Poly(α-hydroxyl acids)/hydroxyapatite porous composites for bone-tissue engineering. I. Preparation and morphology

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Abstract: Tissue engineering has shown great promise for creating biological alternatives for implants. In this approach, scaffolding plays a pivotal role. Hydroxyapatite mimics the natural bone mineral and has shown good bone-bonding properties. This paper describes the preparation and morphologies of three-dimensional porous composites from poly(L-lactic acid) (PLLA) or poly(D,L-lactic acid-co-glycolic acid) (PLGA) solution and hydroxyapatite (HAP). A thermally induced phase separation technique was used to create the highly porous composite scaffolds for bone-tissue engineering. Freeze drying of the phase-separated polymer/HAP/solvent mixtures produced hard and tough foams with a co-continuous structure of interconnected pores and a polymer/HAP composite skeleton. The microstructure of the pores and the walls was controlled by varying the polymer concentration, HAP content, quenching temperature, polymer, and solvent utilized. The porosity increased with decreasing polymer concentration and HAP content. Foams with porosity as high as 95% were achieved. Pore sizes ranging from several microns to a few hundred microns were obtained. The composite foams showed a significant improvement in mechanical properties over pure polymer foams. They are promising scaffolds for bone-tissue engineering. © 1999 John Wiley & Sons, Inc. J Biomed Mater Res, 44, 446–455, 1999.

Key words: hydroxyapatite; polymer scaffold; tissue engineering; bone; composite

INTRODUCTION

Tissue engineering offers a promising new approach to repair of bone fractures with bone loss, fractures that do not heal, and fractures due to bone tumors.[1–3] In this approach, a porous scaffold that seeds the cells and serves as a template for tissue regeneration is necessary. The design of a scaffolding material can significantly affect the cell seeding and growth both *in vitro* and *in vivo*.[4] Scaffolding materials should be biocompatible and biodegradable. The degradation products should be nontoxic and must be easily excreted by metabolic pathways. The scaffolds should be osteoconductive so that osteoblasts attach and migrate on them, and they should be mechanically strong to maintain their structural integrity during culture. The ideal scaffolding materials also should be easy to fabricate into a desired shape, and they should have a controlled porous architecture to allow for cell growth, tissue regeneration, and vascularization.

Poly(α-hydroxyl acids), such as poly(L-lactic acid) (PLLA), poly(glycolic acid) (PGA), and poly(D,L-lactic acid-co-glycolic acid) (PLGA), satisfy many of these material requirements and already have been fabricated into scaffolds for cell transplantation and tissue engineering.[4–8] One of the disadvantages of these materials is that the degradation products reduce the local PH value, which, in turn, may accelerate the polyesters' degradation rates[9] and induce an inflammatory reaction. Another disadvantage is that the mechanical properties of the highly porous scaffolds made from poly(α-hydroxyl acids) are relatively weak, which limits their use for bone-tissue regeneration, especially in the *in vivo* implant site.

Hydroxyapatite (HAP) has been investigated as a bone replacement for a long time since the material mimics the natural bone mineral.[10–13] HAP has been studied extensively for cell cultures and has been found to possess good osteoconductive properties.[14–17] Compact PLLA/HAP composites (with little porosity) as bone fillers or implant materials have shown good bone-bonding properties.[18,19] By monitoring for 24 weeks the PH value variations during their incubation in phosphate-buffered saline (PBS), it has been found that the PH value of PLLA/HAP composite is more stable than that of pure PLLA and pure HAP.[20] The incorporation of synthetic HAP into a po-

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rous biodegradable poly(α-hydroxyl acid) is expected, for the following reasons, to result in a promising composite scaffold for bone-tissue engineering: (1) a better cell seeding and growth environment is expected because of the good osteoconductive properties provided by HAP; (2) the acidic degradation byproducts from polyesters may be buffered; and (3) the mechanical properties may be improved.

For cell transplantation and tissue engineering, a scaffold must be fabricated into a three-dimensional structure with a high porosity and an appropriate pore size. Various techniques have been utilized to prepare such highly porous scaffolds.[4–7,21,22] Recently, a new procedure for preparing polymer foam from polymer solution by thermally inducing phase separation (TIPS) and subsequent sublimation of solvent has generated considerable interest.[23,24] In this procedure, two phases—a polymer-rich phase and a polymer-lean phase—are formed by cooling down the polymer solution to induce liquid–liquid phase separation. The solvent then is removed by solvent extraction or sublimation *in vacuo* to form pores. In this paper, we report on the preparation and the characteristic pore morphologies of highly porous poly(α-hydroxyl acids)/HAP composite foams by solid–liquid phase separation of polymer/HAP/dioxane suspensions.

MATERIALS AND METHODS

Materials

Poly(L-lactic acid) with an inherent viscosity of approximately 1.6 was purchased from Boehringer Ingelheim (Ingelheim, Germany). Poly(D,L-lactic acid-co-glycolic aicd; 75/25) with an inherent viscosity of 0.5~0.65 was obtained from Medisorb Technologies International L. P. (Cincinnati, Ohio). These polymers were used without further purification. Dioxane and hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] were obtained from Aldrich Chemical Company (Milwaukee, Wisconsin).

Preparation of polymer/hydroxyapatite mixture

PLLA or PLGA was weighed accurately into a flask, and then an accurately measured amount of dioxane or dioxane/water mixture was added to the flask to make a solution with a desired concentration of from 1% to 7.5% (w/v). The mixture was stirred at 50°C for 2 h to obtain a homogeneous polymer solution. HAP powder was added into the prepared solution to make a polymer/HAP mixture. The final composition of PLLA/HAP composite foam was determined by the concentration of the polymer solution and HAP content in the mixture.

Polymer/HAP composite foam fabrication

The PLLA/HAP/composite foam was prepared by solid–liquid phase separation and subsequent sublimation of solvent. Typically, a foam was prepared with the following steps: 10 mL of PLLA/HAP/dioxane mixture was put into a beaker (30 mL, prewarmed to 50°C). The beaker containing the mixture then was rapidly transferred into a refrigerator or a freezer at a preset temperature to solidify the solvent and induce solid–liquid phase separation. The solidified mixture was maintained at that temperature for 2 h and then immersed into liquid nitrogen to deep freeze the mixture. The frozen mixture was transferred into a freeze-drying vessel at a temperature between −5°C and −10°C in an ice/salt bath. The samples were freeze dried at 0.5 mmHg for at least 4 days to completely remove the solvent. Ninety nine percent of the solvent was removed in one day, and a constant sample weight was achieved within 4 days. The foam samples then were stored in a desiccator until characterization.

Characterization

The density and porosity values of the composite foams prepared from polymer/HAP/dioxane mixtures were measured by liquid displacement, similar to a published method.[25] In this method water was used as the displacement liquid. However, we found water was difficult to work with because it did not penetrate very easily into the pores, probably due to the hydrophobicity of the polymers. Thus ethanol was used in our procedure because it penetrated easily into the pores and did not induce shrinkage or swelling as a nonsolvent of the polymers. A foam sample of weight W was immersed in a graduated cylinder containing a known volume (V_1) of ethanol. The sample was kept in the ethanol for 5 min, and then a series of brief evacuation–repressurization cycles was conducted to force the ethanol into the pores of the foam. Cycling was continued until no air bubbles were observed emerging from the foam. The total volume of ethanol and the ethanol-impregnated foam then was recorded as V_2. The volume difference, ($V_2 - V_1$) was the volume of the polymer/HAP composite skeleton of the foam. The ethanol-impregnated foam was removed from the cylinder and the residual ethanol volume recorded as V_3. The quantity ($V_1 - V_3$)—the volume of the ethanol held in the foam—was determined as the void volume of the foam. Thus the total volume of the foam was: $V = (V_2 - V_1) + (V_1 - V_3) = V_2 - V_3$. The density of the foam (d) was expressed as:

$$d = W/(V_2 - V_3)$$

and the porosity of the foam (ε) was obtained by:

$$\varepsilon = (V_1 - V_3)/(V_2 - V_3)$$

The porous morphologies of the composite foams were studied with scanning electron microscopy (SEM; S-3200N, Hitachi, Japan) at 15 kV. The specimens were cut with a razor blade after being frozen in liquid nitrogen for 5 min, and then they were coated with gold using a sputter coater

(Desk-II, Denton Vacuum Inc). The gas pressure was less than 50 mtorr, and the current was about 40 mA. The coating time was 200 s.

The compressive mechanical properties of the foams were tested with an Instron 4502 mechanical tester (Instron Co., Canton, Massachusetts). A PLLA/HAP/dioxane mixture was cast into a circular flat-bottom Teflon vial and phase separated to produce circular disks (~16 mm in diameter) for mechanical testing. The top layer of the disk was removed to achieve the desired thickness (~3 mm) and ensure a flat surface. The aspect ratio of these mechanical testing specimens was chosen to match the cell-scaffolding constructs used to engineer bone tissue in our lab. A crosshead speed of 0.5 mm/min was used. The compressive modulus was defined as the initial linear modulus. The yield strength was determined from the cross point of the two tangents on the stress–strain curve around the yield point. At least five specimens were tested for each sample. The averages and standard deviations were graphed. A two-tail Student's t test (assuming equal variances) was performed to determine the statistical significance ($p < 0.05$) of the differences in mechanical properties.

RESULTS

High porosity (low density) PLLA/HAP and PLGA/HAP composite foams have been prepared by solid–liquid phase separation and subsequent sublimation of the solvent (Table I). The density increases with polymer concentration and HAP content. In parallel, porosity decreases with increasing polymer concentration and HAP content. Phase-separation temperature does not show obvious effects on the porosity of the polymer/HAP foams in the composition range studied. The densities of PLGA/HAP foams are slightly higher than those of PLLA/HAP foams prepared from the same polymer concentration, HAP content, and processing conditions.

A typical SEM micrograph of the PLLA/HAP composite foam prepared from 2.5% (w/v) PLLA solution with a quenching temperature of −18°C shows co-continuous structure of interconnected irregular pores and a polymer/HAP composite skeleton [Fig. 1(a)]. The irregular pores range from several microns up to about 300 microns. The walls of the pores are composed of both PLLA and HAP [Fig. 1(b)]. The HAP platelets ranging from 10 to 100 μm in size [Fig. 1(c)] are randomly distributed in the PLLA matrix.

The morphology of PLLA/HAP foam is much different from pure PLLA foam [Fig. 1(d)] prepared with the same procedure. The PLLA foam prepared from solid–liquid phase separation of the PLLA/dioxane solution has a highly anisotropic tubular morphology with an internal ladder-like structure. This ladder-like structure is a characteristic morphology of the foams formed by solid–liquid phase separation of a polymer solution.[26] The channels are parallel to the direction of solidification (heat transfer direction). Each channel has repeating partitions with uniform spacing perpendicular to the solidification direction. The diameter of the channels and the spacing between repeating partitions in the channel change with cooling rate and with the polymer concentration. The solid–liquid phase separation is attributed to the crystallization of the solvent. When the temperature of the polymer solution is lower than the freezing point (crystallization temperature) of the solvent, the crystallization of the solvent takes place and the polymer phase is expelled from the crystallization front as ''impurities.'' A continuous polymer-rich phase is formed by aggregation of polymer expelled from every single solvent crystal.

TABLE I
Densities and Porosities of PLLA/HAP and PLGA/HAP Foams Prepared From PLLA/HAP/Dioxane and PLGA/HAP/Dioxane Mixtures

Polymer Concentration	Composition	Quenching Temperature (°C)	Density g/cm^3	Porosity
2.5%(w/v)	PLLA/HAP: 100/0	−18	0.045	94.8%
2.5%(w/v)	PLLA/HAP: 90/10	−18	0.049	93.4%
2.5%(w/v)	PLLA/HAP: 70/30	−18	0.060	92.5%
2.5%(w/v)	PLLA/HAP: 50/50	−18	0.090	89.9%
2.5%(w/v)	PLLA/HAP: 30/70	−18	0.120	85.1%
5.0%(w/v)	PLLA/HAP: 100/0	−18	0.083	92.7%
5.0%(w/v)	PLLA/HAP: 90/10	−18	0.086	91.7%
5.0%(w/v)	PLLA/HAP: 70/30	−18	0.110	91.0%
5.0%(w/v)	PLLA/HAP: 50/50	−18	0.144	89.2%
5.0%(w/v)	PLLA/HAP: 30/70	−18	0.203	86.6%
2.5%(w/v)	PLLA/HAP: 100/0	liquid nitrogen	0.043	92.5%
2.5%(w/v)	PLLA/HAP: 50/50	liquid nitrogen	0.085	88.0%
2.5%(w/v)	PLLA/HAP: 100/0	8	0.047	95.6%
2.5%(w/v)	PLLA/HAP: 50/50	8	0.090	90.9%
2.5%(w/v)	PLGA/HAP: 50/50	−18	0.126	87.5%
5.0%(w/v)	PLGA/HAP: 50/50	−18	0.151	85.7%

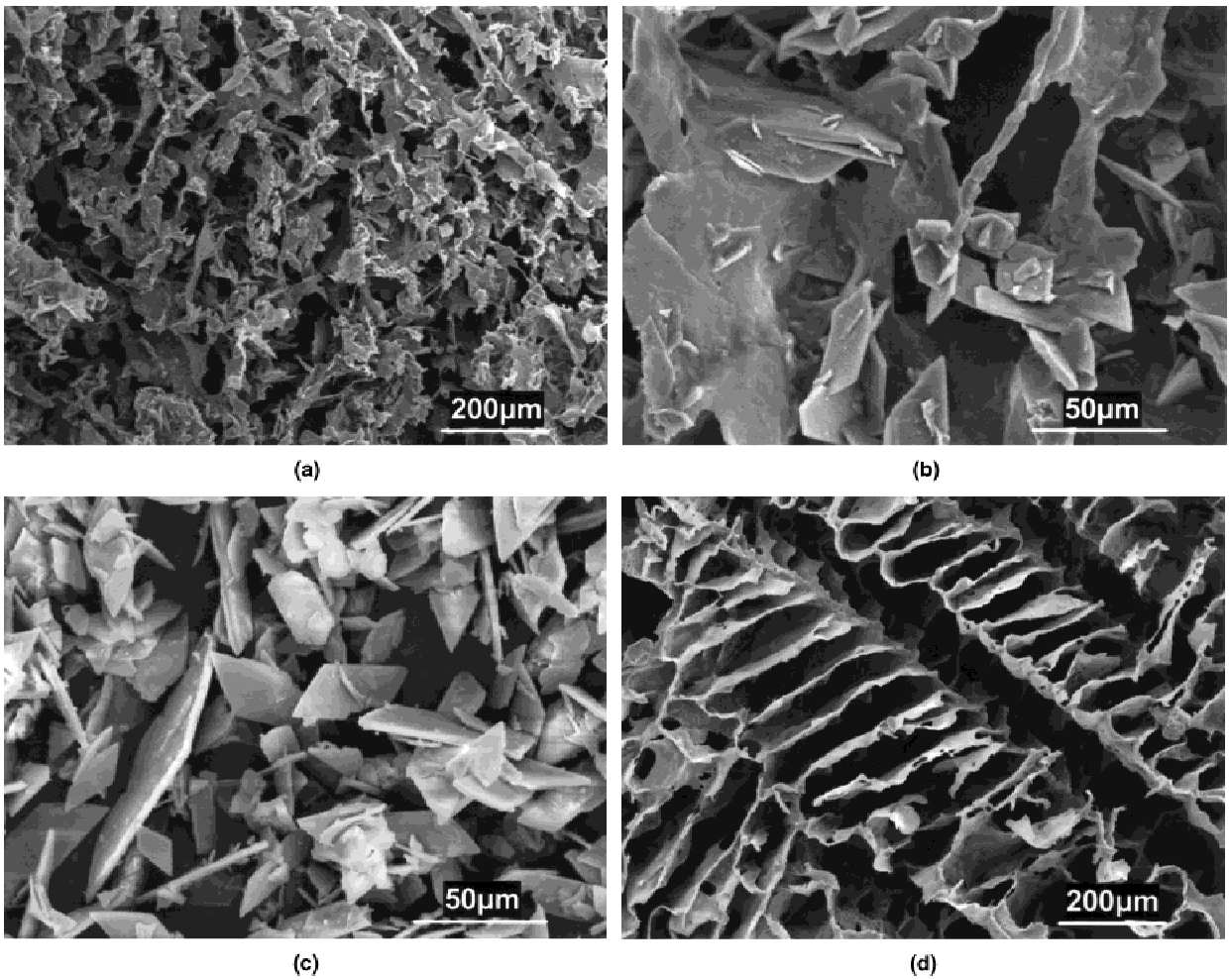

Figure 1. SEM micrographs of PLLA/HAP foam (PLLA/HAP: 50/50) prepared from 2.5% (w/v) PLLA/HAP/dioxane mixture (quenching temperature: −18°C), PLLA foam from 2.5% (w/v) PLLLA/dioxane solution (quenching temperature: −18°C), and HAP particles. Original magnifications: (a) PLLA/HAP, ×100; (b) PLLA/HAP, ×500; (c) HAP, ×500; (d) PLLA, ×100.

After solvent crystals have been sublimated, foam is formed with pores, similar to the geometry of solvent crystals. The temperature gradient along the solidification direction (from sample surface to sample center) may have led to the highly anisotropic pore structure.

When HAP is introduced into the PLLA/dioxane solution, the crystallization of solvent is perturbed by the existing solid HAP particles. The randomly distributed HAP particles change the solvent crystallization front by impeding the crystal growth, making the crystals of the solvent more irregular. On the other hand, both polymer and HAP particles are expelled from the crystallization front, forming a PLLA/HAP-rich phase. After sublimation of the solvent, this polymer/HAP-rich phase forms a continuous skeleton for the PLLA/HAP foam, and the spaces taken by solvent crystals become pores of the foam. As a result of irregular solvent crystal growth, the pores become irregular (more isotropic), and no channel structure or repeating partitions are observed.

Good adhesion between PLLA matrix and HAP particles also is observed [Fig. 1(b)]. Most of the HAP particles bonded to PLLA matrix are on the surfaces of the solid walls of the pores. These HAP surfaces may provide a better environment for osteoblast attachment and growth when used as scaffolding for bone tissue engineering.

To study the effect of polymer concentration on the foam structure, a series of PLLA/HAP composite foams were prepared from PLLA/HAP/dioxane mixtures, with PLLA concentration ranging from 1.0% to 7.5% (w/v). The ratio of PLLA to HAP was kept at one. The composite foam made from 1.0% PLLA solution was composed of bonded very thin PLLA leaflets [Fig. 2(a,b)]. Almost all the HAP particles settled at the bottom of the sample, presumably due to the low viscosity of the PLLA solution. The foams prepared from 5.0% and 7.5% PLLA solution were very hard and tough. SEM observation shows that the pore structure of the foam from the 7.5% PLLA solution is

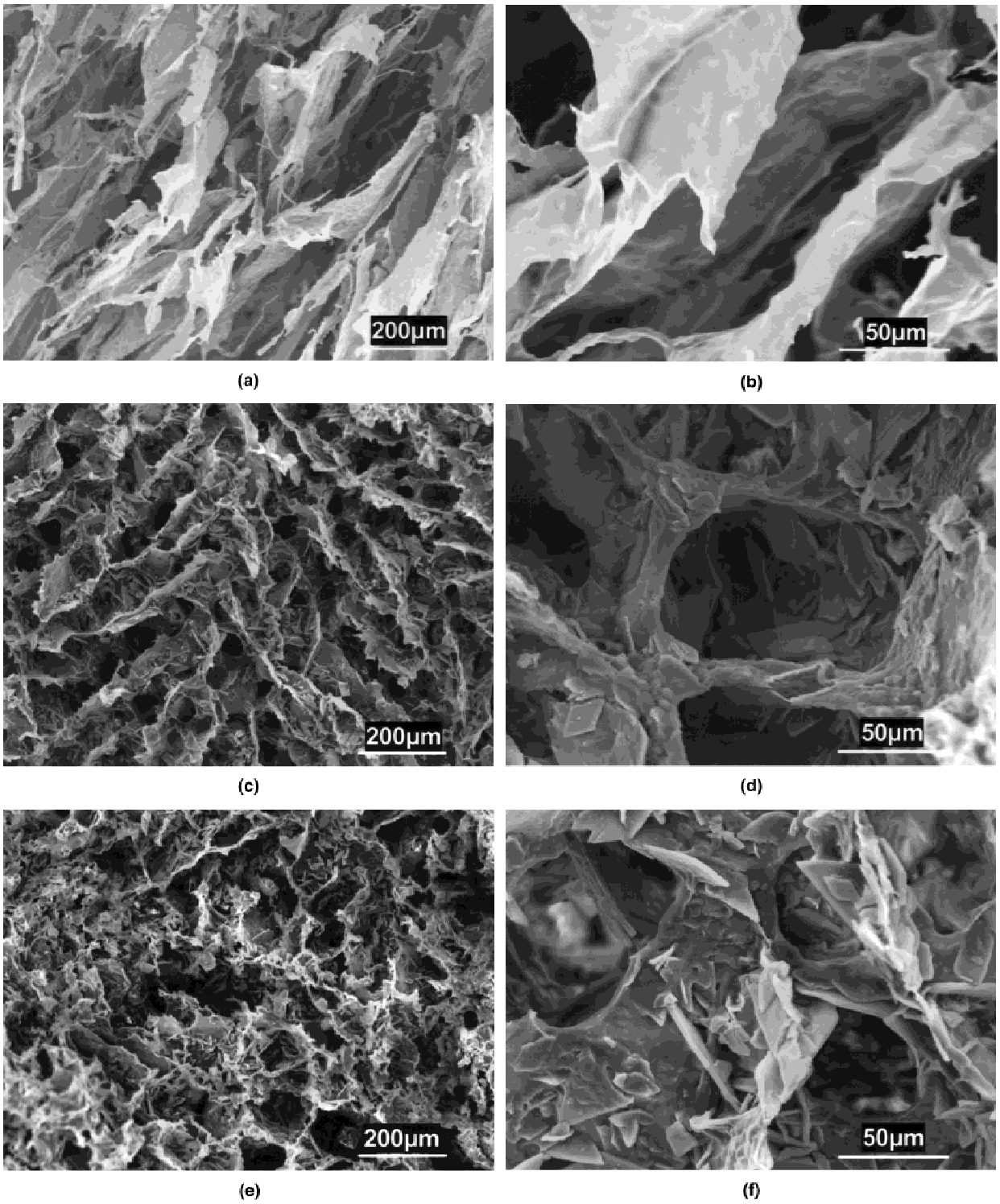

Figure 2. SEM micrographs of PLLA/HAP foams (PLLA/HAP: 50/50) prepared from PLLA/HAP/dioxane mixtures with different PLLA concentrations (quenching temperature: −18°C). Original magnifications: (a) 1.0% (w/v) PLLA/dioxane, ×100; (b) 1.0% (w/v) PLLA/Dioxane, ×500; (c) 5.0% (w/v) PLLA/dioxane, ×100; (d) 5.0% (w/v) PLLA/dioxane, ×500; (e) 7.5% (w/v) PLLA/dioxane, ×100; (f) 7.5% (w/v) PLLA/dioxane, ×500.

almost the same as the foam from the 5.0% PLLA solution [Fig. 2(c–f)], and the HAP particles are uniformly distributed. The foams from 5.0% and 7.5% PLLA solutions have morphologies slightly different from the foam made from a 2.5% PLLA solution [Fig. 1(a,b)]. The pore structure is more uniform, with pore sizes ranging from about 50 to 200 microns, and the pore walls are thicker than that of foam from the 2.5%

PLLA solution. Compared with the pore structure of a foam from the 5.0% PLLA solution, the pore size of the foam derived from the 7.5% PLLA solution is smaller and the walls of the pores are thicker.

The effects of HAP content on the structure of PLLA/HAP foams also have been investigated by varying the HAP amount in the PLLA/HAP foams while maintaining the PLLA concentration constant. SEM observation shows that the micropore structure of the foam changes considerably with the HAP content (Fig. 3). When HAP content is low, regular channels and a ladder-like structure similar to those in pure PLLA foam are observed. With increasing HAP content, the pore structure becomes more and more irregular. When the HAP/polymer ratio is higher than 1:1 (50 wt % HAP content), the pore structure becomes so irregular that no regular channels or ladder-like structure is observed. These results have demonstrated that the pore structure of the PLLA foam can be modified by the incorporation of HAP.

In the preparation of polymer foam by solid–liquid phase separation from polymer solution, quenching temperature (cooling rate) is an effective factor in controlling the morphology of the foam since the crystalline morphology of a solvent depends on the crystallization temperature. In this work, the effects of quenching temperature on the structure of PLLA/HAP foam from PLLA/HAP/dioxane mixtures are studied. The crystallization process induces two stages—nucleation and growth. Generally, a higher degree of supercooling (at a lower temperature) induces a high nucleation rate and a low crystal growth rate, which leads to the formation of a large number of small crystals. In contrast, a relatively lower degree of supercooling (at a higher temperature) includes a low nucleation rate and a high crystal growth rate, which leads to a small number of large crystals. The freezing point of dioxane is about 12°C. When the temperature of the PLLA/HAP/dioxane mixture is lower than this temperature, crystallization of dioxane takes place.

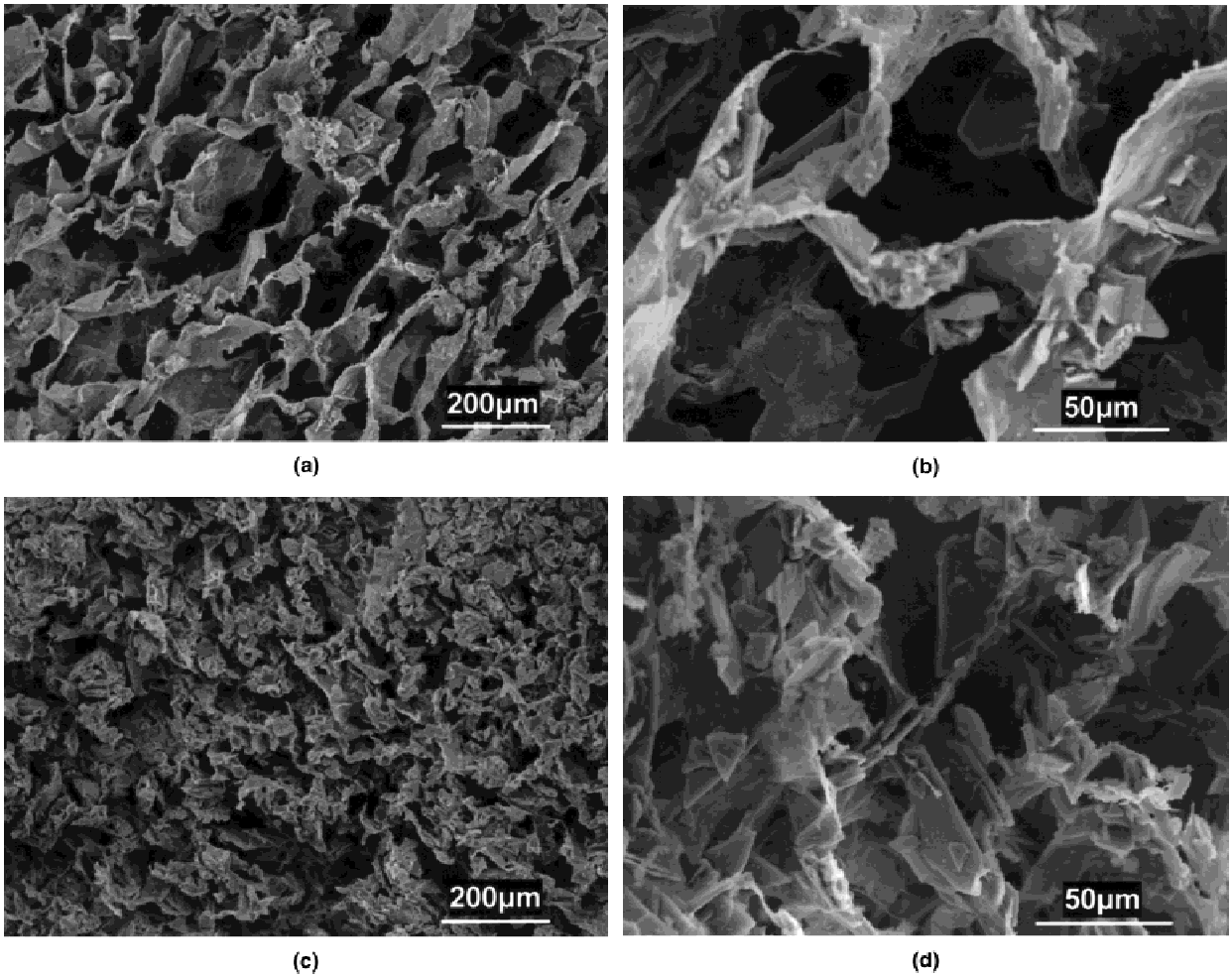

Figure 3. SEM micrographs of PLLA/HAP foams prepared from 2.5% (w/v) PLLA/HAP/dioxane mixtures with different HAP contents (quenching temperature: −18°C). Original magnifications: (a) PLLA/HAP: 70/30, ×100; (b) PLLA/HAP: 70/30, ×500; (c) PLLA/HAP: 30/70, ×100; (d) PLLA/HAP: 30/70, ×500.

Figure 4(a,b) shows the SEM micrographs of PLLA/HAP foam formed by quenching the mixture to 8°C, which is slightly lower than the freezing point of dioxane. At this temperature, the degree of supercooling of the dioxane is very low, so the crystallization of the dioxane has a low nucleation and a high growth rate, which leads to large solvent crystal formation and thereby a PLLA/HAP foam with large pore sizes, up to 600 microns. The pore wall of the foam is thicker than those prepared at lower temperatures. When the PLLA/HAP/dioxane mixture is quenched with liquid nitrogen, the microstructure of the foam formed is much different from that of the foams prepared at higher temperatures. In contrast to the regular ladder-like microstructure prepared from pure PLLA solution at the same temperature, small channels with relatively irregular rows parallel to the solidification direction but few regular horizontal partitions are observed for the PLLA/HAP composites [Fig. 4(c,d)]. The HAP particles randomly intersect the PLLA channels. The high nucleation rate and low crystal growth rate of dioxane at this quenching temperature and the greater temperature gradient in the direction of solidification may be responsible for the foam morphology.

The composite foams can be prepared from HAP and poly(α-hydroxyl acids) other than PLLA with the aforementioned procedure. Figure 5 shows the micropore morphology of the PLGA/HAP foam produced from a PLGA/HAP/dioxane mixture with a PLGA concentration of 2.5% (w/v). The microstructure of the PLGA/HAP foam is similar to that of PLLA/HAP foam from the same polymer concentration. The pore size, ranging from 30 to 100 microns, is slightly smaller than that of PLLA/HAP foam prepared from the same concentration and at the same temperature [Fig. 1(a,b)]. The densities of the PLGA/HAP foams are higher than those of corresponding PLLA/HAP foams. These results show that the shrinkage of the PLGA/HAP foam is higher than that of the PLLA/HAP foam. The shrinkage is attributed to the molecu-

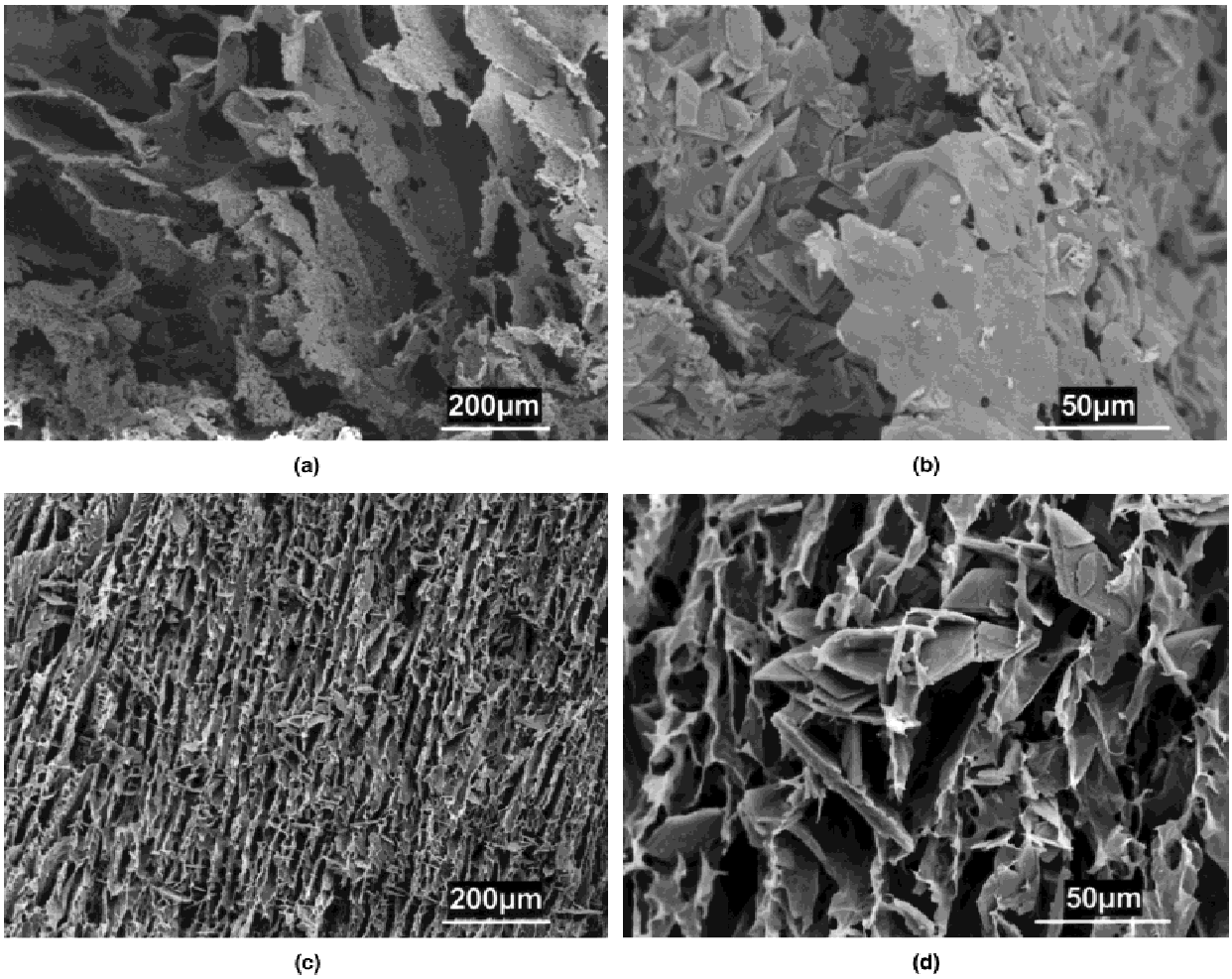

Figure 4. SEM micrographs of PLLA/HAP foams prepared form 2.5% (w/v) PLLA/HAP/dioxane mixtures by different quenching temperatures. Original magnifications: (a) 8°C, ×100; (b) 8°C, ×500; (c) liquid nitrogen, ×100; (d) liquid nitrogen, ×500.

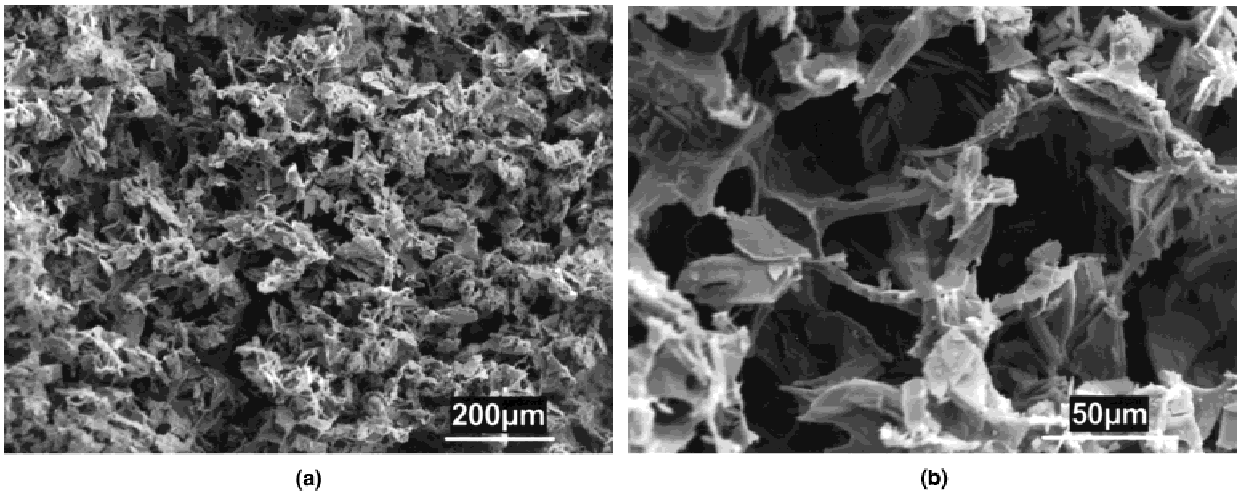

Figure 5. SEM micrographs of PLGA/HAP foam (PLGA/HAP: 50/50) prepared from 2.5% (w/v) PLGA/HAP/dioxane mixtures (quenching temperature: −18°C). Original magnifications: (a) PLGA/HAP: 50/50, ×100; (b) PLGA/HAP: 50/50, ×500.

lar rearrangement of the polymer chains that occurs during freeze drying. The amorphous PLGA may rearrange more easily and is less stable than the semicrystalline PLLA during freeze drying. In any event, the shrinkage of most poly(α-hydroxyl acids)/HAP composite foams prepared is less than 10%.

Solvent effects on the structure of polymer/HAP foams are studied by comparing the PLLA/HAP foams prepared from dioxane solution with those prepared from a dioxane/H_2O mixture solution. A very different morphology of the foams prepared from PLLA/HAP/dioxane/H_2O (dioxane/H_2O = 90/10) mixtures is shown in Figure 6. A fiber-like network intersected by HAP particles is obtained. The pores are relatively uniform, with a pore size of about 10 microns. The addition of H_2O (a nonsolvent) reduces the solubility of PLLA, and the liquid–liquid phase separation of the PLLA solution may have occurred before solvent freezing (solid–liquid phase separation) during the cooling process. The interesting fiber-like network microstructure may be attributed to the liquid–liquid phase separation. The fibers anchor onto the HAP particles [Fig. 6(b)], showing good adhesion between PLLA and HAP.

The mechanical properties of a porous PLLA/HAP composite are compared to those of the corresponding PLLA foam (Fig. 7). Both the compressive modulus and the compressive yield strength of the composite foam are significantly higher than those of the PLLA foam ($p < 0.05$). These data demonstrate the positive effects of the hydroxyapatite in enhancing the mechanical performance of the scaffolds.

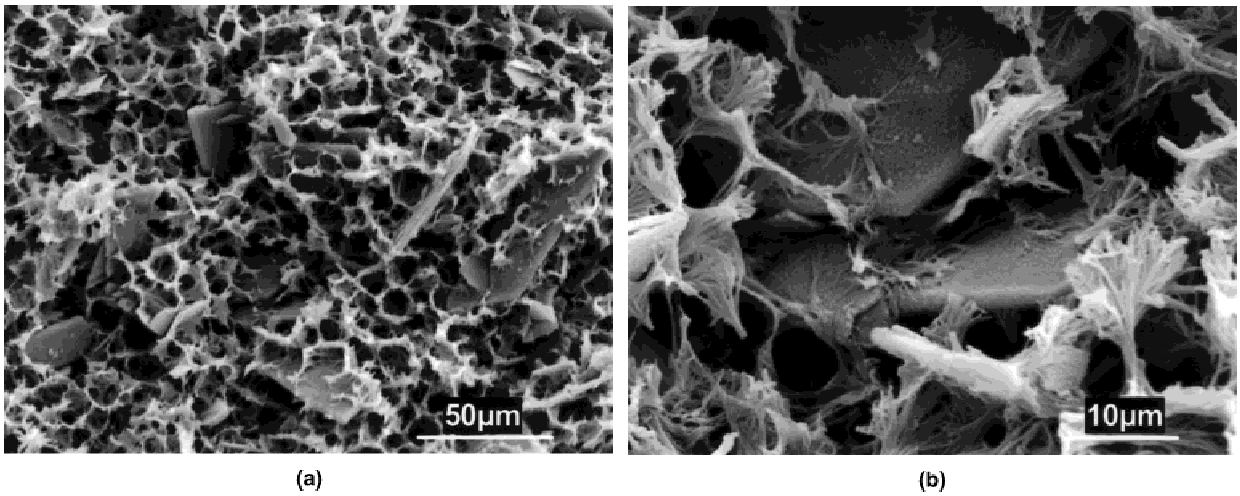

Figure 6. SEM micrographs of PLLA/HAP foam (PLLA/HAP: 50/50) prepared from 5.0% (w/v) PLLA/HAP/dioxane/H_2O (dioxane/H_2O: 90/10) mixtures (quenching temperature: −18°C). Original magnifications: (a) PLLA/HAP: 50/50, ×500; (b) PLLA/HAP: 50/50, ×2000.

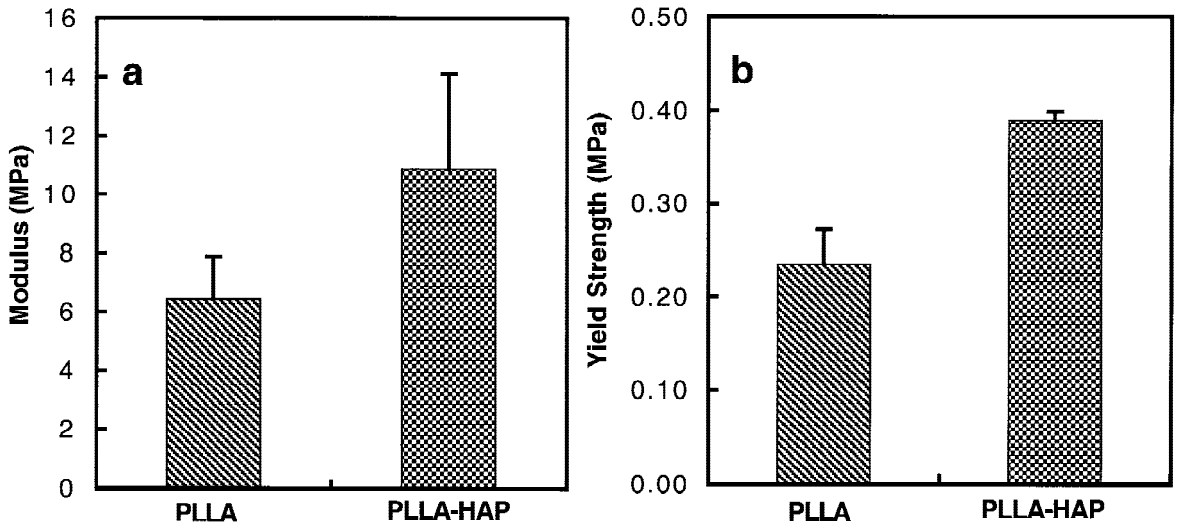

Figure 7. The compressive modulus and yield strength of the PLLA foam and PLLA/HAP composite foam prepared from 5% PLLA/dioxane solution: (a) PLLA foam; (b) PLLA/HAP (50/50) composite foam.

DISCUSSION

The effort to find bone substitutions for the repair of seriously damaged human body parts dates back centuries. Metals have been the primary materials for bone replacement in spite of the dangerous ions release *in vivo* from many of those metallic alloys. Only in the past quarter century have ceramics and polymers gained increasing attention as bone replacement and repair materials. The mineral fraction of the bone is constituted of hydroxyapatite. Therefore, hydroxyapatite, along with other bioceramics and bioglasses, has been studied extensively as a bone repairing material and is used as a coating for implanted prostheses to enhance the direct adhesion of bone tissue.[27]

Biodegradable polymers, such as PGA, PLLA, and PLGA, have been studied widely as scaffolding materials for tissue engineering,[4–8,28–30] including bone-tissue engineering.[29,30] A salt-leaching technique has been well documented for the generation of porous foams of these degradable polymers.[4,5,31,32] A PLGA (75:25) was processed into porous foam and cultured with stromal osteoblastic cells by Ishaug et al.[30] The cells proliferated and secreted mineralized matrix. One of the limitations was that the mineralized matrix was formed only at the surface layer of the foam (~240 μm). Composite foam of PLGA (50:50) and hydroxyapatite was fabricated with the salt-leaching technique by Laurencin and co-workers.[33,34] In a 21-day osteoblast culture on the PLGA/HAP composite matrix, the cell attachment, function, and mineral formation showed some promising features of the HAP-containing matrix. However, the matrix porosity was quite low (probably around 30% porosity, based on the mixture used to make the matrix: PLGA:HAP:NaCl = 1:1:1), which might not be ideal for long-term cell survival, proliferation, and tissue formation due to mass transport limitations.

In this work, a novel solid–liquid phase separation technique was used to create highly porous biodegradable poly(α-hydroxyl acids)/hydroxyapatite composite scaffolds. It is demonstrated that the porosity, pore size, and pore morphology of these composite foams can be controlled by the polymer concentration, chemical structure of the polymer, hydroxyapatite content, phase-separation temperature, and solvent used. The high porosity is expected to better satisfy the cell penetration and mass transport requirements (for nutrient, metabolites, and soluble signals) for scaffolding for tissue engineering.[4,6,7] Improved mechanical properties of the composite scaffolds over the pure polymer foams also are demonstrated. A more detailed study of structure–property relationships is underway and will be reported separately together with the degradation behavior, osteoblast function, and bone tissue formation in these novel composite scaffolds.

CONCLUSIONS

New highly porous composites composed of biodegradable poly(α-hydroxyl acids) and hydroxyapatite have been developed for bone-tissue engineering by thermally induced solid–liquid phase separation and subsequent solvent sublimation. A series of characteristic interconnected open pore microstructures with pore sizes ranging from several microns to a few hundred microns were created. Porosity as high as 95% was achieved. The microstructure of the composite

foams can be controlled by varying: (1) the concentration of the polymer solution, (2) the content of the HAP, (3) the quenching temperature (cooling rate), and (4) the polymer and solvent utilized. The composite foams showed enhanced mechanical properties over the pure polymer foams. The degradation behavior, detailed structure–property relationships, cell adhesion, and growth onto the composite scaffolds are under investigation and will be reported separately.

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References

1. Yaszemski M, Payne R, Hayes W, Langer R, Mikos A. Evolution of bone transplantation: Molecular, cellular and tissue strategies to engineer human bone. Biomaterials 1996;17(2): 175–185.
2. Crane G, Ishaug S, Mikos A. Bone tissue engineering. Nat Med 1995;1(12):1322–1324.
3. Vacanti C, Vacanti J. Bone and cartilage reconstruction with tissue engineering approaches. Otolaryngol Clin N Am 1994;27(1):263–276.
4. Ma PX, Langer R. Fabrication of biodegradable polymer foams for cell transplantation and tissue engineering. In: Yarmush M, Morgan J, editors. Tissue engineering. Totowa, New Jersey: Humana Press Inc.; 1998.
5. Mikos AG, Thorsen AJ, Czerwonka LA, Bao Y, Langer R, Winslow DN, Vacanti JP. Preparation and characterization of poly(L-lactic acid) foams. Polymer 1994;35(5):1068–1077.
6. Ma PX, Langer R. Degradation, structure and properties of fibrous nonwoven poly(glycolic acid) scaffolds for tissue engineering. In: Mikos AG, Leong KW, Yaszemski MJ, Tamada JA, Radomsky ML, editors. Polymers in medicine and pharmacy. Materials Research Society; Pittsburgh: 1995. p 99–104.
7. Ma PX, Schloo B, Mooney D, Langer R. Development of biomechanical properties and morphogenesis of in vitro tissue-engineered cartilage. J Biomed Mater Res 1995;29(12):1587–1595.
8. Shinoka T, Ma PX, Shum-Tim D, Breuer CK, Cusick RA, Zund G, Langer R, Vacanti JP, Mayer JE Jr. Tissue-engineered heart valves. Autologous valve leaflet replacement study in a lamb model. Circulation 1996;94(9):164–168.
9. Vert M, Mauduit J, Li S. Biodegradation of PLA/GA polymers: Increasing complexity. Biomaterials 1994;15(15):1209–1213.
10. Akao M, Aoki H, Kato K. Mechanical properties of sintered hydroxyapatite for prosthetic applications. J Mater Sci 1981;16: 809–812.
11. Dewith G, van Dijk HJA, Hattu N, Prijs K. Preparation, microstructure and mechanical properties of dense polycrystalline hydroxyapatite. J Mater Sci 1981;6:1592–1598.
12. Driessen AA, Klein CP, de Groot K. Preparation and some properties of sintered beta-whitlockite. Biomaterials 1982;3(2): 113–116.
13. Best S, Bonfield W. Processing behaviour of hydroxyapatite powders with contrasting morphology. J Mater Sci: Mater Med 1994;5(8):516–521.
14. Akao M, Sakatsume M, Aoki H, Takagi T, Sasaki S. In vitro mineralization in bovine tooth germ cell cultured with sintered hydroxyapatite. J Mater Sci: Mater Med 1993;4(6):569–574.
15. Puleo DA, Holleran LA, Doremus RH, Bizios R. Osteoblast responses to orthopedic implant materials in vitro. J Biomed Mater Res 1991;25(6):711–723.
16. Klein CP, Patka P, Wolke JG, de Blieck–Hogervorst JM, de Groot K. Long-term in vivo study of plasma-sprayed coatings on titanium alloys of tetracalcium phosphate, hydroxyapatite and alpha-tricalcium phosphate. Biomaterials 1994;15(2):146–150.
17. Jansen JA, van der Waerden JP, Wolke JG. Histologic investigation of the biologic behavior of different hydroxyapatite plasma-sprayed coatings in rabbits. J Biomed Mater Res 1993;27(5):603–610.
18. Higashi S, Yamamuro T, Nakamura T, Ikada Y, Hyon SH, Jamshidi K. Polymer–hydroxyapatite composites for biodegradable bone fillers. Biomaterials 1986;7(3):183–187.
19. Flahiff CM, Blackwell AS, Hollis JM, Feldman DS. Analysis of a biodegradable composite for bone healing. J Biomed Mater Res 1996;32(3):419–424.
20. Verheyen CCPM, Klein CPAT, Blieck–Hogervorst JMAD, Wolke JGC, van Blitterswijn CA. Evaluation of hydroxyapatite/poly(l-lactide) composites: Physico–chemical properties. J Mater Sci: Mater Med 1993;4:58–65.
21. Mikos A, Bao Y, Cima L, Ingber D, Vacanti J, Langer R. Preparation of poly(glycolic acid)-bonded fiber structures for cell attachment and transplantation. J Biomed Mater Res 1993;27(2):183–189.
22. Wintermantel E, Mayer J, Blum J, Eckert KL, Luscher P, Mathey M. Tissue engineering scaffolds using superstructures. Biomaterials 1996;17(2):83–91.
23. Lo H, Ponticiello MS, Leong KW. Fabrication of controlled release biodegradable foams by phase separation. Tiss Eng 1995;1(1):15–28.
24. Schugens C, Maquet V, Grandfils C, Jerome R, Teyssie P. Polylactide macroporous biodegradable implants for cell transplantation. II. Preparation of polylactide foams by liquid–liquid phase separation. J Biomed Mater Res 1996;30(4):449–461.
25. Hsu YY, Gresser JD, Trantolo DJ, Lyons CM, Gangadharam PR, Wise DL. Effect of polymer foam morphology and density on kinetics of in vitro controlled release of isoniazid from compressed foam matrices. J Biomed Mater Res 1997;35(1):107–116.
26. Whinnery LL, Even WR, Beach JV, Loy DA. Engineering the macrostructure of thermally induced phase separated polysilane foams. J Polym Sci, Polym Chem 1996;34:1623–1627.
27. Ravaglioli A, Krajewski A. Bioceramics. London: Chapman & Hall, 1992. 422 p.
28. Shinoka T, Shum-Tim D, Ma PX, Tanel RE, Isogai N, Langer R, Vacanti JP, Mayer JE Jr. Creation of viable pulmonary artery autografts through tissue engineering. J Thorac Cardiovasc Surg 1998;115(3):536–545.
29. Vacanti C, Kim W, Upton J, Vacanti M, Mooney D, Schloo B, Vacanti J. Tissue-engineered growth of bone and cartilage. Transplant Proc 1993;25:1019–1021.
30. Ishaug SL, Crane GM, Miller MJ, Yasko AW, Yaszemski MJ, Mikos AG. Bone formation by three-dimensional stromal osteoblast culture in biodegradable polymer scaffolds. J Biomed Mater Res 1997;36(1):17–28.
31. Cusick RA, Lee H, Sano K, Pollok JM, Utsunomiya H, Ma PX, Langer R, Vacanti JP. The effect of donor and recipient age on engraftment of tissue-engineered liver. J Ped Surg 1997;32(2): 357–360.
32. Kim TH, Lee HM, Utsonomiya H, Ma P, Langer R, Schmidt EV, Vacanti JP. Enhanced survival of transgenic hepatocytes expressing hepatocyte growth factor in hepatocyte tissue engineering. Transplant Proc 1997;29(1–2):858–860.
33. Laurencin CT, Attawia MA, Elgendy HE, Herbert KM. Tissue-engineered bone regeneration using degradable polymers: The formation of mineralized matrices. Bone 1996;19(1):93S–99S.
34. Attawia MA, Herbert KM, Laurencin CT. Osteoblast-like cell adherence and migration through 3-dimensional porous polymer matrices. Biochem Biophys Res Comm 1995;213(2):639–644.