

# Freeze Casting of Porous Hydroxyapatite Scaffolds. I. Processing and General Microstructure

Qiang Fu,<sup>1</sup> Mohamed N. Rahaman,<sup>1</sup> Fatih Dogan,<sup>1</sup> B. Sonny Bal<sup>2</sup>

<sup>1</sup> Department of Materials Science and Engineering, University of Missouri-Rolla, Rolla, Missouri 65409

<sup>2</sup> Department of Orthopaedic Surgery, University of Missouri-Columbia, Columbia, Missouri 65212

Received 24 May 2007; revised 10 September 2007; accepted 1 October 2007

Published online 20 December 2007 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jbm.b.30997

**Abstract:** Freeze casting of aqueous suspensions on a cold substrate was investigated as a method for preparing hydroxyapatite (HA) scaffolds with unidirectional porosity. In the present paper, we report on the ability to manipulate the microstructure of freeze-cast constructs by controlling the processing parameters. Constructs prepared from aqueous suspensions (5–20 volume percent particles) on a steel substrate at  $-20^{\circ}\text{C}$  had a lamellar-type microstructure, consisting of plate-like HA and unidirectional pores oriented in the direction of freezing. Sintering for 3 h at  $1350^{\circ}\text{C}$  produced constructs with dense HA lamellas, porosity of  $\sim 50\%$ , and inter-lamellar pore widths of 5–30  $\mu\text{m}$ . The thickness of the HA lamellas decreased but the width of the pores increased with decreasing particle concentration. Decreasing the substrate temperature from  $-20^{\circ}\text{C}$  to  $-196^{\circ}\text{C}$  produced a finer lamellar microstructure. The use of water-glycerol mixtures (20 wt % glycerol) as the solvent in the suspension resulted in the production of finer pores (1–10  $\mu\text{m}$ ) and a larger number of dendritic growth connecting the HA lamellas. On the other hand, the use of water-dioxane mixtures (60 wt % dioxane) produced a cellular-type microstructure with larger pores (90–110  $\mu\text{m}$ ). The ability to produce a uniaxial microstructure and its manipulation by controlling the processing parameters indicate the potential of the present freeze casting route for the production of scaffolds for bone tissue engineering applications. © 2007 Wiley Periodicals, Inc. *J Biomed Mater Res Part B: Appl Biomater* 86B: 125–135, 2008

**Keywords:** biomaterials; scaffolds; hydroxyapatite; freeze casting; porous ceramics

## INTRODUCTION

The development of new scaffolds and their processing into structures that have properties tailored for specific applications, such as the repair of large defects in load-bearing bones, are becoming increasingly important. In addition to the scaffold having adequate mechanical properties for supporting physiological loads, tissue infiltration and facile integration of the scaffold with surrounding tissue are required for ultimate clinical application.

Certain synthetic and natural polymers, such as poly(lactic acid), PLA, poly(glycolic acid), PGA, copolymers of PLA and PGA, and collagen are biodegradable, so the scaffold can be gradually replaced by new bone matrix synthesized by tissue-forming cells.<sup>1–4</sup> However, the use of degradable polymers for replacing load-bearing bones is often challenging because of their relatively weak mechanical strength.<sup>1</sup> To improve the load-bearing properties of biode-

gradable polymer scaffolds, attempts have been made to reinforce the polymers with particles or short fibers. The use of reinforcing materials such as hydroxyapatite (HA) and bioactive glass provides scaffolds that are biodegradable as well as bioactive.<sup>5–7</sup>

Certain compositions of glasses, glass-ceramics, and ceramics, referred to as bioactive ceramics, have been widely investigated for healing bone defects, due to their ability to enhance bone formation and to bond to surrounding tissue.<sup>8</sup> Cell-seeded bioactive ceramics are also of interest as potential scaffolds for bone tissue engineering because they are osteoconductive as well as osteoinductive, and have the potential for providing higher mechanical strength than the aforementioned polymers. As the main mineral constituent of bone, hydroxyapatite (HA) has been widely investigated as a scaffold material for bone tissue engineering. Scaffolds of HA, both synthetic and natural, are biocompatible as well as osteoconductive. They produce no systemic toxicity or immunological reactions, and show biological affinity for bony tissues.<sup>9</sup> A disadvantage of HA is its brittleness, which limits the scaffolds to applications subjected to mainly compressive loading. One

Correspondence to: M. N. Rahaman (e-mail: rahaman@umr.edu)

approach to this mechanical limitation is the use of HA as the bioactive phase in composites, mainly polymer composites.<sup>9,10</sup> By stiffening a compliant biocompatible synthetic polymer such as polyethylene (PE) with HA particles (40 vol %), composites with mechanical properties comparable to those of cortical bone, coupled with bone-bonding ability due to the HA phase, have been developed.<sup>10</sup>

A variety of methods have been used to produce porous scaffolds for bone repair and regeneration, including consolidation with a pore-producing fugitive phase such as starch or PVA,<sup>11,12</sup> the use of foaming agents,<sup>13,14</sup> and solid freeform fabrication.<sup>15,16</sup> Freeze casting is a technique which has been used to produce polymeric and ceramic parts.<sup>17–25</sup> It has been applied to the production of synthetic and natural polymer scaffolds for tissue engineering applications,<sup>17–19</sup> and its application to the production of porous bioceramic scaffolds is now receiving some interest.<sup>26–29</sup>

For the production of porous bioceramic scaffolds, such as HA, the freeze casting route involves rapid freezing of a colloiddally stable suspension of HA particles in a nonporous mold, and sublimation of the frozen solvent under cold temperatures in a vacuum. After drying, the porous green samples are sintered to improve their mechanical strength. The microstructure and, hence, the properties of the final sintered body are determined essentially by the suspension parameters (such as particle size, particle concentration, and colloid stability of the suspension), the freezing conditions (rate and direction of freezing), and the sintering conditions (temperature and time). By controlling the growth direction of the ice crystals during freezing, it is possible to achieve preferential orientation of the porosity, leading to anisotropic microstructure and properties, mimicking the structural characteristics of natural materials such as bone.

Recent investigations have explored how the processing conditions, such as suspension concentration, freezing rate, and sintering conditions, influence the microstructure and compressive strength of HA scaffolds.<sup>28</sup> Directional freezing of aqueous HA suspensions in a cylindrical mold placed on a cold substrate produced porous constructs with three distinct zones, each characterized by a different shape and dimensions of the pores, described as fully dense, cellular, and lamellar. In the lamellar region, which formed the major portion of the construct, an increase in the freezing rate of the suspension, from 1°C/min to 10°C/min resulted in a reduction of the pore width from ~40  $\mu\text{m}$  to ~10  $\mu\text{m}$ .

A way to further manipulate the microstructure of the freeze cast construct is to control the freezing (or solidification) behavior of the liquid in the suspension, but this has received little attention. Certain liquids, such as glycerol and 1,4-dioxane (referred to hereafter simply as dioxane), are miscible with water, and when added to water modify the local structure of the aqueous liquid. As a result, they modify the nucleation and growth of the ice crystals, thereby modifying the freezing behavior of the aqueous liquid. Glycerol effectively binds to water molecules and disrupts the com-

plete crystallization of ice. This leads to a localized structure, resulting in a reduced size of the growing ice crystals and inhibiting solute rejection.<sup>30,31</sup> The addition of glycerol (10–30 wt %) to the aqueous solvent reduced the viscosity of  $\text{Al}_2\text{O}_3$  suspensions stabilized with an anionic dispersant, ammonium polymethacrylate, and produced more homogeneous microstructures after freeze casting and sintering.<sup>23</sup> The effects were explained in terms of the ability of glycerol to both enhance the colloidal stability of the suspensions and modify the freezing behavior of water. Dioxane is miscible with water at any composition under ambient conditions. A study of the local solvent structure of dioxane-water mixtures<sup>32</sup> indicated that the structure changed at three specific compositions when the mole fraction ( $\chi$ ) of dioxane in the mixture was 0.13, 0.3, and 0.7. For  $\chi < 0.13$ , the mixture consisted mainly of water molecules with some water-dioxane complexes. For  $0.13 < \chi < 0.3$ , the system consisted mainly of small clusters of water-dioxane molecules, whereas for  $\chi > 0.3$ , the water-dioxane clusters gradually disappeared and the mixture evolved gradually to the inherent solvent structure of pure dioxane.

The objective of the present work was to explore the microstructural manipulation of freeze-cast HA constructs by controlling the processing parameters. Using aqueous suspensions as the baseline system, the effects of particle concentration and temperature of the cold substrate on the microstructure were studied. Further manipulation of the microstructure was explored by controlling the freezing (solidification) of the liquid in the suspension through the use of water-glycerol and water-dioxane mixtures. In Part II, the microstructure of the sintered constructs and its effect on the mechanical behavior are described. An understanding of the relationship between the processing parameters and the resulting microstructure is useful for manipulating the microstructure and properties of scaffolds for bone repair and replacement.

## MATERIALS AND METHODS

### Processing of HA Suspensions

HA powder (Alfa Aesar, Haverhill, MA) with an average particle size of  $<0.5 \mu\text{m}$ , was used in the present work. The particle size was measured using a laser diffraction particle size analyzer (Model LS 13 320; Beckman Coulter Inc., Fullerton, CA). Aqueous suspensions were prepared by dispersing HA powder in deionized water containing a dispersant and an organic binder to improve the rheological behavior. Four anionic and one nonionic dispersants were investigated in an attempt to determine an effective dispersant for the HA particles used in the present work. The anionic dispersants were: ammonium polymethacrylate (Darvan C, molecular weight MW = 10,000–16,000; R.T. Vanderbilt Co., Norwalk, CT), sodium polyacrylate (Darvan 811, MW = 5000; R.T. Vanderbilt Co.), ammonium polyacrylate (Darvan 821A, MW = 3500; R.T. Vanderbilt Co.),

ammonium polycarbonate (Targon 1128, MW = 13,000–15,000; BK Ladenburg GmbH, Ladenburg, Germany). The nonionic dispersant was an ethoxylated acetylenic diol surfactant (Dynol 604, Air Products & Chemicals Inc., Allentown, PA). The effectiveness of the dispersants for stabilizing the suspension against flocculation was investigated by measuring the viscosity of the suspensions as a function of dispersant concentration, HA particle concentration, and shear rate using a rotating cylinder viscometer (Model VT500; Haake Inc., Paramus, NJ).

Based on the viscosity data, suspensions containing 0.75 wt % of Dynol 604 and 1.5 wt % of an organic binder poly (vinyl alcohol), PVA, (DuPont Elvanol<sup>®</sup> 90-50, DuPont, DE), based on the dry weight of the HA powder, and 5–20 vol % HA particles were prepared by ball-milling for 48 h in polypropylene containers using Al<sub>2</sub>O<sub>3</sub> grinding media. The suspensions were de-aired by ball-milling at a low speed prior to freeze casting.

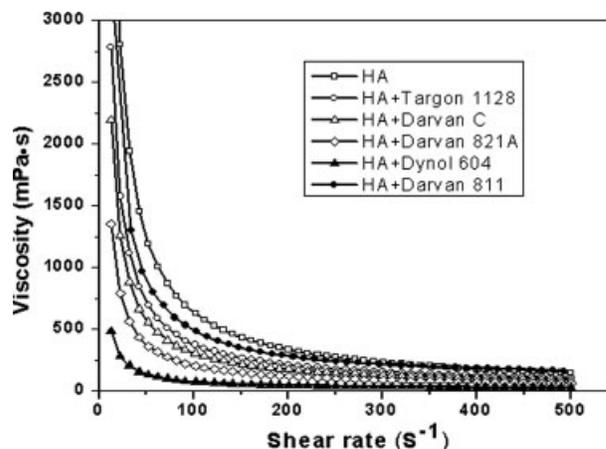
To explore the effects of a modifier on the solidification of the solvent during freeze casting, aqueous suspensions containing 5–20 wt % of glycerol (Fisher Scientific, Pittsburg, PA) or 40–70 wt % dioxane (Fisher Scientific), were also prepared using the same procedure.

### Freeze Casting of Suspensions

Freeze casting was performed by pouring the suspensions into polyvinyl chloride (PVC) tubes (~10 mm internal diameter × 20 mm long) placed on a cold steel substrate kept at a fixed temperature using a freeze dryer (Genesis 25 SQ Freeze Dryer, VirTis Co., Gardiner, NY) or cooled by liquid nitrogen. Polyurethane foam was used to cover the PVC tubes to reduce heat transfer from the surrounding environment to the slurry, ensuring optimum directional freezing of the system. The arrangement did not provide a method for providing a fixed cooling rate. Instead, it allowed the substrate to be kept at a fixed temperature. Three substrate temperatures were used in the experiments: –196°C, –50°C, and –20°C. The frozen constructs were subjected to a vacuum of 4 Pa for 48 h in a freeze dryer (Genesis 25 SQ Freeze Dryer) to cause sublimation of the frozen solvent. The green constructs were sintered in air for 3 h at temperatures between 1250°C and 1375°C, with a heating and cooling rate of 3°C/min.

### Microstructural Characterization of Porous HA Constructs

The microstructure of the cross-sections of the green and sintered constructs, in the planes parallel to and perpendicular to the direction of freezing, was observed using scanning electron microscopy, SEM (S-4700, Hitachi Co., Tokyo, Japan). Since the green constructs (after sublimation of the frozen liquid) were too weak for handling, most of the characterization experiments were carried out on the sintered samples. The volume of open porosity in the sintered samples was measured using the Archimedes method.



**Figure 1.** Viscosity of aqueous HA suspension (20 vol %) as a function of shear rate for different dispersants.

In addition, the porosity and pore size distribution of open pores were measured using mercury intrusion porosimetry (Poremaster, Quantachrome, Boynton Beach, FL).

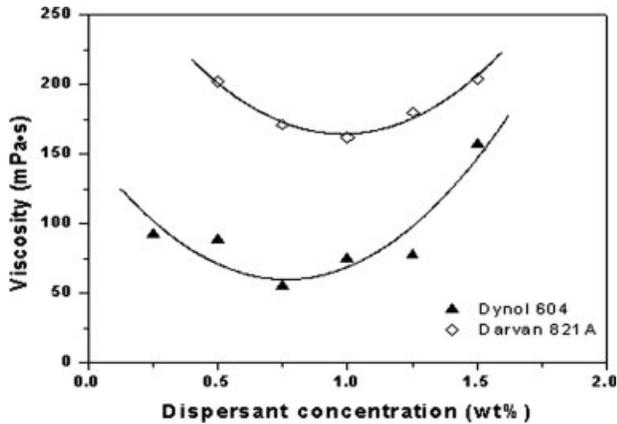
The width of the pores and the thickness of the HA lamellas in the sintered constructs were determined from SEM micrographs of cross-sections in the plane parallel to the direction of freezing. For each construct, at least 5 SEM micrographs were taken at random positions of the cross-section, and at least 10 measurements of the pore width and lamella thickness were made on each micrograph, to provide a mean value and a standard deviation.

## RESULTS

### Rheological Behavior of HA Suspensions Used for Freeze Casting

Figure 1 shows data for the viscosity as a function of shear rate for aqueous HA suspensions (20 vol % particles) stabilized with the dispersants Darvan 811, Darvan 821A, Darvan C, Targon 1128, and Dynol 604. The concentration of each dispersant was 1.0 wt %, based on the dry mass of the particles. At a given shear rate, and particularly at shear rates <100 s<sup>-1</sup>, Darvan 821A and Dynol 604 reduced the viscosity of the suspension more effectively, indicating their ability to better stabilize the particles against flocculation. As with other suspension-based forming methods, the colloidal stability of the suspension is important for producing homogeneous green microstructures. In the directional freeze casting route used in the present work, a high rate of flocculation can lead to zones with different microstructures from the bottom to the top of the sample.

The optimum concentration of Darvan 821A or Dynol 604 required to stabilize the HA particles was determined from data for the viscosity of the suspension (20 vol % particles) as a function of the dispersant concentration at a constant shear rate of 100 s<sup>-1</sup> (Figure 2). The data indicated a minimum viscosity at ~0.75 wt % Dynol 604 and at ~1.0 wt % Darvan 821A. Furthermore, at these minima,



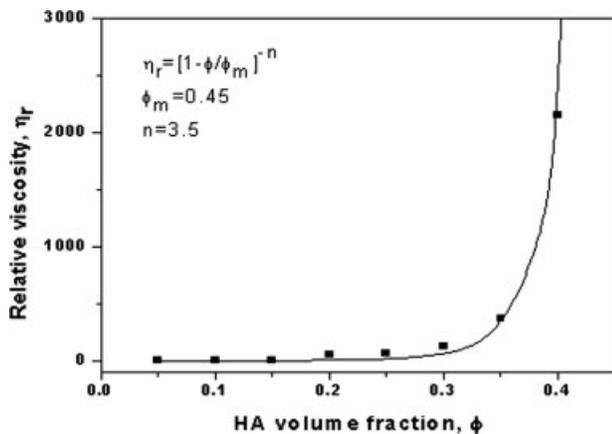
**Figure 2.** Viscosity of aqueous HA suspension (20 vol %) as a function of dispersant concentration for a shear rate of  $100 \text{ s}^{-1}$ .

the viscosity of the suspension stabilized with Dynol 604 was almost three times lower than the viscosity for the suspension stabilized with Darvan 821A. Similar trends were found for suspensions containing 5–15 vol % particles, but the data are omitted for the sake of brevity. The data in Figure 2 indicated that the viscosity of the suspensions was reduced more effectively using Dynol 604, allowing the incorporation of a higher concentration of particles for the same given viscosity. Unless stated otherwise, henceforth the suspensions described are those stabilized with 0.75 wt % Dynol 604.

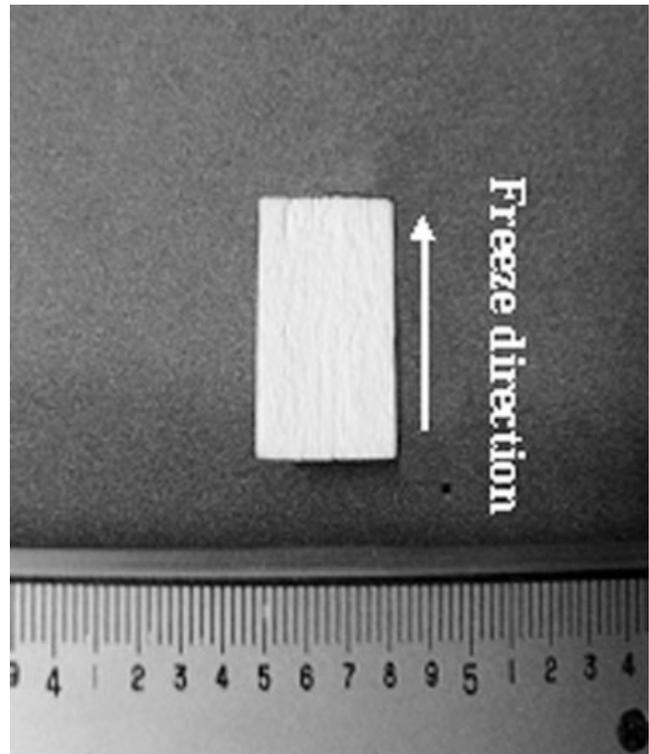
Data for the relative viscosity of the suspensions (shear rate =  $100 \text{ s}^{-1}$ ) as a function of HA particle concentration are shown in Figure 3. The particle concentration had a limited effect on the viscosity for volume fractions lower than  $\sim 30 \text{ vol } \%$  but above this value, the viscosity started to increase steeply. The data can be well fitted by a modified Krieger-Dougherty equation<sup>33,34</sup> of the form:

$$\eta_r = \left(1 - \frac{\phi}{\phi_m}\right)^{-n}$$

where  $\eta_r$ , the relative viscosity, is defined as the viscosity of the suspension,  $\eta$ , divided by the viscosity of the solvent



**Figure 3.** Relative viscosity at a shear rate of  $100 \text{ s}^{-1}$  versus volume fraction of HA particles stabilized by Dynol 604.



**Figure 4.** Optical image of a sintered HA sample, showing the shape uniformity. (Scale is in inches.)

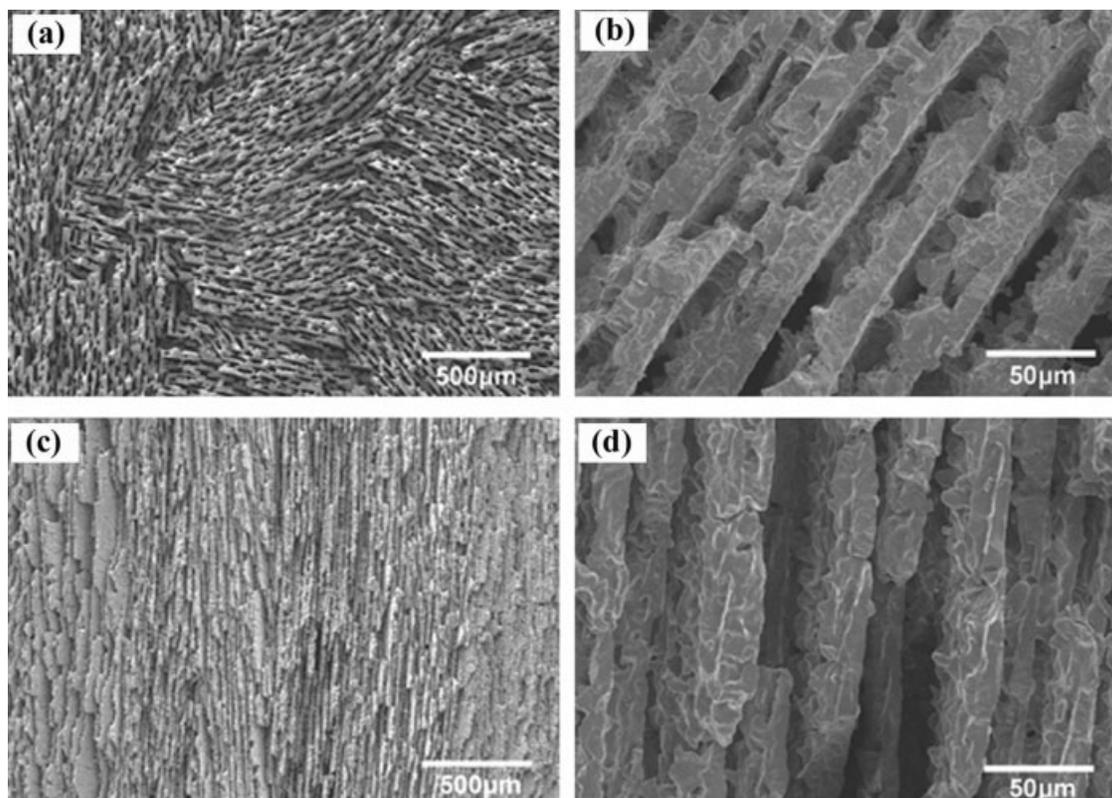
(water)  $\eta_L$ ,  $\phi$  is the volume fraction of the particles,  $\phi_m$  is the volume fraction of particles at which the viscosity becomes practically infinite, and  $n$  is a fitting parameter. The maximum HA particle concentration predicted by this model was  $\phi_m = 45 \text{ vol } \%$ , with  $n = 3.5$ . In the present experiments, the suspensions became difficult to pour for particle volume fractions greater than  $\sim 25 \text{ vol } \%$ . As a result, the highest particle concentration used was 20 vol % (equal to 45 wt %).

### Microstructure of Freeze-Cast HA Constructs

#### Constructs Prepared From Aqueous Suspensions.

After freeze casting and sintering, the constructs maintained a uniform cylindrical shape (Figure 4). The green constructs, after sublimation of the frozen liquid, were too weak for handling, so most of the microstructural characterization studies were performed on constructs after they were sintered. As described later (Part II), sintering at temperatures between  $1250^\circ\text{C}$  and  $1350^\circ\text{C}$  produced an increase in the density of the HA phase of the constructs, but did not alter the general features of the microstructure. The microstructural features are therefore described for constructs sintered under the same sintering conditions (3 h at  $1350^\circ\text{C}$ ).

Figure 5 shows SEM images of constructs prepared from suspensions with 20 vol % HA particles on a substrate at  $-20^\circ\text{C}$ . A uniform lamellar-type microstructure was observed throughout the whole construct, with the



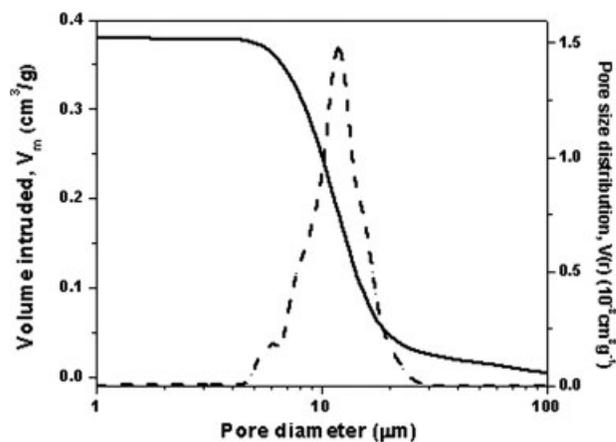
**Figure 5.** SEM images of the HA sample cross section perpendicular (a, b) and parallel (c, d) to the freezing direction. The sample was sintered for 3 h at 1350°C.

pores oriented in the direction of freezing. This lamellar-type microstructure is quite similar to the microstructures of polymeric materials such as collagen prepared for tissue engineering applications.<sup>19,20</sup> For the present system, the plate-like HA lamellas and the inter-lamellar macropores were oriented in the direction of freezing. The HA lamellas had a thickness of  $25 \pm 5 \mu\text{m}$  and a length of 100–250  $\mu\text{m}$  in cross section [Figure 5(a,b)] and extended from the bottom of the construct to the top [Figure 5(c–d)]. The average pore width, determined from SEM micrographs, was  $15 \pm 10 \mu\text{m}$ . Dendritic structures were present on the surfaces of the HA lamellas, and in some cases, they formed bridges between adjacent HA lamellas.

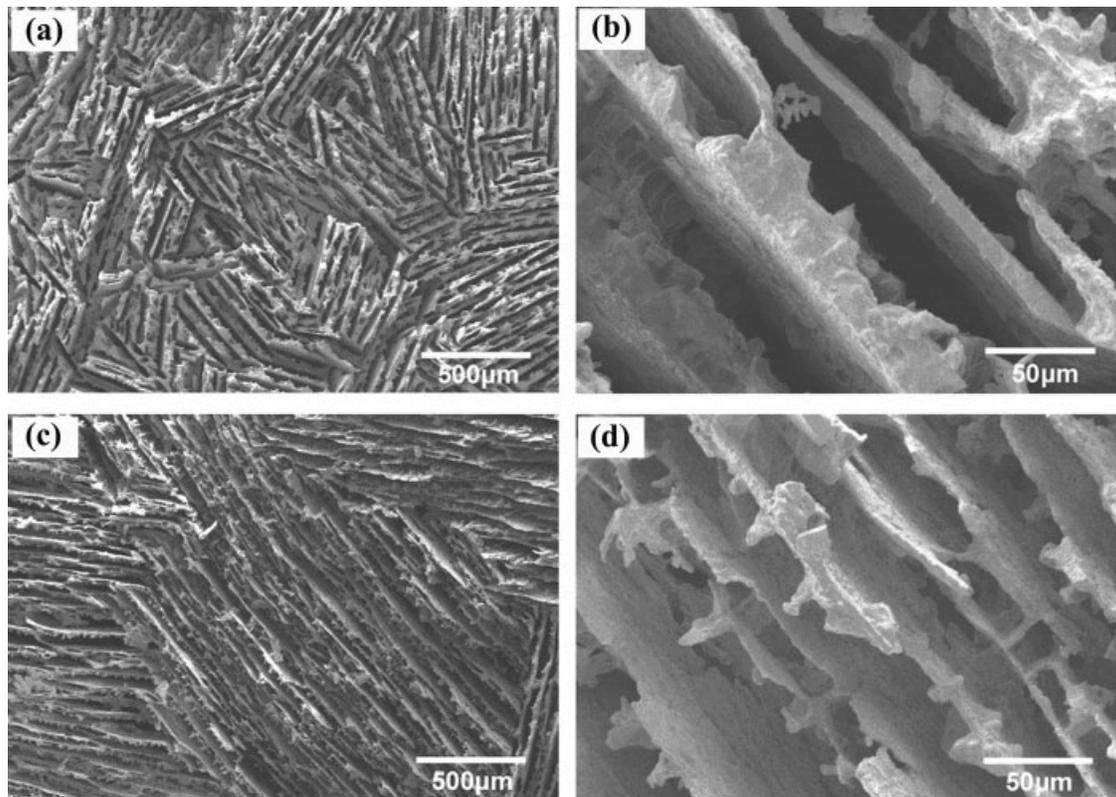
Mercury porosimetry data (Figure 6) for HA scaffolds sintered for 3 h at 1350°C showed a narrow pore size distribution, with pore widths in the range 5–30  $\mu\text{m}$ , in good agreement with the pore widths found in the SEM images (Figure 5). From the total volume of pores intruded, the open porosity was  $\sim 55\%$ , in good agreement with the porosity (52%) found by the Archimedes method. The data showed that almost all the open pores in the sample were in this narrow range, indicating the good homogeneity of the microstructure produced by the present freeze casting process.

A reduction of the particle concentration of the suspension or the temperature of the cold substrate did not change the general lamellar-type microstructure, but produced changes in the thickness of the HA lamellas, the porosity,

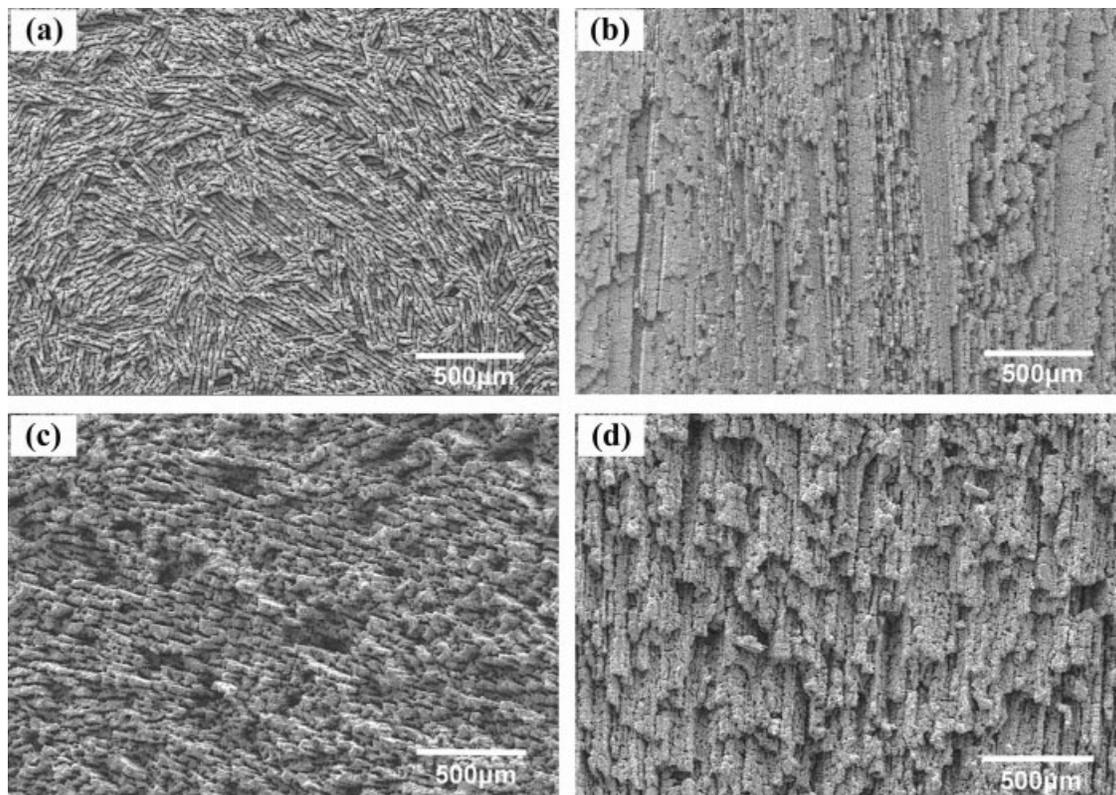
and the pore width. With decreasing particle concentration (from 20 vol % to 10 vol % and 5 vol %), the HA lamellas became thinner, the porosity was higher, and the pore width increased (Figure 7). Decreasing the substrate temperature during freezing from  $-20^\circ\text{C}$  to  $-50^\circ\text{C}$  and  $-196^\circ\text{C}$  resulted in pores with finer cross sections (Figure 8). The lamellar thickness, porosity, and pore width, determined from SEM micrographs of the sample cross-sections, are summarized in Table I.



**Figure 6.** Mercury porosimetry data for the sintered HA sample, showing the pore volume and the pore size distribution as functions of the pore diameter.



**Figure 7.** SEM images of the sintered HA sample fabricated from suspensions with different particle concentrations: (a), (b), 10 vol %; (c), (d), 5 vol %. (The cross section is perpendicular to the freezing direction.)



**Figure 8.** SEM images of the cross section perpendicular (a), (c), and parallel (b), (d), to the freezing direction, for sintered HA samples fabricated by freezing the suspension on substrates at (a), (b)  $-50^{\circ}\text{C}$ ; (c), (d)  $-196^{\circ}\text{C}$ .

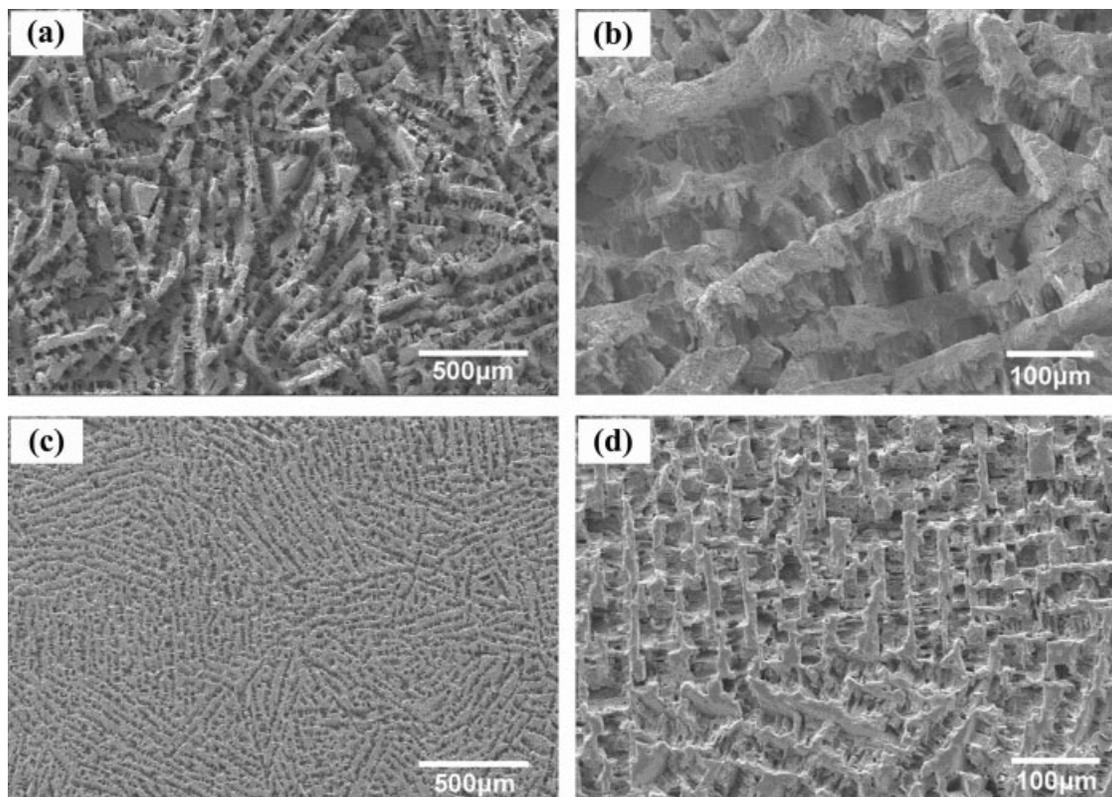
**TABLE I. Summary of Lamellar Thickness, Porosity, and Pore Width for HA Constructs Prepared From Aqueous Suspensions With 5, 10, and 20 vol % particles**

HA Concentration (vol %)	Lamellar Thickness ( $\mu\text{m}$ )	Porosity (%)	Pore Width ( $\mu\text{m}$ )
5	$8 \pm 2$	$85 \pm 2$	$50 \pm 10$
10	$15 \pm 5$	$70 \pm 5$	$25 \pm 5$
20	$25 \pm 5$	$55 \pm 2$	$15 \pm 10$

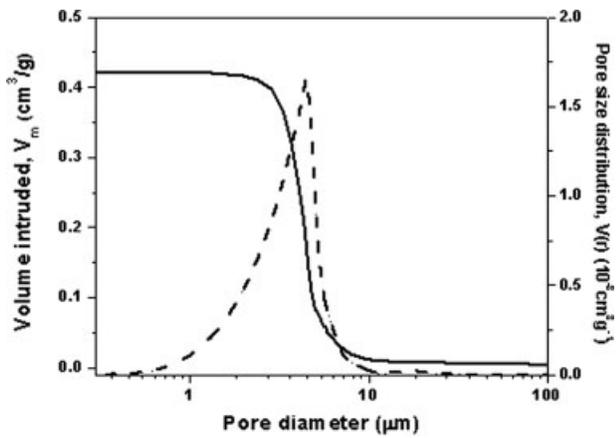
**Constructs Prepared From Suspensions With Water-Glycerol and Water-Dioxane Mixtures.** Figure 9 shows the microstructure of sintered HA constructs formed from suspensions (20 vol % HA particles) in which the solvent consisted of a mixture of water with 5 and 20 wt % of glycerol. The freeze-cast green bodies were sintered for 3 h at  $1350^{\circ}\text{C}$ . Compared to the constructs prepared without glycerol addition (Figure 5), the presence of 5 wt % glycerol in the suspensions had the effect of thickening the cross-section of the HA lamellas and reducing the homogeneity of the microstructure [Figure 9(a,b)]. With higher additions of glycerol, the microstructure became finer and more uniform. In general, the glycerol additions had the effect of increasing the number of dendritic bridges between the HA lamellas. This increased to such an extent that the cross-section of the pores changed from a plate-

like morphology to a more rectangular shape. Consequently, with the increasing concentration of glycerol in the aqueous solvent, the microstructure became more uniform, the pores became smaller, and the number of dendritic bridges between the HA lamellas increased [Figure 9(c,d)]. Mercury porosimetry data (Figure 10) of the construct prepared from suspensions with 20 wt % glycerol confirmed the narrow size distribution of the pores. The pore widths were in the range 1–10  $\mu\text{m}$ , compared to values of 5–30  $\mu\text{m}$  for similar constructs prepared without glycerol (Figure 6).

Microstructures of constructs prepared from suspensions (10 vol % particles) in which the solvent consisted of water with 30 wt % and 60 wt % dioxane are shown in Figure 11. With 30 wt % dioxane [Figure 11(a,b)], a coarse lamellar-type microstructure was obtained. However, the microstructure changed with increasing dioxane concentration, giving a cellular type microstructure [Figure 11(c,d)], with pore diameters of  $100 \pm 10 \mu\text{m}$ , much larger than the pore width of constructs prepared from aqueous suspensions (i.e., 100% water). Increasing the dioxane concentration to 70 wt % did not change the general cellular microstructure, but the pore diameter decreased substantially, to  $50 \pm 5 \mu\text{m}$ . Table II summarizes the microstructural parameters of the constructs prepared from suspensions with aqueous solvent (100% water), water-glycerol mixtures, and water-dioxane mixtures.



**Figure 9.** Effects of glycerol concentration on the microstructure of the sintered HA sample (a), (b) 5 wt %; (c), (d) 20 wt %. (The cross section is perpendicular to the freezing direction.)



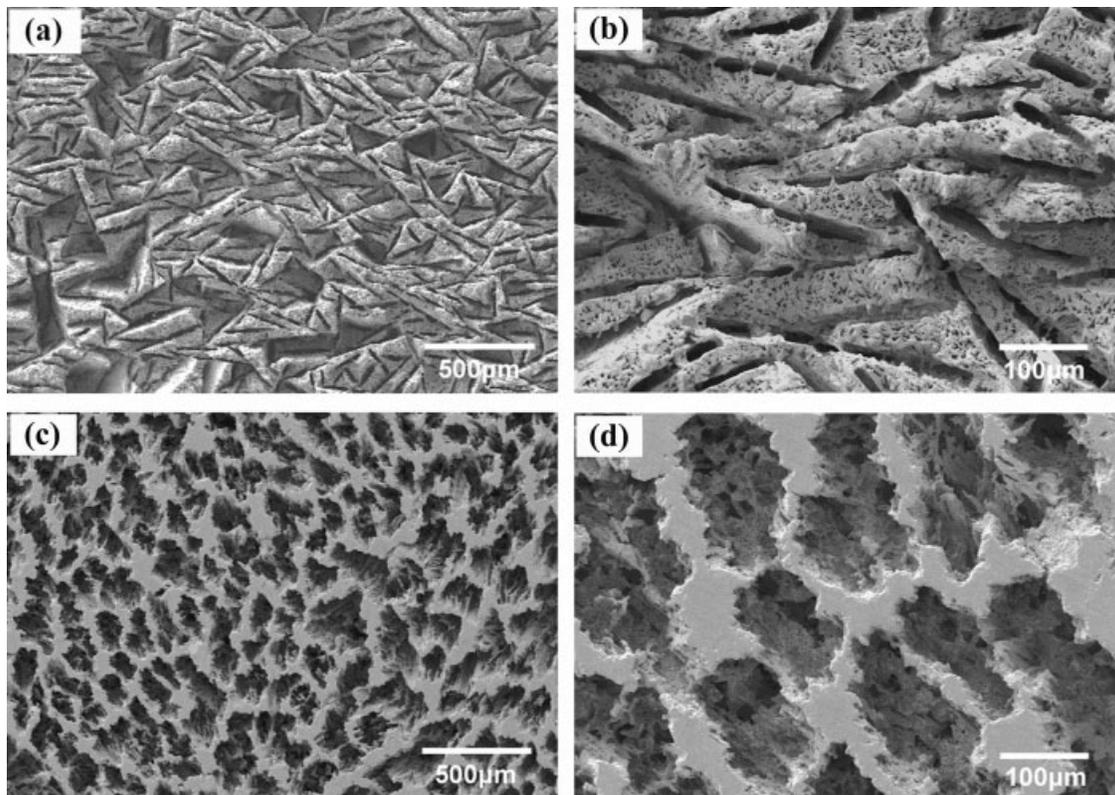
**Figure 10.** Mercury porosimetry data for the sintered HA sample prepared from suspensions with 20 wt % glycerol, showing the pore volume and the pore size distribution as functions of the pore diameter.

## DISCUSSION

For freeze-cast constructs prepared from aqueous suspensions (no glycerol or dioxane additions), the present work showed the ability to achieve a uniform lamellar-type microstructure throughout the whole construct, with the lamellas oriented in the direction of freezing. Compared to the recent work of Deville et al.,<sup>28</sup> in which three microstructural zones were observed from the bottom to the top

of the sample (described as fully dense, cellular, and lamellar), no fully dense or cellular zones were observed. A number of factors may be responsible for the difference in microstructure observed in this study and the work of Deville et al. They include differences in the freeze casting method and the colloidal properties of the HA suspensions used in the two investigations. For example, in the present work, the freezing of the suspension was determined by the temperature of the cold substrate, whereas Deville et al. used an arrangement that allowed greater control of the freezing rate. The HA particle size in the present study ( $<0.5 \mu\text{m}$ ) was much finer than that ( $2.4 \mu\text{m}$ ) used by Deville et al., and a different dispersant was used to achieve a stable suspension.<sup>35</sup> The finer particle size used in the present work, coupled with the stable suspension, presumably reduced the tendency for particle settling and improved the ease with which particles were expelled from the frozen liquid during the freeze casting step, giving a homogeneous microstructure throughout the construct. It should be noted that a homogenous microstructure, such as that obtained in the present work, is desirable in many applications because it generally leads to more reliable and controllable properties of the construct.

The results also showed the ability to manipulate the microstructure of the freeze cast constructs by changing the processing conditions. For aqueous suspensions, lower particle concentration resulted in the production of constructs



**Figure 11.** Effects of dioxane concentration on the microstructure of the sintered HA sample (a), (b) 30 wt %; (c), (d) 60 wt %. (The cross section is perpendicular to the freezing direction.)

**TABLE II. Comparison of Porosity, Pore Widths and General Microstructure for Constructs Prepared From Aqueous Suspensions, Suspensions With Glycerol, and Suspensions With Dioxane**

Solvent Composition	HA Concentration (vol %)	Pore Width ( $\mu\text{m}$ )	Porosity (%)	General Microstructure
Aqueous	10	$25 \pm 5$	$70 \pm 5$	Lamellar
	20	$15 \pm 10$	$55 \pm 2$	Lamellar
Water–Glycerol (20 wt %)	10	$10 \pm 5$	$67 \pm 2$	Rectangular
	20	$4 \pm 2$	$46 \pm 2$	Rectangular
Water–dioxane (60 wt %)	10	$100 \pm 10$	$65 \pm 2$	Cellular
	20	$90 \pm 10$	$37 \pm 2$	Cellular

with higher porosity, larger pore width, and thinner HA lamellas (Table I). A lower HA particle concentration allows larger separation distances between the HA lamellas since the dendritic growth of ice crystals is relatively less hindered as the particles are pushed at the solidification front. Particles in more dilute suspensions travel longer distances and become immobilized when the highest particle packing density is achieved between two growing dendritic crystals. The lower HA particle concentration also resulted in the fewer particles rejected by the ice lamella, leading to thinner lamellas in the sintered HA sample. From a thermodynamic point of view, a lower substrate temperature means a larger driving force for the nucleation of ice crystals, which results in the formation of a larger number of ice nuclei and an increased growth rate of the ice crystals. By adjusting the particle concentration and substrate temperature, changes in the scale of the microstructural features can be achieved without drastically altering the general lamellar-type microstructure.

Far greater changes in the microstructure of the constructs were obtained by modifying the freezing (or solidification) behavior of the solvent through the use of liquid mixtures. Glycerol, with the ability to form hydrogen bonds, has been found to influence the freezing behavior of aqueous suspension by destroying the molecular order in water and reducing the size of ice crystals during the freezing process.<sup>30</sup> Glycerol, acting as a cryoprotectant, effectively binds to natural water molecules and disrupts the complete crystallization of ice, which leads to a localized amorphous structure, resulting in the reduced size of growing ice crystals and inhibiting solute rejection.<sup>23,31</sup> A small amount (5 wt %) of glycerol addition to the suspension was capable of reducing the size of ice crystals and, hence, the pore diameter in the sintered construct, which in turn resulted in the thickening of the HA lamellas [Figures 9(a,b)]. With increasing glycerol addition, more localized ice crystals were formed with less solute rejection during the solidification process. The large lamellar ice crystals eventually disappeared due to the formation of the localized ice crystals and smaller crystals were produced in the frozen samples, which corresponded to fine dendritic pores in the sintered HA samples [Figure 9(c,d)]. Furthermore, the well-dispersed HA suspension enabled the formation of the uniform ice crystals in the frozen sample and, hence, the homogenous pore structure in the sintered body.

Dioxane has been shown to break the hydrogen bonds in water,<sup>36,37</sup> forming stable dioxane (Diox)-water ( $\text{H}_2\text{O}$ ) complexes  $(\text{Diox})_m \cdot (\text{H}_2\text{O})_n$ .<sup>38,39</sup> According to studies of the local structure,<sup>32</sup> these complexes are more prevalent when the mole fraction of dioxane  $\chi$  is between  $\sim 0.13$  and  $\sim 0.30$  ( $\sim 40$ – $70$  wt %). The change in microstructure of the freeze-cast constructs may be attributed to the presence of these complexes. Below 40 wt % dioxane, the structure of the solvent was dominated by hydrogen-bonded water molecules, leading to a lamellar-type microstructure (Figure 11(a,b)), not drastically different from the microstructure observed for aqueous solvents. For the suspension with 60 wt % dioxane, the freezing of the  $(\text{Diox})_m \cdot (\text{H}_2\text{O})_n$  complexes resulted in a *cellular* microstructure with pore diameters of  $100 \pm 10 \mu\text{m}$ .

The microstructures obtained in the present work for HA are similar in nature to those observed in porous constructs prepared by unidirectional freezing of collagen suspensions,<sup>17–19</sup> solutions of biodegradable polymers, such as poly(lactic acid), PLA, poly(glycolic acid), PGA, and their copolymers, PLGA,<sup>5</sup> and suspensions of polymers and particles, such as collagen and HA.<sup>20,26,40</sup> The use of suspensions with a low concentration of collagen (typically  $<5$  wt %) commonly resulted in lamellar or cellular microstructures such as those shown in Figure 7 and Figure 11(c,d), but with higher porosity (typically  $>80$ – $85\%$ ). Pore sizes (diameter or width) in the range  $50$ – $500 \mu\text{m}$  have been reported, depending on the freezing rate.<sup>18,41</sup> Unidirectional microstructures with tubular pores were obtained using a thermally induced phase separation (TIPS) technique during freezing of PLA and PLGA solutions.<sup>5</sup> Constructs with porosity of  $88$ – $97\%$  and average pore diameter of  $30$ – $115 \mu\text{m}$  were produced, depending on the concentration of the polymer solution ( $2.5$ – $10$  wt %) and the freezing rate. To mimic the microstructure and mineral composition of bone, the production of unidirectional composites of HA with collagen or a biodegradable polymer (PLA, PGA, or PLGA) has also been investigated.<sup>20,26,40</sup> Typically, these composite scaffolds have microstructures similar to those of the unreinforced polymer constructs.

Scaffolds intended for the repair and regeneration of large defects in load-bearing bones should have sufficient strength to serve a mechanical function, as well as the requisite microstructure to support tissue ingrowth into the pores. In Part II,<sup>42</sup> the mechanical behavior of the con-

structs prepared in the present work is reported. The microstructural parameters that influence tissue ingrowth include the porosity, the pore diameter, the pore shape and the pore interconnectivity. Interconnected pores with a mean diameter or width of 100  $\mu\text{m}$  or greater, and open porosity of >50% are generally considered the minimum requirements to permit tissue ingrowth and function in porous scaffolds.<sup>43,44</sup> For constructs prepared from aqueous suspensions (5–20 vol % particles), the mean pore widths obtained in the present work and by Deville et al.<sup>28</sup> are <40  $\mu\text{m}$ . The ability to produce larger pores (100  $\pm$  10  $\mu\text{m}$ ), by modifying the freezing behavior of the liquid, as demonstrated in this work for water-dioxane mixtures (Table II), provides an important approach for the production of HA scaffolds with the requisite microstructure for bone repair and regeneration.

## CONCLUSIONS

Freeze casting of aqueous suspension of HA particles on a cold substrate produced porous constructs with a uniform, lamellar-type microstructure in which plate-like HA lamellas were oriented in the direction of freezing. Changes in the particle concentration of the suspension and temperature of the cold substrate did not drastically alter the lamellar-type microstructure but changed the porosity, the pore width, and thickness of the HA lamellas. With increasing particle concentration in the suspension (5–20 vol %), the porosity and pore cross section decreased, but the thickness of the HA lamellas increased. A decrease in the temperature of the cold substrate ( $-20^{\circ}\text{C}$  to  $-196^{\circ}\text{C}$ ) caused a reduction in the size of the HA lamellas and the pore cross section. The addition of glycerol ( $\sim 20$  wt%) to the aqueous solvent used in the suspensions produced finer pores and a larger number of dendritic structures connecting the HA lamellas. On the other hand, the addition of 60 wt % dioxane produced a cellular microstructure with much larger pores.

## REFERENCES

1. Goldstein SA, Patil PV, Moalli MR. Perspectives on tissue engineering of bone. *Clin Orthop* 1999;(357 Suppl):S419–S423.
2. Kneser U, Schaefer DJ, Munder B, Klemm C, Andree C, Stark GB. Tissue engineering of bone. *Min Invas Ther Allied Technol* 2002;11:107–116.
3. Griffith LG. Polymeric biomaterials. *Acta Mater* 2000;48:263–277.
4. Borden M, El-Almin SF, Attawia M, Laurencin CT. Structural and human cellular assessment of a novel microsphere-based tissue engineered scaffold for bone repair. *Biomaterials* 2003;24:597–609.
5. Zhang R, Ma PX. Poly( $\alpha$ -hydroxyl acids)/hydroxyapatite porous composites for bone-tissue engineering. I. Preparation and morphology. *J Biomed Mater Res* 1999;44:446–455.
6. Thomson RC, Yaszemski MJ, Powers JM, Mikos AG. Hydroxyapatite fiber-reinforced poly( $\alpha$ -hydroxy ester) foams for bone regeneration. *Biomaterials* 1998;19:1935–1943.

7. Roether JA, Gough JE, Boccaccini AR, Hench LL, Maquet V, Jérôme R. Novel bioresorbable and bioactive composites based on bioactive glass and polylactide foams for bone tissue engineering. *J Mater Sci Mater Med* 2002;13:1207–1214.
8. Hench LL, Wilson J. Surface active biomaterials. *Science* 1984;226:630–636.
9. Hench LL. Bioceramics. *J Am Ceram Soc* 1998;81:1705–1728.
10. Huang J, DiSilvio L, Wang M, Tanner KE, Bonfield W. In vitro mechanical and biological assessment of hydroxyapatite-reinforced polyethylene composite. *J Mater Sci Mater Med* 1997;8:775–779.
11. Rodríguez-Lorenzo LM, Vallet-Regí M, Ferreira JMF. Fabrication of porous hydroxyapatite bodies by a new direct consolidation method: Starch consolidation. *J Biomed Mater Res* 2002;60:232–240.
12. Li SH, De Wijn JR, Layrolle P, De Groot K. Synthesis of macroporous hydroxyapatite scaffolds for bone tissue engineering. *J Biomed Mater Res* 2002;61:109–120.
13. Sepulveda P, Binner JG, Rogero SO, Higa OZ, Bressiani JC. Production of porous hydroxyapatite by the gel-casting of foams and cytotoxic evaluation. *J Biomed Mater Res* 2000;50:27–34.
14. Tamai N, Myoui A, Tomita T, Nakase T, Tanaka J, Ochi T, Yoshikawa H. Novel hydroxyapatite ceramics with an interconnective porous structure exhibit superior osteoconduction in vivo. *J Biomed Mater Res* 2002;59:110–117.
15. Roy TD, Simon JL, Ricci JL, Rekow ED, Thompson VP, Parsons JR. Performance of hydroxyapatite bone repair scaffolds created via three-dimensional fabrication techniques. *J Biomed Mater Res A* 2003;67:1228–1237.
16. Wilson CE, de Bruijn JD, van Blitterswijk CA, Verbout AJ, Dhert WJA. Design and fabrication of standardized hydroxyapatite scaffolds with a defined macro-architecture by rapid prototyping for bone-tissue-engineering research. *J Biomed Mater Res A* 2004;68:123–132.
17. Wang HW, Tabata Y, Ikada Y. Fabrication of porous gelatin scaffolds for tissue engineering. *Biomaterials* 1999;20:1339–1344.
18. Schoof H, Bruns L, Fischer A, Heschel I, Rau G. Dendritic ice morphology in unidirectionally solidified collagen suspensions. *J Cryst Growth* 2000;209:122–129.
19. Schoof H, Apel J, Heschel I, Rau G. Control of pore structure and size in freeze-dried collagen sponges. *J Biomed Mater Res Appl Biomater* 2001;58:352–357.
20. Zhang H, Hussain I, Brust M, Butler MF, Rannard S, Cooper AI. Aligned two- and three-dimensional structures by directional freezing of polymers and nanoparticles. *Nat Mater* 2005;4:787–793.
21. Fukasawa T, Ando M, Ohji T, Kanzaki S. Synthesis of porous ceramics with complex pore structure by freeze-dry processing. *J Am Ceram Soc* 2001;84:230–232.
22. Dogan F, Hausner H. The role of freeze drying in ceramic processing. In: Messing GL, Fuller ER, Hausner H, editors. *Ceramic Powder Science II*, Ceramic Transactions, Vol. 1. Westerville, OH: The American Ceramic Society; 1988. p 127–134.
23. Sofie SW, Dogan F. Freeze casting of aqueous alumina slurries with glycerol. *J Am Ceram Soc* 2001;84:1459–1464.
24. Araki K, Halloran JW. Porous ceramic bodies with interconnected pore channels by a novel freeze casting technique. *J Am Ceram Soc* 2005;88:1108–1114.
25. Halloran JW. Making better ceramic composites with ice. *Science* 2006;311:479–480.
26. Yunoki S, Ikoma T, Tsuchiya A, Monkawa A, Ohta K, Sotome S, Shinomiya K, Tanaka J. Fabrication and mechanical and tissue ingrowth properties of unidirectionally porous hydroxyapatite/collagen composite. *J Biomed Mater Res Appl Biomater B* 2007;80:166–173.

27. Song JH, Koh YH, Kim HE. Fabrication of a porous bioactive glass-ceramic using room-temperature freeze casting. *J Am Ceram Soc* 2006;89:2649–2653.
28. Deville S, Saiz E, Tomsia A. Freeze casting of hydroxyapatite scaffolds for bone tissue engineering. *Biomaterials* 2006;27:5480–5489.
29. Deville S, Saiz E, Nalla RK, Tomsia A. Freezing as a path to build complex composites. *Science* 2006;311:515–518.
30. Miner CS, Dalton NN. *Glycerol*. New York: Reinhold; 1953. p 270–284.
31. Dogan F, Sofie SW. Microstructural control of complex-shaped ceramics processed by freeze casting. *cfi/Ber. DKG* 2002;79:E35–E38.
32. Wu YG, Tabata M, Takamuku T. A local solvent structure study on 1,4-dioxane-water binary mixtures by total isotropic Rayleigh light scattering method. *J Mol Liq* 2001;94:273–282.
33. Krieger IM, Dougherty M. A mechanism for non-newtonian flow in suspensions of rigid spheres. *Trans Soc Rheol* 1959;3:137–152.
34. Bergström L. Shear thinning and shear thickening of concentrated ceramic suspensions. *Colloids Surf A: Physicochem Eng Aspects* 1998;133:151–155.
35. Rao RR, Kannan TS. Dispersion and slip casting of hydroxyapatite. *J Am Ceram Soc* 2001;84:1710–1716.
36. Reakins D, O'Neill RD, Waghorne WE. Washburn numbers, Part 5. Relative solvent transport numbers for ion constituents in the dioxan + water and dimethylsulphoxide + water systems. *J Chem Soc Faraday Trans 1* 1983;79:2289–2298.
37. Mazurkiewicz J, Tomsasik P. Why 1,4-dioxane is a water-structure breaker. *J Mol Liq* 2006;126:111–116.
38. Zoidis E, Yarwood J, Tassing T, Danten Y, Besnard M. Vibrational spectroscopic studies on the state of aggregation of water in carbon tetrachloride, in dioxane and in the mixed solvent. *J Mol Liq* 1995;64:197–210.
39. Buzko VY, Sukhno IV, Panytushkin VT, Ramazanova DN. Theoretical investigation of 1,4-dioxane complexes with water in the chair conformation by semiempiric MNDO/PM3 method. *J Struct Chem* 2005;46:596–602.
40. Wei G, Ma PX. Structure and properties of nano-hydroxyapatite/polymer composite scaffolds for bone tissue engineering. *Biomaterials* 2004;25:4749–4757.
41. Buttafoco L, Engers-Buijtenhuijs P, Poot AA, Dijkstra PJ, Daamen WF, van Kuppevelt TH, Vermes I, Feijen J. First steps towards tissue engineering of small-diameter blood vessels: preparation of flat scaffolds of collagen and elastin by means of freeze drying. *J Biomed Mater Res B Appl Biomater* 2006;77:357–368.
42. Fu Q, Rahaman MN, Dogan F, Bal BS. Freeze casting of porous hydroxyapatite scaffolds. II. Sintering, microstructure, and mechanical behavior. *J Biomed Mater Res B Appl Biomater* 2007. Submitted for publication.
43. Hulbert SF, Young FA, Mathews RS, Klawitter JJ, Talbert CD, Stelling FH. Potential of ceramic materials as permanently implantable skeletal prostheses. *J Biomed Mater Res* 1970;4:433–456.
44. Hollinger JO, Brekke J, Gruskin E, Lee D. Role of bone substitutes. *Clin Orthop Relat Res* 1996;324:55–65.