

Manipulation of Porous Bioceramic Microstructures by Freezing of Suspensions Containing Binary Mixtures of Solvents

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In our previous work, using a unidirectional freeze-casting method, it was observed that the addition of certain polar organic solvents, such as 1,4-dioxane and glycerol, to aqueous suspensions of hydroxyapatite (HA) resulted in drastic changes to the microstructure. The objective of the present work is to provide a rationalization of the observed microstructures. Phase separation of the binary water–dioxane and water–glycerol mixtures during freezing, and the physical chemistry of the solvent mixtures, such as the ability of the polar organic solvents to modify the hydrogen bonding of water, are the key factors influencing the freeze-cast microstructures. The porous HA constructs could be applied as scaffolds for bone repair.

I. Introduction

FREEZE casting has been used widely to produce porous polymer substrates for biomedical engineering applications,^{1–3} and its application to the production of porous bioceramics for bone repair is now receiving interest.^{4–10} For the production of porous bioceramics, such as hydroxyapatite (HA), the method involves rapid freezing of aqueous suspensions in a nonporous mold, sublimation of the frozen solvent, and sintering. The microstructure of the sintered material is determined essentially by the suspension, freezing, and sintering parameters.

Control of the growth direction of the ice crystals during freezing leads to preferential orientation of the porosity, and to anisotropic microstructure and properties.^{4–8,11} Recent studies of unidirectional freeze casting have explored the effect of processing conditions on the microstructure and compressive strength of porous HA.^{4,7} The use of nonaqueous eutectic mixtures for near-room-temperature freezing has been investigated to avoid the use of low freezing temperatures.^{9,10} A unidirectional freeze-casting route resulted in the production of HA constructs with a lamellar-type microstructure,^{4–7} high compressive strength (e.g., 65 MPa; 56% porosity) in the direction parallel to the freezing direction, and interlamellar pore size (diameter or width) in the range of 10–40 μm .^{4,6}

Our previous work,^{6–8} using a unidirectional freeze-casting method, showed that the addition of polar organic solvents such as 1,4-dioxane (referred to hereafter simply as dioxane) and glycerol to aqueous suspensions of HA resulted in drastic changes to the microstructure. Lamellar-, cellular-, and rectangular-type microstructures with a wider range of pore widths (10–100 μm) were obtained, depending on the composition and the concentration of the organic liquid in the mixture.

The objective of the present work is to present a rationalization of the microstructures obtained by unidirectional freezing

of suspensions with binary mixtures of solvents. Our previous work^{6–8} was extended to cover a wider range of water–dioxane mixtures (0–100 mol%), and cooling curves of specific water–dioxane and water–glycerol mixtures were obtained to understand the solidification of the binary mixtures.

The use of binary mixtures of solvents can lead to additional effects, such as phase separation and modification of the hydrogen bonding (H bonding) of water, for microstructural manipulation. Dioxane ($\text{C}_4\text{H}_8\text{O}_2$) is miscible with water at any composition under ambient conditions. The phase diagram¹² for water–dioxane mixtures at ambient pressure (Fig. 1(a)) shows the formation of a simple eutectic with a dioxane molar concentration (χ_d) \approx 0.15 at -16°C . The local solvent structure of water–dioxane mixtures was found to change at three specific compositions¹³: (1) mainly of water molecules with some water–dioxane complexes for $\chi_d < 0.13$, (2) small clusters of water–dioxane molecules for $0.13 < \chi_d < 0.3$, and (3) gradual disappearance of the water–dioxane clusters and evolution to the inherent structure of pure dioxane for $\chi_d > 0.3$.

Glycerol has a melting point of 18°C , but it is well known for its ability to supercool and to vitrify below -90°C into a rigid glass.¹⁴ A phase diagram¹⁵ for the water–glycerol mixture at ambient pressure (Fig. 1(b)) shows the formation of a eutectic at -45°C with a glycerol molar concentration (χ_g) \approx 0.30. Glycerol is important technologically as a cryoprotective agent.¹⁶ Molecular dynamics simulations and infrared spectroscopy indicated that glycerol affected the organization and H bonding of water.¹⁷ The average number of H bonds in bulk water decreased from ~ 4 at $\chi_g = 0$ to ~ 1 at $\chi_g = 0.05$, and to almost zero at $\chi_g = 0.15$, whereas the number of H bonds between glycerol and water molecules in the first hydration shell increased in an almost opposite manner. With increasing χ_g , the bulk-like water is depleted, water in the first hydration shell becomes concentrated around the polar groups of glycerol, the alkyl groups of glycerol self-associate, and glycerol–glycerol H bonds become the dominant interaction.

II. Experimental Procedure

HA powder (Alfa Aesar, Haverhill, MA), with an average particle size of $< 0.5 \mu\text{m}$, was used in the present work. Methods for preparing aqueous HA suspensions, freezing the suspensions, and sintering the freeze-dried constructs are described in detail elsewhere.⁶ Briefly, the aqueous suspensions used in the present work consisted of 10 vol% HA particles, 0.75 wt% dispersant (Dynol 604; Air Products & Chemicals Inc., Allentown, PA), and 1.5 wt% binder, poly(vinyl alcohol) (DuPont Elvanol[®] 90-50, DuPont, DE). Suspensions containing 0–100 mol% dioxane (Fisher Scientific, St. Louis, MO) or 0–5 mol% glycerol (Fisher Scientific) were prepared using the same method. Higher glycerol concentrations were not used because of the rapid increase in the viscosity, which limited processing, or the low temperatures required for complete freezing.

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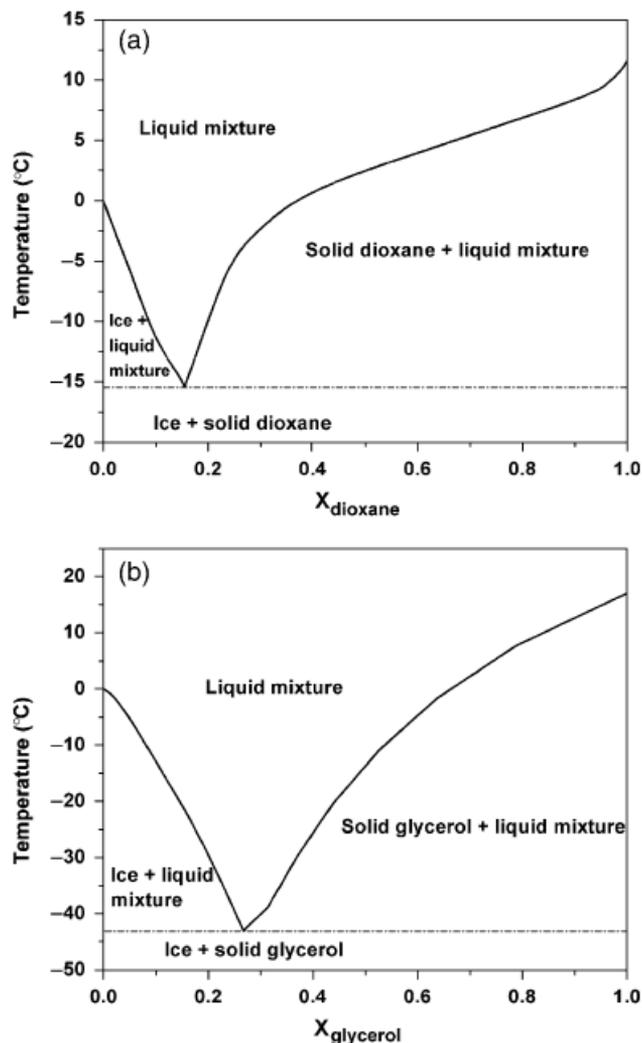


Fig. 1. Phase diagram for (a) the water–dioxane system (from Unkovskaya¹²) and (b) the water–glycerol system (from reference¹⁵).

Unidirectional freezing was performed by pouring the suspensions into poly(vinyl chloride), PVC, tubes (~ 10 mm internal diameter \times 20 mm long) placed on a cold steel substrate at -20°C in a freeze dryer (Genesis 25 SQ Freeze Dryer, VirTis Co., Gardiner, NY). After sublimation of the frozen solvent in a freeze dryer (Genesis 25 SQ), the constructs were sintered in air, for 3 h, at 1350°C (heating and cooling rate = $3^\circ\text{C}/\text{min}$).

Cooling curves (temperature versus time) for binary mixtures of water–dioxane and water–glycerol were measured under conditions approximating those used for freezing the suspensions. A flat-bottomed vial (diameter ≈ 3 cm) containing 20 cm^3 of the binary mixture was placed on a steel substrate at -20°C in a freeze dryer (Genesis 25 SQ). The temperature was measured as a function of time using a thermocouple placed near the center of the mixture.

Scanning electron microscopy (SEM) (S-4700; Hitachi Co., Tokyo, Japan) was used to observe the microstructures of the sintered constructs. The volume of open porosity in the sintered samples was measured using the Archimedes method. The width of the pores and the thickness of the HA lamellae in the sintered constructs were determined from SEM micrographs of cross sections in the plane perpendicular to the direction of freezing.

III. Results and Discussion

Table I summarizes the microstructure and porosity of sintered HA constructs prepared from the suspensions. Microstructures showing sections perpendicular to the freezing direction are given in Fig. 2. No marked changes in the microstructure were

Table I. Effect of Solvent Composition on the General Microstructure and Pore Characteristics (Where Determined) of Sintered HA Constructs Prepared by Unidirectional Freezing of Suspensions (10 vol% Particles)

Solvent composition	Microstructure	Pore width (μm)	Porosity (%)
Water	Lamellar	25 ± 5	70 ± 5
Water+dioxane [†] (mol%)			
8	Lamellar	15 ± 5	45 ± 5
12	Lamellar	20 ± 10	68 ± 5
23	Cellular	100 ± 10	65 ± 2
32	Cellular	50 ± 5	63 ± 1
64	Lamellar	—	—
Water+glycerol [‡] (mol%)			
1.25	Rectangular	15 ± 10	68 ± 2
5	Rectangular	10 ± 5	67 ± 2

[†]Concentration of dioxane in the mixture. [‡]Concentration of glycerol in the mixture.

observed along the length of the sample, and so each cross section was, in general, representative of the whole construct. Freezing of aqueous suspensions resulted in a lamellar-type microstructure (Fig. 2(a)), with the HA lamellae and interlamellar pores oriented in the direction of freezing. Dendritic structures were present on the surfaces of the lamellae, and in some cases, they formed bridges between adjacent lamellae. The lamellar microstructure resulted from the unidirectional growth of ice crystals, which expelled the fine HA particles from the growing crystals.^{4–6} The pore morphology was determined by the hexagonal crystal structure of ice.

Over the range of compositions studied ($\chi_d = 0–1$), dioxane additions resulted in remarkable changes to the microstructure. Below $\chi_d \approx 0.12–0.17$, dioxane additions did not change the general lamellar-type microstructure (Fig. 2(b)), although changes in the dimensions of the pores and lamellae were apparent. As χ_d increased to ~ 0.23 , the microstructure changed markedly to a cellular-type structure. The pores were approximately circular in cross section (Fig. 2(c)), with a diameter of $100 \pm 10\ \mu\text{m}$, much larger than the pore widths of constructs prepared from aqueous suspensions ($25 \pm 5\ \mu\text{m}$). An increase in χ_d to 0.32 did not change the cellular-type microstructure, but the pore diameter decreased substantially to $50 \pm 5\ \mu\text{m}$. For $\chi_d > 0.32$, a heterogeneous lamellar-type microstructure was obtained.

According to Fig. 1(a), the hypoeutectic liquid with $\chi_d = 0.08$ (30 wt% dioxane) starts to freeze at -10°C , and ~ 40 vol% of the liquid is filled with dendritic ice crystals before the eutectic freezes. The microstructure (Fig. 2(b)) shows the lamellar structure resulting from the ice dendrites. The hypereutectic liquid mixture with $\chi_d = 0.23$ (60 wt% dioxane) starts to freeze at -5°C . Proeutectic dioxane starts to solidify, and ~ 20 vol% of the liquid is filled with dioxane crystals before the eutectic freezes. The microstructure shows the cellular structure resulting from columnar dioxane crystals, which presumably acted as templates for subsequent solidification of the eutectic liquid consisting predominantly of water–dioxane complexes (Fig. 2(c)). For suspensions with $\chi_d = 0.32$ (70 wt% dioxane), the liquid starts to freeze at 0°C , and $\sim 40\%$ of the liquid volume is filled with dioxane crystals before the eutectic freezes. A cellular microstructure with smaller pores was formed (Table I), which presumably resulted from a larger number of finer dioxane crystals, coupled with the solidification of a smaller volume of eutectic liquid onto the dioxane crystals. The hypereutectic liquid with $\chi_d = 0.64$ starts to freeze at 5°C and ~ 80 vol% of the liquid solidifies as dioxane crystals when the eutectic liquid freezes. Well before the freezing of the eutectic liquid, the concentration of the particles in the liquid presumably became high enough (e.g., > 30 vol%) to limit the unidirectional freezing and expulsion of the particles from the solidifying liquid. The result was an inhomogeneous lamellar-type microstructure.

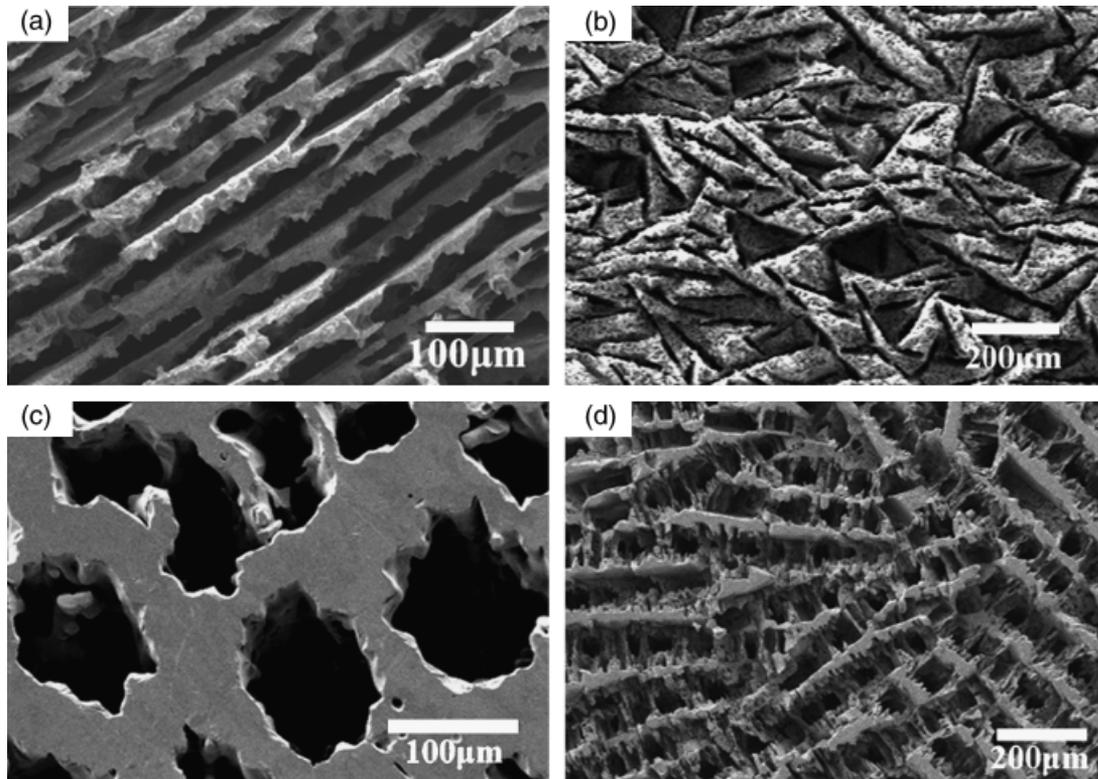


Fig. 2. Scanning electron microscopic images of HA constructs prepared from aqueous suspensions (a), and suspensions with 8 mol% (30 wt%) dioxane (b), 23 mol% (60 wt%) dioxane (c), and 1.25 mol% (5 wt%) glycerol (d).

Cooling curves of water–dioxane mixtures (Fig. 3(a)) showed marked changes in their slopes, indicative of phase changes, at temperatures that corresponded to the liquidus (T_l) and eutectic (T_s) temperatures (Fig. 1(a)). The cooling curves provided further support for the interpretation of the microstructural changes in terms of the phase separation of the solvent mixture. The HA particles in the suspension provided a more or less passive phase, and were pushed aside during the freezing of the binary liquid mixture, and so the resulting microstructure was basically patterned by the phase separation of the liquid.

Freezing of hypereutectic water–dioxane mixtures (without HA particles) in glass vials on a substrate at -20°C showed the formation of crystals with different morphologies, depending on the dioxane concentration. Although the frozen material could not be photographed with sufficient clarity, crystals with a columnar-type morphology were visible in the mixture with $\chi_d = 0.23$, whereas crystals with a lamellar-type morphology were visible in the mixture with $\chi_d = 0.64$. These crystal morphologies were qualitatively similar to the cellular and lamellar-type pore shapes observed in the HA microstructures formed from suspensions with these χ_d values.

Freezing of the suspensions containing glycerol also resulted in marked changes in the microstructure. As the glycerol concentration χ_g increased, the number of dendritic bridges between the HA lamellae also increased, and the pore cross section became finer, with an approximately rectangular shape (Fig. 2(d) and Table I). While the phase diagrams for the water–glycerol and water–dioxane mixtures are generally similar (Fig. 1), the freeze-cast microstructures were different (Figs. 2(b) and (d)). According to Fig. 1(b), a hypoeutectic liquid mixture with $\chi_g = 0.05$ (20 wt% glycerol) starts to freeze at -5°C , and 50–60 vol% of the liquid is filled with ice crystals at -20°C , the temperature of the cold substrate. The mixture consists of ice and a water–glycerol mixture into which the HA particles are expelled. A cooling curve (Fig. 3(b)) for this composition showed a change in slope at -6°C , which corresponded to the liquidus for this composition (Fig. 1(b)).

The phase diagram (Fig. 1(b)) and the present experiments indicated that the water–glycerol mixture in the suspensions was

not completely frozen at -20°C , the temperature of the substrate. To determine whether the partially frozen liquid influenced the resulting HA microstructure, additional experiments were performed in which HA suspensions (glycerol concentration = 1.25 mol%) were frozen on a substrate at -50°C to ensure complete freezing of the liquid. SEM observations showed a rectangular-type microstructure, approximately similar to that in Fig. 2(d), indicating that the partial freezing at -20°C did not have a marked effect on the resulting microstructure, and that the change in microstructure from the lamellar type (Fig. 2(a)) to the rectangular type (Fig. 2(d)) was caused mainly by the presence of glycerol.

The finer, rectangular-type pores obtained from suspensions with glycerol might result from the cryoprotective properties of glycerol. The addition of glycerol changes the H bonding of water, and may therefore influence the crystallization of ice. As χ_g increases to ~ 0.05 (20 wt% glycerol), the H bonding of bulk water is rapidly reduced, with more water concentrating in the first hydration shell around the polar groups of glycerol.¹⁷ The percolative nature of the water network is disrupted, presumably leading to the formation of smaller ice crystals. Addition of glycerol to water also leads to a rapid increase in the viscosity of the mixture,¹⁸ resulting in limited diffusion of water molecules to the ice lattice. As the growth of ice crystals in an aqueous solution is determined by the diffusion of water molecules from the solution adjacent to the ice crystals, the limited diffusion leads to smaller ice crystals.

The technique used in the present work could be applied to manipulate the microstructure of porous materials for a variety of applications. Our main interest is the preparation of novel substrates for *in vitro* cell culture and scaffolds for bone repair. Scaffolds intended for bone repair should have interconnected pores with a mean pore diameter (or width) of 100 μm or greater, and an open porosity of $>50\%$. HA constructs prepared from suspensions with 23 mol% dioxane fulfilled these minimum requirements. *In vitro* cell culture experiments in progress indicated the ability of these constructs to support the proliferation of osteoblastic cells into the pores. On the other hand, cell proliferation occurred predominantly on the surface of constructs prepared from aqueous suspensions.

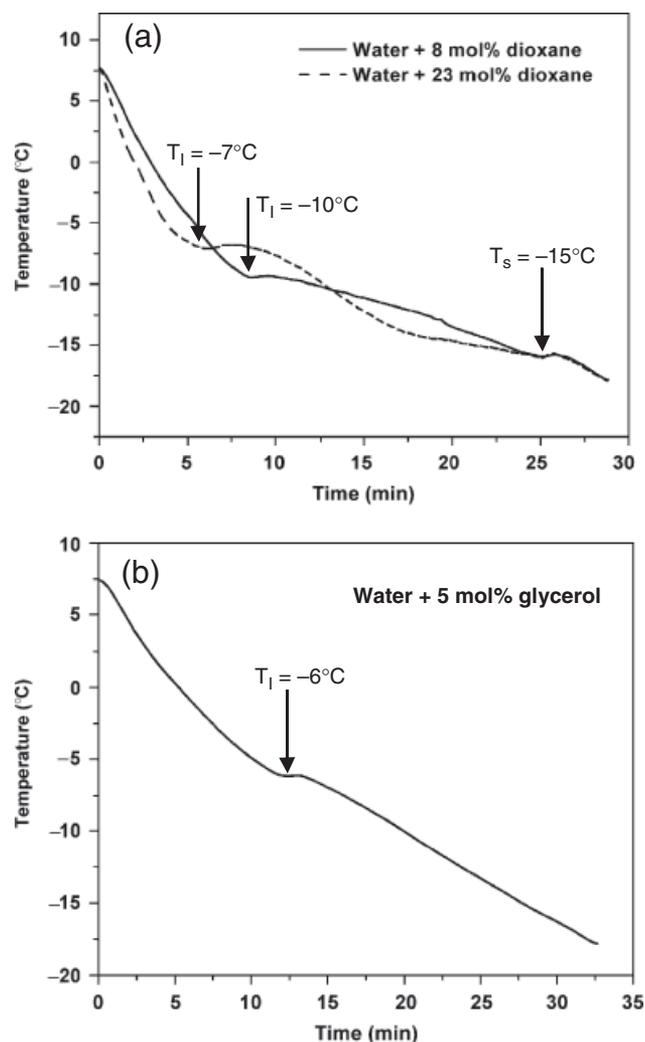


Fig. 3. Cooling curves for (a) water–dioxane mixtures with 8 mol% (30 wt%) dioxane and 23 mol% (60 wt%) dioxane, and (b) water–glycerol mixture with 5 mol% (20 wt%) glycerol. (T_l and T_s correspond to the liquidus and eutectic temperatures, respectively, in Fig. 1.)

IV. Conclusion

Microstructures obtained by unidirectional freezing of HA suspensions with water–dioxane solvents were rationalized in terms of the phase separation of the binary mixture on cooling. While the phase diagrams for the water–glycerol and water–

dioxane systems are generally similar, the microstructures were different. Suspensions with hypoeutectic mixtures produced a lamellar-type microstructure for the water–dioxane system but a rectangular-type microstructure for the water–glycerol system. This rectangular-type microstructure cannot be rationalized in terms of phase separation only. The influence of glycerol on the hydrogen bonding of water and on the viscosity of the solvent may have additional effects.

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