Preparation of Biodegradable Polymer Scaffolds with Dual Pore System for Tissue Regeneration

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Summary: Biodegradable polymer scaffolds that have dual pores on the matrix were successfully prepared using a combination of gas foaming and phase separation methods. PLLA and PLGA were dissolved in dioxane/water at the ratios of 90/10, 87/13, or 85/15 (v/v), respectively. Sodium bicarbonate was then added to the polymer solution and freezed-dried, then subjected to a gas foaming process. Dual pore scaffolds have two distinct pore sizes: large pores in 200∼300 μm and small ones in 5∼20 μm, with the interconnected structure. Their porosities were ranged from 92 to 98%, significantly higher than those of unipore scaffold (90%). Two variables, water content (10, 13, and 15%) and freeze-drying temperature (FDT; −196, −70, and −20 °C), were introduced during the scaffold preparation. Mechanical compressive strength decreased as the polymer solution was gradually diluted with water (100/0 to 85/15, v/v). Earlier weight loss of the different scaffolds was found with FDT at −20 °C but the final result was the same, regardless of different FDTs used. These dual pore polymer scaffolds may thus be useful in tissue engineering applications.

Keywords: biodegradable polymer; dual pore scaffold; gas foaming; sodium bicarbonate/water; tissue engineering

Introduction

Since the inception of tissue engineering, it has been devoted to making artificial tissues and organs, i.e., cartilage, bone, skin, tendon, liver, ureter, intestine, blood vessel, and peripheral nerve.[1] In general, three major components in tissue engineering are cell, growth factor, and scaffold. Among them, biodegradable polymer scaffolds are of importance as a three-dimensional (3D) structural platfom, where cells can attach, spread, proliferate, and differentiate. The main role of scaffolds is to deliver specific cells to a needed site in the body or to grow them into a target tissue in an *in vitro* condition. An implanted scaffold is to be fully replaced with a regenerated tissue, along with a timely biodegradation of scaffold itself. Polyesters, such as poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA), and their copolymer, poly(L-lactic-co-glycolic acid) (PLGA) are typical synthetic polymers, from which scaffolds are fabricated. While criteria for an optimal scaffold are rather dependent of the type of tissue to be regenerated, some structural requirements are waranted. Scaffold matrix should be highly porous with an interconnective pore structure to accomodate mass transport of large molecules and diffusional supply of nutrients around the matrix. Higher surface area is also advantageous for better cell attachment on the surface. Several methods have been developed to fabricate porous scaffolds: solvent casting and particulate leaching (SC/PL), fabrication of non-woven sheet, phase separation, gas foaming, emulsion freeze drying, and

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3D printing.[2–9] Scaffolds can also take various forms, such as nonwoven mesh, sponge, and micro/nanospheres. In this study, gas foaming and phase separation techniques were applied to fabricate a dual pore sponge-type scaffolds, in which they have two distinct pore sizes, large (200~300 μm) in the matrix and small (~20 μm) pores on the interconnecnted walls. As compared to single-sized unipore scaffolds, it is assumed that due to its structural feature, dual pore system may hold some advantages for, such as cell attachment, mass transport of macromolecules, and nutrient diffusion. For this purpose, several parameters, i.e., different 1,4-dioxane/water ratios and varying quenching temperatures were tested to find an optimized preparation condition of dual pore scaffolds.

Materials and Methods

Materials

Poly(L-lactic acid) (PLLA, Mw = 110,000) and poly(lactic-co-glycolic acid) (PLGA, 85:15 (wt/wt), Mw = 120,000) were purchased from Boehringer Ingelheim (Germany). Sodium bicarbonate, citric acid, and 1,4-dioxane were purchased from Aldrich (USA). Other chemicals were of reagent grade and used as received.

Preparation of Dual Pore Scaffolds

PLLA or PLGA was dissolved in dioxane/water solvent, which has different volume ratios, i.e., 90/10, 87/13, or 85/15 (v/v) and evenly agitated using a magnetic bar to obtain 5% polymer solutions. For unipore scaffold as a control, only dioxane (100/0, v/v) was used. Sodium bicarbonate was then put into the solution at the ratio of porogen-to-polymer to 20:1 (wt/wt). Homogeneously mixed, the viscous pastes were transferred to a disc-shaped silicon mold and freeze-dried at different temperatures of −196, −70, or −20 °C. After the disks were then briefly sonicated for 30 sec in 50% aqueous ethanol solution and they were immersed, stirred in 20% citric acid solution for 24 hr for gas foaming. The polymer scaffolds were washed several times with distilled water and vacuum-dried.

Characterization of Dual Pore Scaffolds

Surface morphology of the dual pore scaffolds was examined using a scanning electron microscopy (SEM, Hitachi, Tokyo, Japan). Samples were gold-coated in a sputter-coater (Eiko IB3, Tokyo, Japan) before the operation at 15 KV. To estimate the porosity of the dual pore scaffolds, a mercury intrusion porosimeter (PMI 60K, Porous Materials, Inc, USA) was used. For surface characterization, water contact angle was also measured using an optical bench type contact angle goniometer (VCA Optima XE Video Contact Angle System, Crest Technology Inc., USA). Mechanical test for the compressive strength of the dual pore scaffolds was carried out using an Instron machine (Model 5567, Canton, MA, USA). Disc-shaped samples that have a dimension of 2 cm in height and 1 cm in diameter were placed on the platen and the load was uniaxially applied. The maximum load was obtained when the scaffolds were compressed to 50% of its original thickness. Each compressive strength was calculated, based on the peak loads and surface areas of the samples. An accelerated biodegradation of the dual pore scaffolds was tested with phosphate-buffered saline (PBS) at 85 °C. Once the initial weights of the scaffolds were measured, they were immersed in the PBS solution and the remaining weights were measured at 1, 2, 3, and 4 days, respectively.

Results and Discussion

Characteristics of Dual Pore Scaffolds

Once dual pore scaffolds were prepared, their porosities were determined and compared in terms of varying solvent system of dixoane/water and multiple freeze-drying temperatures (FDT). Overall porosity was ranged from 92 to 98% for dual pore scaffolds and 90% for unipore ones (Table 1). When the FDT was fixed at −196 °C, more water content contributed to

Table 1.
Porosity of biodegradable scaffolds.

	PLLA		PLGA	
Pore system	FDT (Dioxane/H_2O, v/v)	Porosity (%)	FDT (Dioxane/H_2O, v/v)	Porosity (%)
Dual Pore	−196 °C (90/10)	93	−196 °C (90/10)	92
	−196 °C (87/13)	96	−196 °C (87/13)	93
	−196 °C (85/15)	98	−196 °C (85/15)	98
	−70 °C (90/10)	97	−70 °C (90/10)	96
	−20 °C (90/10)	98	−20 °C (90/10)	98
Unipore	−196 °C (100/0)	90	−196 °C (100/0)	90

the increased porosity and there was no significant difference with either PLLA or PLGA. It was interesting that lower FDT led to a higher porosity at the solvent ratio of 90/10 (v/v).

The ratio of dioxane/water was 85:15 (v/v), the pore size was irregular and the pore structure was poorly formed (Figure 1c). When the ratio was 90:10 (v/v), the scaffold had rather large pores but

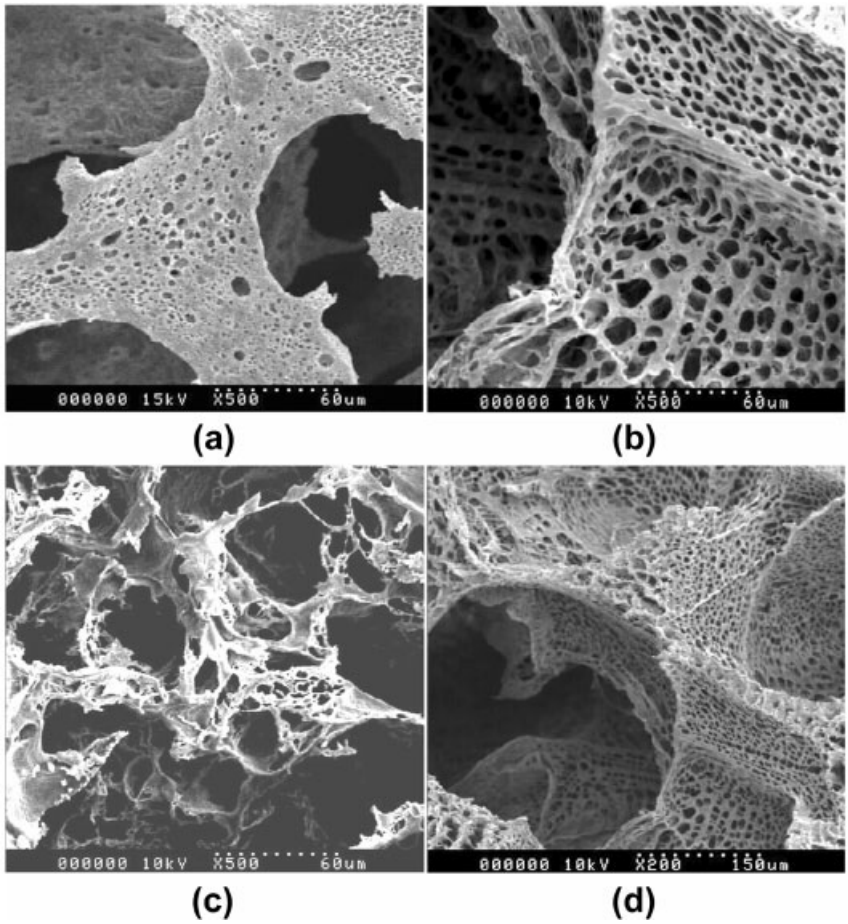

Figure 1.
SEM images of dual pore PLLA scaffolds. Each scaffold was prepared using different ratios (v/v) of dioxane and water: (a) 90:10, (b) 87:13, (c) 85:15, and (d) 87:13 (lower magnification).

its architecture does not appear to be well-defined in making pores on the interconnected walls (Figure 1a). It seemed that the ratio of 87:13 (v/v) was an optimal condition to produce a dual pore PLLA scaffold: the large pores (200∼300 μm) were uniformly distributed in the matrix by sodium bicarbonate, along with the smaller pores (∼20 μm) on the interconnecting wall by H_2O (nonsolvent) (Figure 1b & 1d).

It is postulated that while large pores were created in the gas foaming process, smaller ones could be developed during phase separation by the added nonsolvent, water. Since the network of small pores was created around the large pores, this unique architecture may provide much higher surface areas, which should be beneficial for cell seeding, cell attachment, nutrient diffusion, mass transport of macromolecules, and even vascular ingrowth into the implanted scaffold. The pore interconnectivity is greatly enhanced by the presence of the small pores, which form channels between the large pores. In fact, the final pore morphology is determined by the thermodynamic state of polymer solution prior to freeze-drying. As a nonsolvent volume fraction increases, polymer-diluent interaction becomes weaker and it would induce the formation of greater droplet domains. Therefore, increased water content in the solvent system tends to generate larger pores. Having appropriate mechanical strength is essential in scaffold material. When the mechanical property of the dual pore scaffolds was tested, the ratio of dioxane/water was a critical determinant for individual compressive strength (Figure 2). It was apparent that as the water content decreased, the strength significantly increased, regardless of the FDT. For instance, the strength of PLLA or PLGA at 85/15 (v/v) in dioxane/water was half that of 100/0 (v/v).

This result is closely related to the increased porosity and consequently reduced polymer content in the scaffold. Speaking of the relationship between porosity and mechanical property, there may be a conflict to harmonize the balance of the two. It is generally believed that a scaffold possess a porosity of 90% to not only provide sufficient area for cell adhesion but to allow diffusion and mass transport during tissue formation.[4] Goldstein et al., however reported that porosity of PLGA scaffold should not exceed 80% to be used for orthopaedic implants as it would otherwise compromise the mechanical stability of the scaffold.[10] These contradictory findings suggest that scaffolds need to be designed and fabricated to fit the requirement of target tissue.

In vitro Biodegradation

To evaluate the biodegradation behavior of dual pore polymer scaffolds, an accelerated

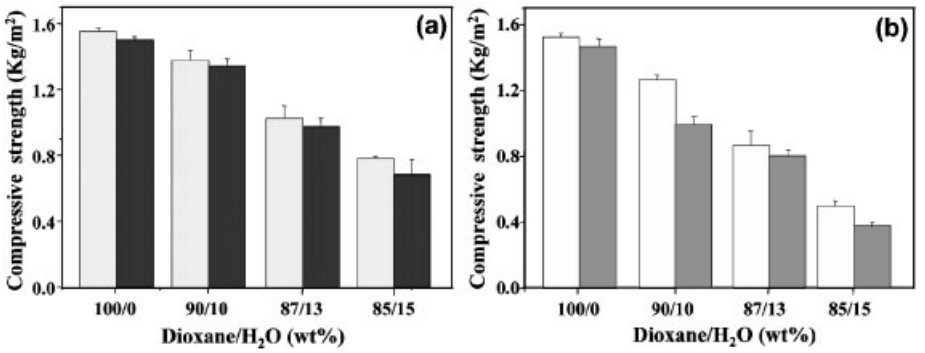

Figure 2.
Compressive strength of PLLA and PLGA scaffolds. Scaffolds in different ratios of dioxane and water were tested, with the given FDT at (a) −196 °C and (b) −70 °C. PLLA and PLGA are indicated in light (left) and dark (right) bars, respectively.

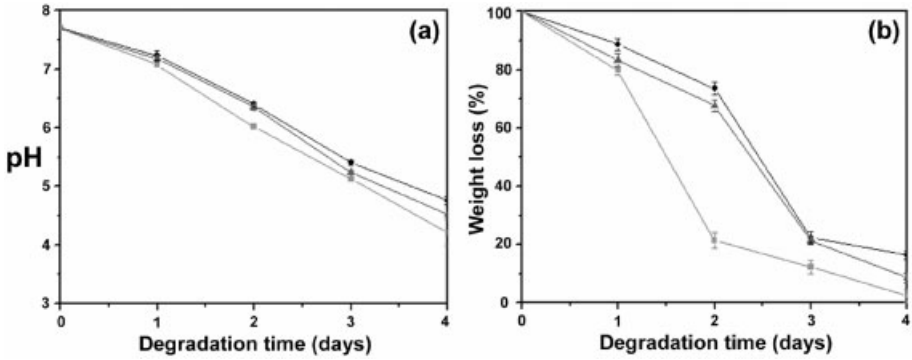

Figure 3.
Biodegradation test of dual pore PLLA scaffold: (a) pH change and (b) weight loss. Freeze-dried at −196 °C, scaffolds in different ratios (v/v) of dioxane and water were screened for 4 days: 90:10 (●), 87:13 (▲), and 85:15 (■).

degradation condition was used in hot PBS solution. Due to the byproduct of PLLA hydrolysis, lactic acid, pH change of PLLA gradually occurred, becoming more acidic during 4 days (Figure 3a). The difference of pH was barely noticed with varying ratios of dioxane/water. In addition to the pH measurement, the weight loss of each scaffold was also evaluated during the biodegradation test (Figure 3b). The notable is that the solvent ratio of 85/15 (v/v) experienced the largest loss of the initial weight as compared to the others. It occurred sharply in between 1 and 2 days, remaining 20% of the orignial mass, whereas the other ratios, 90/10 and 87/13 (v/v) showed relatively a gradual degradation for 2 days and then dropped sharply as well.

Finally, the extent of the weight loss appeared to be rather proprotional to the water content in the solvent system, leaving little mass for 85/15 (v/v) at 4 days. When the PLLA scaffolds were also tested for another variable, FDT, the weight loss was found ocurring earlier in between 1 and 2 days at −20 °C, but there was no significant effect of FDTs on the weight loss at the end of 4 days (Figure 4a). When PLGA scaffolds were examined in the same condition, as compared to PLLA, it was the most notable that the degradation was complete in 4 days, leaving no mass of PLGA (Figure 4b).

Upon the degradation profiles of both PLLA and PLGA, the condition at −20 °C appeared worse in the control of biodegradation than the other two FDTs, −70 and −196 °C. The control of biodegradation rate is of importance in making tissue-engineerd product *in vitro* as well as *in vivo*. Too fast degradation cause an early collapse of tissue construct, due to the loss of mechanical support. On the other hand, too late or nonbiodegradation could hinder tissue growth and maturation, acting as a defect in engineered tissues. As a matter of fact, the degradation behavior of scaffolds would be significantly affected by change of their porosity. Agrawal et al. found that PLGA (50/50, wt/wt) scaffold with a lower porosity (80 and 87%) could be degraded faster than higher one (92%) in static as well as in a fluid flow condition.[11] The test was carried out at 37 °C in PBS and the mass reduction was notabley visible past 2 weeks. At 4 weeks, 75 and 25% of the initial weight were left, when the scaffold porosities were 92 and 80%, respectively. In addition, Yoon et al. followed the degradation of porous PLGA (>90%) for up to 84 days and found the PLGA (50/50, wt/wt) weight at 4 weeks left in 70%.[7] The two studies share very similar results. However,

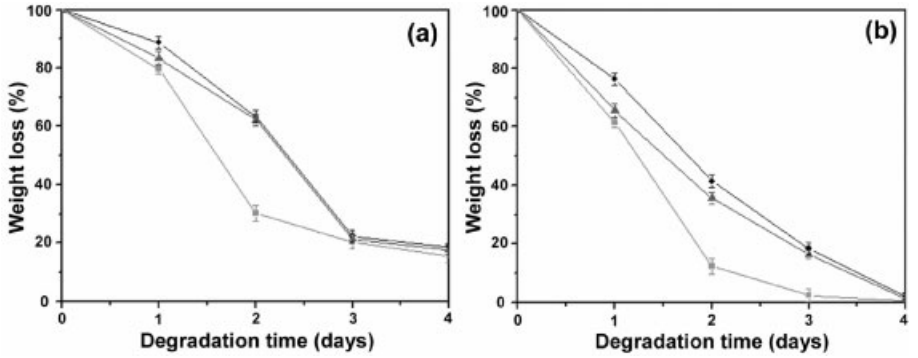

Figure 4.
Weight loss of dual pore PLLA (a) and PLGA (b) scaffolds. Scaffolds in different temperatures of freeze-drying were screened for 4 days: −196 °C (●), −70 °C (▲), and −20 °C (■).

those seem to be rather conflicting as compared to our results (Figure 3b), in which the higher porosity scaffold (85/15, v/v in dioxane/water) showed faster degradation in the early time points, presumably due to the structual features of the dual pore system and different temperature condition. It is noteworthy that biodegradation *in vitro* may not be in parallel with the behavior *in vivo*, owing to totally different environment in the body.

Conclusion

Biodegradable dual pore polymer scaffolds were successfully fabricated through gas foaming and phase separation techniques. Dual pore system carried two distinct pores: large pores of 200∼300 μm and small ones of 5∼20 μm. Their porosities were ranged from 92 to 98%, significantly higher than unipore scaffold (90%). When some variables during the preparation of scaffolds were tested, an optimal condition was set: dioxane/water ratio at 87/13 (v/v) and FDT at −196 °C. It was obvious that increased water content in the solvent system had a negative effect on biodegradation and mechanical property of scaffolds. The present work suggests that due to higher porosity and surface areas, the dual pore scaffold may be a beneficial platform for better cell attachment, advanced nutrient supply, and mass transport of macromolecules.

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