The present study reports an innovative way to produce large pore channels with a size >100 μm for applications in bone tissue engineering using the camphene-based freeze casting method, and using an unusually high freezing temperature, which is close to the solidification temperature of the slurry, in order to allow the formation of excessively overgrown camphene dendrites due to the extremely low solidification velocity. To accomplish this, hydroxyapatite (HA)/slurries with various solid loadings (10, 15, and 20 vol%) were frozen at 35°C for 20 h. The frozen samples were freeze dried and sintered at 1250°C for 3 h. All of the fabricated samples showed highly porous structures with large pore channels >100 μm in size and dense HA walls without any noticeable defects, such as cracks or pores. As the initial solid loading was increased from 10 to 20 vol%, the porosity of the sample decreased linearly from 76% to 55%, while the pore channels became narrower. However, the compressive strength was remarkably improved from 2.5 to 16.7 MPa.

I. Introduction

Freeze casting using either water or camphene as the freezing vehicle has recently received increasing interest, as it can endow porous ceramics with well-defined pore structures. Particularly, the use of camphene as the freezing vehicle makes it possible to freeze a ceramic particles slurry at near room temperature owing to the moderate melting temperature of camphene (T_m = 44–48°C), thus allowing for more flexible processing. In this method, a ceramic/camphene slurry is prepared by warm ball milling at temperatures above its melting temperature, typically 55–60°C, and then poured into a mold below its solidification temperature. During freezing, the molten camphene solidifies in the form of dendrite, and, at the same time, the ceramic particles are rejected by the growing camphene and become concentrated between the camphene dendrites. This freezing behavior results in the formation of a bicontinuous structure, in which each separated phase (camphene or concentrated ceramic powder network) is interconnected in three-dimensional space. Pore channels are readily produced by removing the frozen camphene dendrites via freeze drying. The pore sizes obtained using the camphene-based freeze casting method are typically in the range of several tens of micrometers, which would allow the resulting porous materials to be efficiently used for bone tissue engineering. To accomplish this, hydroxyapatite (HA)/camphene slurries with various solid loadings (10, 15, and 20 vol%) were frozen at 35°C for 20 h, whereupon the camphene dendrites excessively overgrew owing to the extremely low solidification velocity. The characteristics of the fabricated HA scaffolds, including their pore structures and compressive strengths, are reported. The possible mechanisms for the formation of the large pore channels are also discussed.

II. Experimental Procedure

(1) Starting Materials
Commercially available hydroxyapatite (HA) powder (Ca_{10}(PO_4)_6(OH)_2); Alfa Aesar Co., Milwaukee, WI) was used as the bioactive material because of its excellent biocompatibility. Commercial camphene (C_{10}H_{16}; Alfa Aesar/Avocado Organics, Ward Hill, MA) was used without further purification as the freezing vehicle. The as-received HA powder was calcined at 900°C for 1 h in air to improve the rheological behavior of the HA/camphene slurry. After calcination, the specific surface area was notably reduced from 63 to 16 m²/g. In addition, oligomeric polyester (Hypermer KD-4; UniQema, Everburg, Belgium) was used as a dispersant.

(2) Freeze Casting
The freezing temperature and freezing time were examined as the freezing parameters, in order to control the pore size. Firstly, HA/camphene slurries with various initial solid loadings (10, 15, and 20 vol %) were prepared by ball milling at 60°C with the aid of 6 wt% of dispersant. The prepared warm slurries were then poured into polyethylene (PE) molds with a diameter of 12.5 mm and left to stand at controlled temperatures, ranging from 0°C to 35°C, for 2 h. In addition, some of the cast slurries were placed in a drying oven at 35°C and kept at this temperature for various times, ranging from 2 to 50 h, in order to investigate the growth of the camphene dendrites during freezing. Before demolding, all of the frozen bodies were placed in a cool...
temperatures were 36°C with an increase in solidification velocity as slow as possible. To accomplish this, we firstly investigated the effect of the freezing temperature on the solidification velocity caused by the smaller degree of undercooling, as shown in Figs. 2(A)–(D). The size of the pore channels created by freezing the slurry at 35°C approached nearly 100 μm (Fig. 2(D)), implying that this freezing temperature would be very promising for the production of large pore channels.

Based on these observations, the cast bodies were frozen at 35°C for various freezing times, ranging from 2 to 50 h, in order to examine the effect of the freezing time on the pore. The pore sizes were roughly measured using a linear intercept method. We observed that the pore size notably increased from 86 to 194 μm, as the freezing time increased from 2 to 20 h, after which, it further increased up to 229 μm after 50 h, as shown in Fig. 3. Although the mold was capped, the sublimation of some of the solid camphene during freezing at 35°C, particularly at the mold walls, was inevitable. After taking into consideration the pore size that was obtained and the sublimation of the solid camphene, the optimum freezing time was determined to be 20 h. It should be noted that, as the freezing time was increased, negligible changes in the pore size were observed in the samples frozen at lower temperatures, even at 30°C.

(4) Observation of Pore Morphologies
All of the sintered samples retained their highly porous structures, comprised of large interconnected pore channels and sintered HA walls, as shown in Figs. 5(A)–(C). In each case, the porous structure was uniform throughout the entire sample. However, a relatively dense layer with a thickness of several micrometers was formed on the thin surrounding skins of the sample, which were in contact with the mold during the freeze casting process, but this layer could be removed by gently grinding the surfaces of the sample. It is worth mentioning that the sample produced with an initial solid loading of 10 vol% showed highly aligned pore channels (Fig. 5(A)), while the degree of pore alignment decreased, as the initial solid loading was increased to 15 vol% (Fig. 5(B)) and 20 vol% (Fig. 5(C)).
At higher magnification, the morphologies of the pore channels surrounded by the sintered HA walls are more clearly visible, as shown in Figs. 6(A)–(C). As the initial solid loading was increased, the pore channels became narrower. However, it should be emphasized that the pore size obtained is larger than 100 μm, even in the sample produced with an initial solid loading of 20 vol%. In addition, long-aligned pore channels were observed for the sample with an initial solid loading of 10 vol% (Fig. 6(A)), while mixtures of long aligned and short-elongated channels were observed for the samples produced with the initial solid loadings of 15 vol% (Fig. 6(B)) and 20 vol% (Fig. 6(C)).

(5) Formation of Pits on HA Walls

One interesting finding is that a large number of pits with a size of several micrometers were formed in the sample produced with an initial solid loading of 10 vol%, as shown in Fig. 7(A). Such pits have not previously been observed even in samples produced using the same initial solid loading but a lower freezing temperature.9 These pits were only formed on the surface of the HA walls and were isolated from each other, without any interconnections. In addition, it was observed that the HA walls surrounding the pits were fully densified, as shown in Fig. 7(B). We also observed similar pits in the sample produced with an initial solid loading of 5 vol%, but no pits were found in the samples produced with the higher initial solid loadings of 15 and 20 vol%. It is supposed that these pits were originally occupied...
Fig. 5. Scanning electron microscopy micrographs of the porous hydroxyapatite scaffolds produced with initial solid loadings of (A) 10 vol%, (B) 15 vol%, and (C) 20 vol%, showing highly porous structures throughout the samples.

Fig. 6. Scanning electron microscopy micrographs of the porous hydroxyapatite scaffolds produced with initial solid loadings of (A) 10 vol%, (B) 15 vol%, and (C) 20 vol%, showing the pore morphologies.
by the frozen camphene which formed protrusions between the secondary branches due to the relatively weak preference for the growth of the $\langle100\rangle$ and $\langle010\rangle$ side branches.\textsuperscript{14} Although we did not perform further experiments at this stage, it is believed that the initial solid loading is one of the key factors controlling the generation of pits on the sintered ceramic walls. It is meaningful to mention that such pits on the surfaces of the HA walls would be expected to be highly beneficial to the cellular response.\textsuperscript{19}

(6) Pore Size Distributions

The pore size distributions of the fabricated samples were characterized using mercury porosimetry and plotted in Figs. 8(A)–(C). The sample produced with an initial solid loading of 10 vol% showed a bimodal pore size distribution, as shown in Fig. 8(A). The peaks at $\sim 125$ and $5 \mu m$ were attributed to the three-dimensionally interconnected pore channels and the pits formed on the surfaces of the HA walls, respectively. On the other hand, the samples produced with the initial solid loadings of 15 and 20 vol% showed unimodal distributions, as shown in Figs. 8(B) and (C), respectively, implying that the HA walls did not contain any pits or pores. The peaks were observed at $\sim 75$ and $59 \mu m$ for the samples produced with the initial solid loadings of 15 and 20 vol%, respectively.

It should be noted that the measured pore sizes are smaller than those estimated from the SEM micrographs shown in Fig. 6, wherein a number of pore channels with a size of $>100 \mu m$ are visible even in the sample produced with the initial solid loading of 20 vol%. These results imply that relatively narrow interconnections are formed between the large pore channels, which consequently hinders the accurate measurement of the pore size using mercury porosimetry.\textsuperscript{20} As the intercon-nection sizes achieved in this study are larger than 50 $\mu m$, which is the critical interconnection size for bone ingrowth inside the pores, all of the fabricated HA scaffolds would be expected to find very useful applications in the field of bone tissue engineering.

(7) Densification of HA Walls

Regardless of the initial solid loadings, all of the fabricated samples showed excellent densifications with very fine grains after sintering at 1250°C for 3 h, as shown in Fig. 9(A)–(C), which should endow them with excellent mechanical properties. No noticeable cracks or pores were observed either on the free surfaces or fracture surfaces of the HA walls, except for the pits in the sample produced with an initial HA content of 10 vol%.

The effect of the sintering temperature on the densification of the HA walls was also examined. The samples produced with an initial solid loading of 10 vol% were sintered for 3 h at various temperatures, ranging from 1150°C to 1300°C. As the sintering temperature was increased, the porosity which developed in the sintered HA wall was remarkably decreased, as shown in Figs. 10(A)–(D). The sample sintered at a relatively low temperature of 1150°C showed lots of pores in the sintered HA wall (Fig. 10(A)). As the sintering temperature was increased to 1200°C, the porosity was remarkably decreased (Fig. 10(B)). When the sample was sintered at higher temperatures of 1250°C and 1300°C, a negligible amount of pores was observed (Figs. 10(C) and (D)).

Compared with the fracture surfaces, the free surfaces of the sintered HA walls showed notably enhanced densifications, as shown in Figs. 11(A)–(D). This tendency became obvious for the samples produced with the low sintering temperatures (Figs. 11(A) and (B)). Although lots of pores were observed in the fracture surface of the HA walls sintered at 1150°C, only several small pores were observed in the free surfaces (Fig. 11(A)). In addition, the sample sintered at 1200°C also showed a negligible amount of pores in the free surfaces (Fig. 11(B)). These results indicate that the concentration of the ceramic particles near the pore channels might be higher than that in the interior of the HA wall. In addition, the grain size remarkably increased, as the sintering temperature increased. After taking into consideration the fact that finer grains would be expected to offer higher mechanical properties, the optimum sintering temperature was determined to be 1250°C.

(8) Compressive Strengths

In order to evaluate the mechanical properties of the fabricated samples, compressive strength tests were conducted. The compressive strength was remarkably increased from 2.5 to 16.7 MPa, when the initial solid loading was increased from 10 to 20 vol%, as shown in Fig. 12. These values are comparable or even much higher than those reported in the literature.\textsuperscript{4,9,21,22} It is generally accepted that the strength of a porous ceramic is strongly affected not only by the intrinsic strength of the ceramic wall (or strut), but also by the surface flaws on the strut.\textsuperscript{23,24} Therefore, the high compressive strengths obtained in this study are mainly attributed to the achievement of full densification of the HA walls without any defects, such as pores or cracks. However, it should be noted that a balance between the porosity and compressive strength should be established to fulfill the requirements of specific applications.

IV. Discussion

We herein demonstrated the possibility of using the camphene-based freeze casting method for the production of large pore channels with a size $>100 \mu m$ by adopting an unusually high freezing temperature of 35°C, wherein excessive overgrowth of the camphene dendrites occurs, owing to the extremely slow solidification velocity. However, interestingly, it was observed that the camphene dendrites grew continuously, as the freezing time...
Fig. 8. Pore size distributions for the porous hydroxyapatite scaffolds produced with initial solid loadings of (A) 10 vol%, (B) 15 vol%, and (C) 20 vol%.

Fig. 9. Scanning electron microscopy micrographs of the porous hydroxyapatite (HA) scaffolds produced with initial solid loadings of (A) 10 vol%, (B) 15 vol%, and (C) 20 vol%, showing the densifications of the HA walls.
Fig. 10. Scanning electron microscopy micrographs of the porous hydroxyapatite (HA) scaffolds sintered for 3 h at (A) 1150°C, (B) 1200°C, (C) 1250°C, and (D) 1300°C, showing the fracture surfaces of the HA walls.

Fig. 11. Scanning electron microscopy micrographs of the porous hydroxyapatite (HA) scaffolds sintered for 3 h at (A) 1150°C, (B) 1200°C, (C) 1250°C, and (D) 1300°C, showing the free surfaces of the HA walls.
was increased. This phenomenon is not common and, to the best of our knowledge, has never been reported in the case of the freeze casting method. As the freezing temperature of $35^\circ$C is very close to the solidification temperature of the slurry, it is reasonable to suppose that the partial remelting of the frozen body occurs, which would cause the continuative growth of the camphene dendrites. To clarify this hypothesis, we initially froze the slurries at $20^\circ$C for 20 h, and then placed them in a drying oven at $35^\circ$C, and kept them at this temperature for various times, ranging from 2 to 50 h. It was observed that the pore size significantly increased with increasing freezing time, as shown in Figs. 13(A)–(D). The sample before heat treatment showed a pore size of several tens of micrometers (Fig. 2(B)); however, a pore size larger than 100 $\mu$m was obtained after heat treatment at $35^\circ$C for 20 h (Fig. 13(C)). These observations imply that the excessive overgrowth of the camphene dendrites at $35^\circ$C can be attributed not only to the extremely low solidification velocity caused by the very small degree of undercooling, but also to the partial re-melting of the frozen body caused by the extremely high freezing temperature, which is close to the solidification temperature of the slurry. It is worthy to mention that this method can be applicable to various systems other than the present HA/camphene system.

V. Conclusions

We herein demonstrated how large pore channels could be achieved using the camphene-based freeze casting method, which should allow porous HA scaffolds to find very useful applications for bone tissue engineering. Several concluding remarks can be made:

1. Large pore channels with a size $>100 \mu$m were achieved by freezing the HA/camphene slurries at an unusually high freezing temperature of $35^\circ$C, whereupon the excessive overgrowth of the camphene dendrites occurred, owing to the extremely low solidification velocity and the partial re-melting of the cast body.

2. The porosity could be tailored in the range of 55%–76% by simply adjusting the initial solid loadings (10–20 vol%) employed in the slurries.

3. High compressive strengths were achieved, owing to the construction of dense HA walls without any cracks or defects. The compressive strength was remarkably increased from 2.5 to 16.7 MPa, when the initial solid loading was increased from 10 to 20 vol%.

Fig. 13. Scanning electron microscopy micrographs of the porous hydroxyapatite scaffolds produced using the samples initially frozen at $20^\circ$C for 2 h, followed by heat treatment at $35^\circ$C for various times of (A) 2 h, (B) 10 h, (C) 20 h, and (D) 30 h.
(4) The pits formed in the sample produced with an initial solid loading of 10 vol% would be expected to be highly beneficial to new bone ingrowth.

References