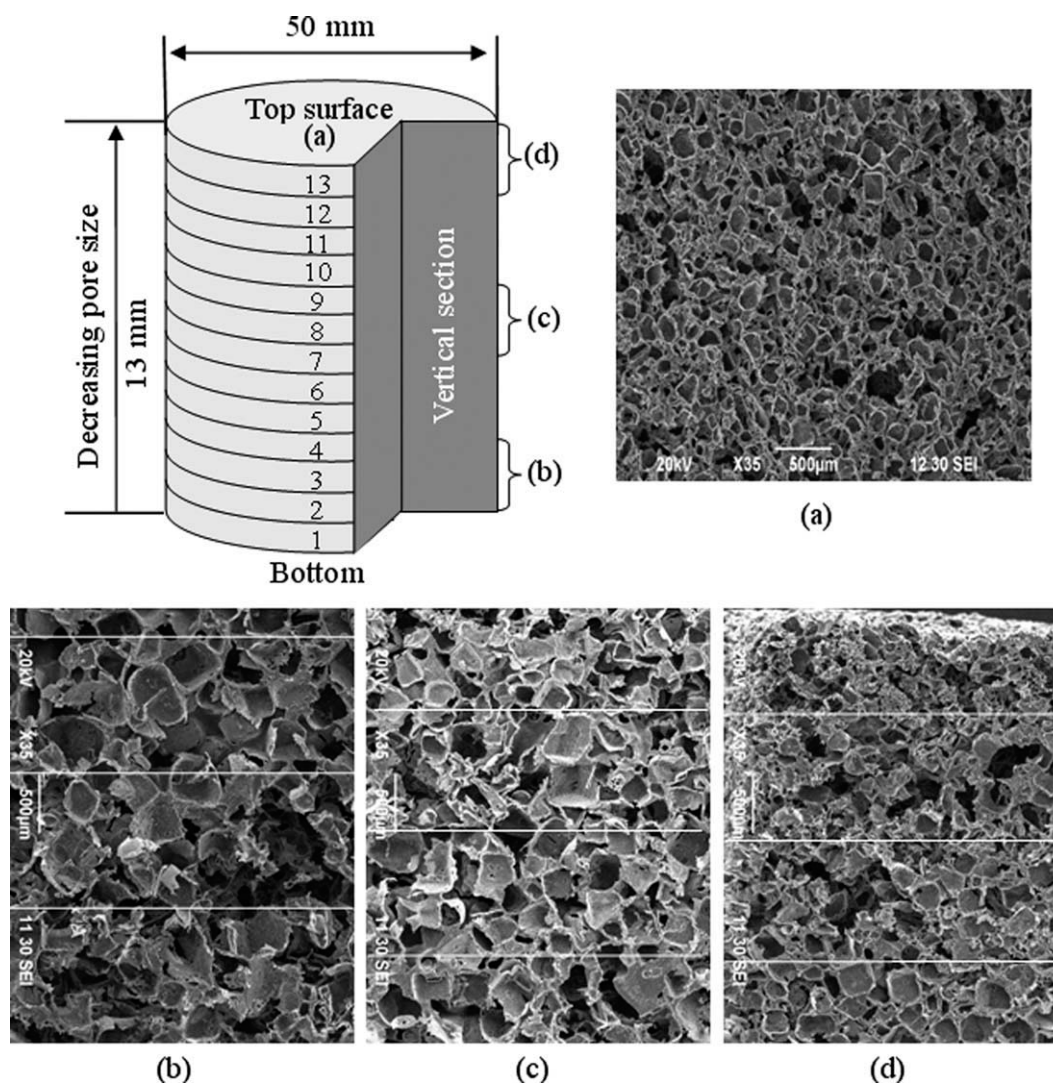


Figure 2 SEM photographs of (a) the top surface and part (b–d) vertical section of PLA scaffold with an axial pore size graded.

and the graded macroporous PLA scaffold produced very similar plots, with the maximum rate of weight loss between 270 and 400°C. When the temperature reached 400°C, the weight loss of three samples were about 100%. The results of TGA confirmed that there was no obvious residual of salts and solvent in the resultant scaffolds.

The porosities and pore sizes of PLA scaffolds which correspond to the 13 different layers are shown in Table II. All layers were highly porous with porosity of more than $87.3\% \pm 0.4\%$. It is noticed that the porosity was slightly decreased with decreasing particle size of the porogen. The pore sizes match the sizes of the NaCl particles incorporated into the layers which confirmed that the pore was formed by porogen leaching. The data of the pore size provided evidence that the pore size showed graded changes from the bottom to the top of the scaffold. The graded PLA scaffold had pore

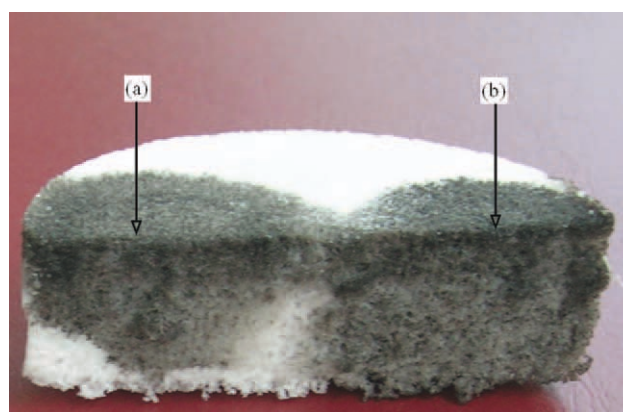


Figure 3 Photo of the ink seepage created by (a) 0.45 mL ink and (b) 0.65 mL ink. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

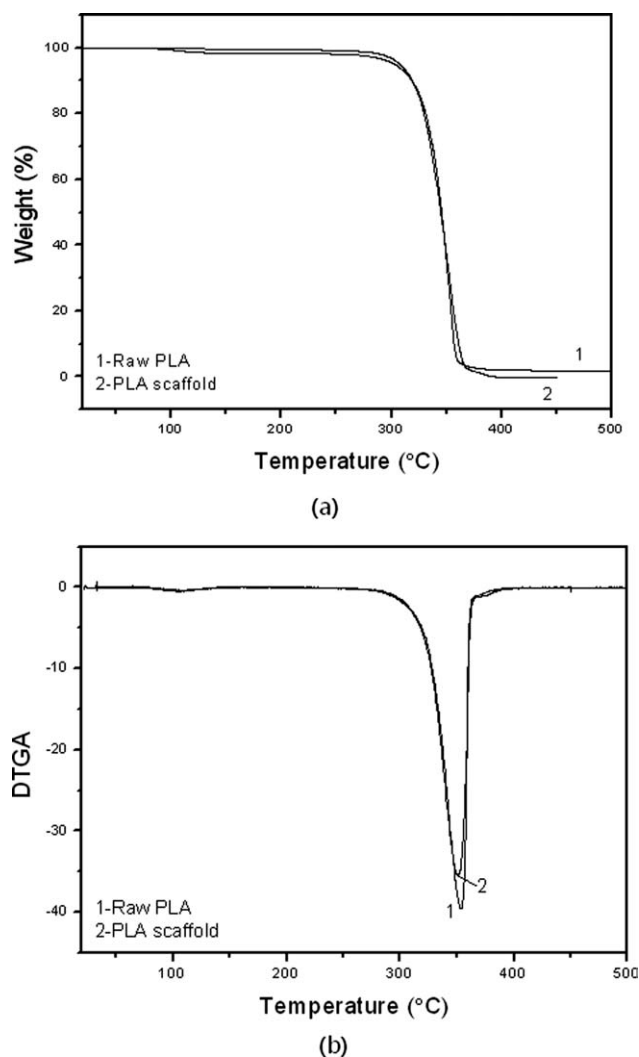


Figure 4 Plots derived from the TGA pyrolysis of raw PLA and PLA scaffolds: (a) TGA curves and (b) DTGA curves.

size of $453 \pm 18 \mu\text{m}$ at the bottom layer that gradually decreased to $166 \pm 47 \mu\text{m}$ at the top layer. It had been reported that this pore size range (from 100 to 500 μm) favors ingrowth of bone tissue cells and subsequent vascularization of calcinations.^{2,10,11}

Figure 5 shows the stress–strain curves of PLA scaffold layers and the whole graded macroporous PLA scaffold. The stress–strain curves were typical ones which showed linear elasticity at low stresses (typically less than 10%) followed by a relatively uniform increase in stress. When the strains were even higher, for example, 30–50%, the stress sharply increased. Figure 6 shows the variations in compressive moduli along the longitudinal direction of the PLA scaffold. The compressive moduli of the PLA scaffold decreased with increasing pore size, which agreed with that reported in the literature.^{7,12,13} The larger pore size leads to more heterogeneous mass distribution of PLA in the scaffold, so that the

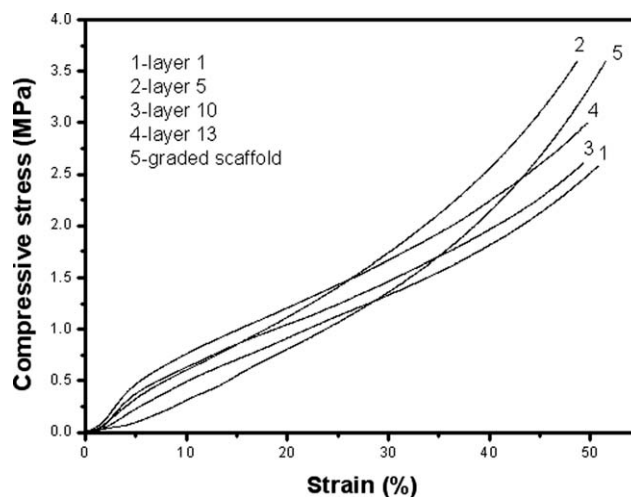


Figure 5 The stress–strain curves of PLA scaffolds.

mechanical property of the scaffold declines. On the other hand, the declining mechanical property was partly contributed to the fact that the porosity of the PLA was slightly increased with the increase of NaCl particle size. The graded PLA scaffold had a compressive modulus of $2.12 \pm 0.08 \text{ MPa}$ at the bottom layer that gradually increased to $2.56 \pm 0.18 \text{ MPa}$ at the top layer. The compressive modulus of the whole graded macroporous PLA scaffold was $2.18 \pm 0.24 \text{ MPa}$. We can deduce that the mechanical properties of the whole graded scaffold depend on the layers with low mechanical properties.

CONCLUSIONS

The PSC/PL approach was demonstrated to be successful for building porous PLA scaffolds with a graded macroporous structure along the longitudinal

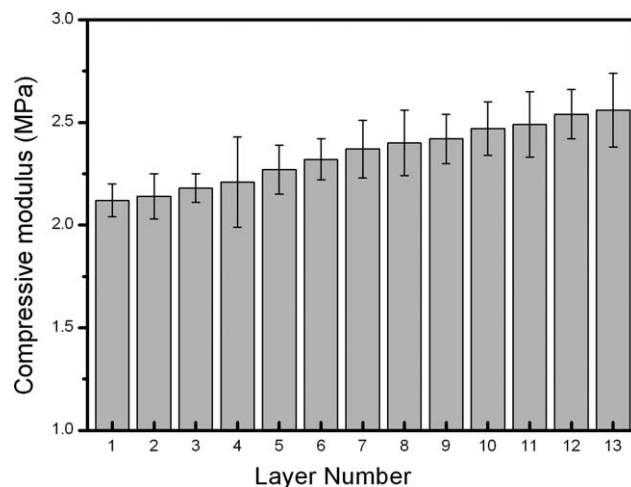


Figure 6 Variations in compressive modulus along the longitudinal direction of PLA scaffold sections.

direction in this study. The volume of PLA/NaCl depends mainly on the volume of NaCl. The porosity and pore size of the resultant scaffolds can be controlled by the salt-to-polymer ratio and the particle size, respectively. The larger pore size led to more heterogeneous mass distribution of PLA in the scaffold, and the porosity of the PLA scaffold layer was slightly increased with the increase of NaCl particle size, so that the compressive moduli of the PLA scaffolds decrease with pore size increasing. The results of TGA confirmed the complete removal of salts and solvent inside the graded macroporous PLA scaffold.

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