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Production of highly porous triphasic calcium phosphate scaffolds with excellent *in vitro* bioactivity using vacuum-assisted foaming of ceramic suspension (VFC) technique

Min-Kyung Ahn[a], Young-Wook Moon[a], Young-Hag Koh[a,*], Hyoun-Ee Kim[b]

[a]*Department of Dental Laboratory Science and Engineering, Korea University, Seoul 136-703, Republic of Korea*
[b]*Department of Materials Science and Engineering, Seoul National University, Seoul 151-742, Republic of Korea*

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Abstract

We produced highly porous triphasic calcium phosphate (CaP) scaffolds, comprising of hydroxyapatite (HA), β-tricalcium phosphate (β-TCP), and α-TCP phases, using vacuum-assisted foaming of a ceramic suspension (VFC) technique. In particular, vigorously foamed CaP green bodies with a composition of ~60 wt% HA and 40 wt% β-TCP were sintered at relatively high temperatures (1200, 1250, 1300, and 1350 °C) to control the amount of three constituent phases. All the produced samples showed a highly porous structure (porosity ~ 83.5–84.5 vol%, pore size ~ 312–338 μm, and interconnection size ~ 61–74 μm) with a number of microchannels in the CaP walls. However, sintering at relatively high temperatures ≥ 1250 °C induced considerable phase transformation of the β-TCP to α-TCP phases. The presence of the more soluble α-TCP phase in the triphasic CaP scaffolds significantly enhanced the in vitro bioactivity of the porous CaP scaffolds, which was assessed in terms of their apatite-forming ability in simulated body fluid (SBF).

Keywords: Porous calcium phosphate; Foaming; Freezing; Bioactivity

1. Introduction

Calcium phosphate (CaP) ceramics are one of the most valuable biomaterials owing to their chemical composition and crystalline structure similar to those of the inorganic phase of calcified tissues (e.g. bones and teeth) [1,2]. In addition, these materials with a porous structure have been widely examined as a scaffold for bone regeneration, since they can provide 3-dimensional space and biocompatible surfaces for favorable bone ingrowth [3]. Fundamentally, the mechanical properties and biological functions of porous CaP scaffolds are strongly affected not only by their porous structure (e.g. porosity, pore size, interconnections between the pores) but also by their physicochemical properties (e.g. microstructure, chemical composition, crystalline structure) [2].

In terms of the porous structure, porous CaP scaffolds are preferred to have high porosity, large pores, and good interconnections between the pores for providing a favorable environment for cell attachment, proliferation, and differentiation, as well as new bone formation [3,4]. Thus far, a variety of manufacturing methods have been developed [5], including sponge replication [6], freeze casting [7–9], and direct foaming techniques [10–14]. Moreover, we recently proposed vacuum-assisted foaming of a ceramic suspension (VFC) as a new manufacturing technique for producing highly porous ceramic scaffolds with large interconnected pores by utilizing the extensive coalescence of air bubbles directly incorporated into a ceramic suspension under reduced pressure [15,16].

As a scaffolding material, a variety of CaP ceramics with different Ca/P ratios have been examined on account of their ability to form strong direct bond with the host bone, including hydroxyapatite (HA), β-tricalcium phosphate (β-TCP), and α-tricalcium phosphate (α-TCP) [17,18]. Fundamentally, HA is the most stable phase under

*Corresponding author. Tel.: +82 2 940 2844; fax: +82 2 909 3502.
E-mail address: kohyh@korea.ac.kr (Y.-H. Koh).

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physiological conditions, while β-TCP and α-TCP are resorbable in the human body [18]. This makes it possible to tailor the biological properties (i.e. bioactivity, bioresorbability, osteoconductivity) of CaP ceramics by combining stable HA and resorbable β-TCP [19–21] or α-TCP [22–25], which are known as biphasic CaP ceramics. However, only a few attempts have been made to produce porous triphasic CaP scaffolds, comprised of HA, β-TCP, and α-TCP phases, although this combination would have great potential for providing enhanced bioactivity and bone regeneration capability [19,23,26–28].

Thus, in this study, we first produced vigorously foamed CaP green bodies with a composition of ~60 wt% HA and 40 wt% β-TCP using VFC technique and then sintered them at relatively high temperatures (1200, 1250, 1300 and 1350 °C) to produce highly porous triphasic CaP scaffolds. The effect of the sintering temperature on the development of the porous structure (e.g. porosity, pore size and interconnections between the pores) and microstructure of CaP walls was examined by field emission scanning electron microscopy (FE-SEM). The crystalline phases and structures of the samples were characterized by X-ray diffraction (XRD). The compressive strength of the samples was measured to determine their structural integrity. The in vitro bioactivity of the samples was evaluated by examining their apatite-forming ability in simulated body fluid (SBF).

2. Experimental procedure

Commercially available CaP powders (NT-BCP, OssGen Co., Korea) with a mean particle size of 0.5 μm were used as the starting material, which were composed of ~60 wt% hydroxyapatite (HA) and 40 wt% β-tricalcium phosphate (β-TCP). First, an aqueous solution was prepared according to a method similar to our previous report [15], wherein polyvinyl alcohol (PVA; Sigma Aldrich, St. Louis, MO, USA) was used as the binder and emulsifying agent to stabilize air bubbles with an assistance of non-ionic surfactant (Hypermer KD-6, UniQema, Everburg, Belgium). Subsequently, the CaP powders (30 vol% in the CaP suspension) were added to the aqueous solutions and stirred for 1 h, which induced vigorous incorporation of air bubbles into the CaP suspensions.

The foamed CaP suspensions were poured into 20 mm diameter polyethylene molds and placed under a reduced pressure of approximately 80 kPa. This induced the extensive expansion of air bubbles, which increased the height of the foamed CaP suspension up to 300%. It should be noted that the degree of expansion can be controlled by adjusting the level of reduced pressure (e.g., 40–80 kPa), which would strongly affect the porous structure (e.g., porosity, pore size, interconnection between the pores) of porous ceramics produced [15]. Subsequently, the vigorously foamed CaP suspensions were rapidly frozen in a cooled ethanol bath at ~−70 °C for 1 h under reduced pressure, followed by freeze drying to remove the ice dendrites. The green bodies were heat-treated at 400 °C for 2 h to remove the organic phases (PVA, polymeric dispersant and glycerol) and sintered at relatively high temperatures (1200, 1250, 1300, and 1350 °C) to achieve three phases (HA, β-TCP, and α-TCP) via the phase transformation of β-TCP to α-TCP.

The effect of the sintering temperature on the pore structure of the porous CaP scaffolds and microstructure of the CaP walls was characterized by field emission scanning electron microscopy (FE-SEM, JSM-6701F, JEOL Techniques, Tokyo, Japan). The overall porosity of the samples was computed by considering their dimensions and weight. The sizes of the pores and interconnections between the pores were measured from the SEM images of the samples [29]. The average grain size was calculated using the liner intercept method on the basis of the SEM images [29].

The crystalline phases and structures of the porous CaP scaffolds sintered at various temperatures (1200, 1250, 1300, and 1350 °C) were characterized by X-ray diffraction (XRD, M18XHF-SRA, MacScience Co., Yokohama, Japan). The effect of the sintering temperature on the phase transformation of β-TCP to α-TCP was roughly examined by estimating the ratio of the relative intensities of the peaks corresponding to the (0210) of β-TCP (JCPDS file No. 9-169) and (170) of α-TCP (JCPDS file No. 9-348) to that of the peak corresponding to the (211) of HA (JCPDS file No. 9-432).

The compressive strength of the porous CaP scaffolds sintered at various temperatures (1200, 1250,1300, and 1350 °C), ~15.5 mm in diameter and ~20 mm in height, was examined using a universal testing machine (OTU-05D, Oriental TM Corp., Korea) at a crosshead speed of 1 mm/min. The stress and strain responses of the samples were monitored during the compressive strength tests. Five samples were tested to obtain the mean and standard deviation.

The in vitro apatite-forming bioactivity of the porous CaP scaffolds sintered at various temperatures (1200, 1250, 1300, and 1350 °C) was tested using simulated body fluid (SBF) that had been prepared according to a similar method reported in the literature [30]. Briefly, the samples were immersed in SBF with an initial pH of 7.40 and placed inside an incubator at a controlled temperature of 37 °C for up to 7 days. The samples were then extracted and washed three times using ethanol absolute and deionized water, and then finally dried at 37 °C for 24 h. The precipitation of the apatite crystals on the surface of the sample was examined by FE-SEM and XRD.

3. Results and discussion

Vigorously foamed CaP green bodies consisting of ~60 wt% HA and 40 wt% β-TCP phases could be successfully produced by vacuum-assisted foaming of a ceramic suspension (VCF) technique. These CaP green bodies were then sintered at relatively high temperatures in the range of

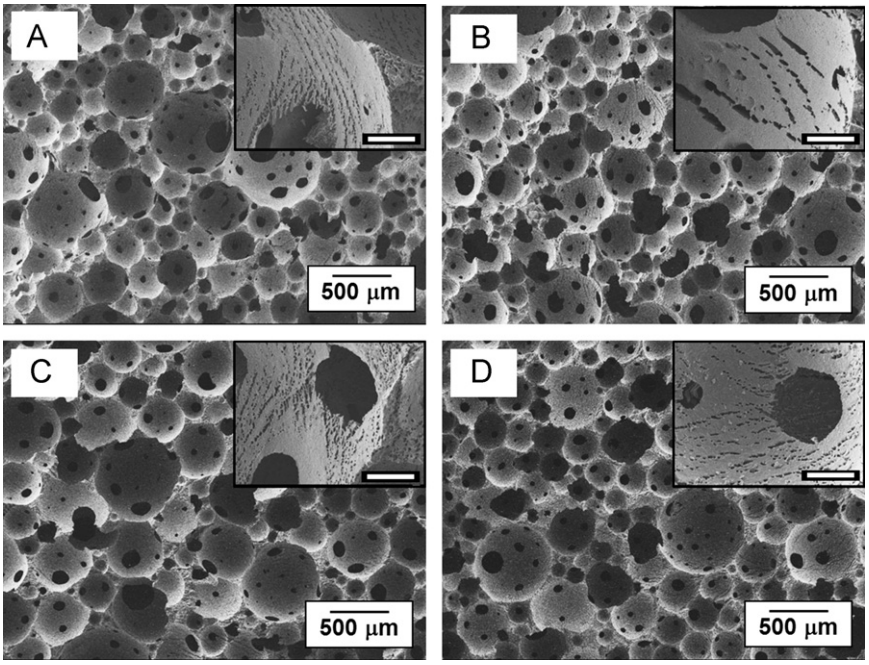

Fig. 1. SEM images showing the porous structures of the porous CaP scaffolds sintered at various temperatures of (A) 1200 °C, (B) 1250 °C, (C) 1300 °C and (D) 1350 °C. Insets show the formation of the microchannels in the CaP walls (scale = 50 μm).

1200–1350 °C to achieve triphasic CaP scaffolds consisting of HA, β-TCP, and α-TCP phases, where α-TCP phase could be newly formed through the phase transformation of β-TCP [17,18]. Fig. 1(A)–(D) show typical SEM images of the porous CaP scaffolds after sintering for 2 h at various temperatures (1200, 1250, 1300, and 1350 °C). All the produced samples showed a highly porous structure with a uniform pore distribution, as well as large interconnected pores. In addition, the CaP walls had a number of microchannels (insets in Fig. 1(A)–(D)) formed as a replica of the ice dendrites that had preferentially grown during freezing of the foamed CaP suspensions [7,15]. These unique microchannels, one of the most striking features of VCF technique [15], would stimulate cell attachment, proliferation, and differentiation, as well as new bone formation when used as a bone scaffold [31]. It should be noted that the sample sintered at a lower temperature of 1150 °C showed a number of micropores in the CaP walls, indicating poor densification (data not shown here), which would result in relatively very low mechanical properties.

The porous structure (e.g. overall porosity, pore size, and interconnection size) of the porous CaP scaffolds after sintering at various temperatures (1200, 1250, 1300, and 1350 °C) are summarized in Table 1. Regardless of the sintering temperature, all the samples showed similar porosity (~83.5–84.5 vol%), pore size (~312–338 μm), and interconnection size (~61–74 μm). These findings suggest that the partial phase transformation of β-TCP to α-TCP [17,18] and decomposition of HA into β-TCP [32,33] during sintering at relatively high temperatures did not deteriorate the highly porous structure of the porous CaP scaffolds. It should be noted that the sizes of pores and interconnections obtained in this study were large enough to effectively induce bone ingrowth when used as a bone scaffold [3,4].

Table 1
Overall porosity, pore size and interconnection size of the porous CaP scaffolds sintered at various temperatures (1200 °C, 1250 °C, 1300 °C, and 1350 °C).

Sintering Temperature [°C]	1200	1250	1300	1350
Overall porosity [vol%]	85.2 ± 0.8	85.6 ± 1.1	85.8 ± 1.3	85.9 ± 1.4
Pore size [μm]	313 ± 135	317 ± 89	338 ± 128	312 ± 156
Interconnection size [μm]	69 ± 34	70 ± 37	74 ± 46	61 ± 33

The effect of the sintering temperature on the microstructure of the CaP walls was examined by SEM, as shown in Fig. 2(A)–(D). Regardless of the sintering temperature, all the produced samples exhibited good densification with negligible micropores. However, the grains became notably larger with increasing sintering temperature. The average grain size, which was estimated by the linear intercept method on the basis of the SEM images, increased 1.1 ± 0.4 μm to 3.8 ± 1.8 μm with increasing sintering temperature from 1200 °C and 1350 °C, as summarized in Table 2.

The crystalline phases and structures of the porous CaP ceramics after sintering at various temperatures (1200, 1250, 1300, and 1350 °C) were characterized by XRD, as shown in Fig. 3(A)–(D). The sample sintered at 1200 °C showed only peaks that corresponded to those of

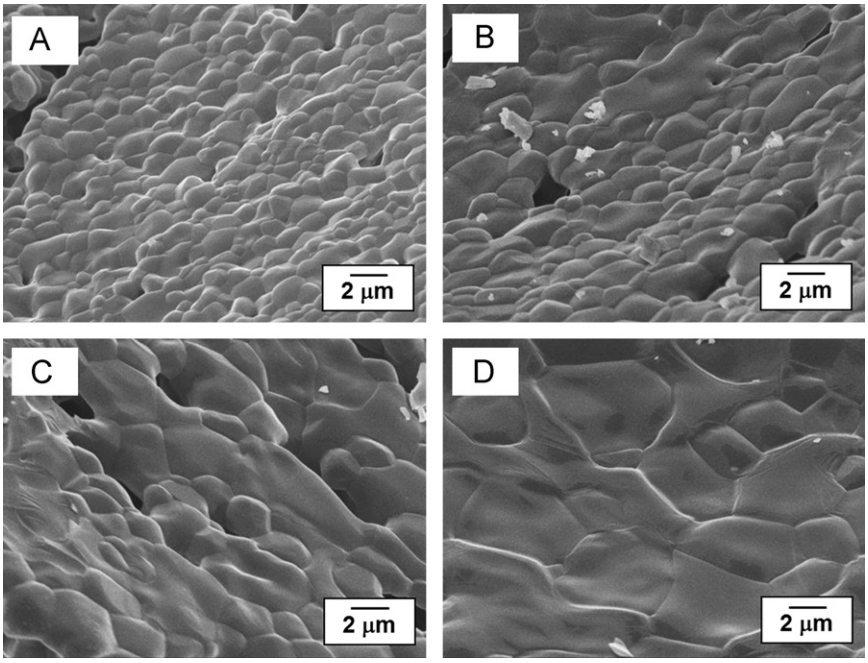

Fig. 2. SEM images showing the microstructures of the CaP walls after sintering at various temperatures of (A) 1200 °C, (B) 1250 °C, (C) 1300 °C and (D) 1350 °C.

Table 2
Average grain size of the porous CaP scaffolds sintered at various temperatures (1200 °C, 1250 °C, 1300 °C, and 1350 °C).

Sintering temperature [°C]	1200	1250	1300	1350
Average grain size [μm]	1.1 ± 0.4	1.8 ± 0.8	2.5 ± 1.0	3.8 ± 1.8

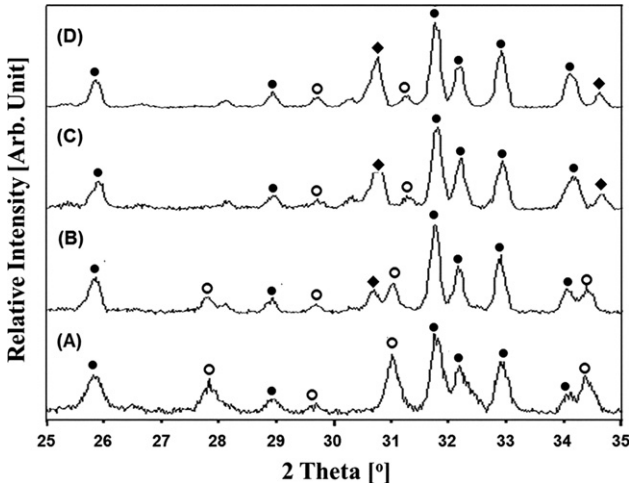

Fig. 3. Typical XRD patterns of the porous structures of the porous CaP scaffolds sintered at various temperatures of (A) 1200 °C, (B) 1250 °C, (C) 1300 °C and (D) 1350 °C. (●: HA, ○: β-TCP, ♦: α-TCP).

crystalline HA (JCPDS file No. 09-0432) and β-TCP (JCPDS file No. 09-0169) (Fig. 3(A)). On the other hand, when the samples were sintered at relatively high temperatures ≥ 1250 °C, additional peaks corresponding to crystalline α-TCP (JCPDS file No. 09-0348) appeared (Fig. 3(B)–(D)). This was mainly attributed to the phase transformation of β-TCP during sintering at relatively high temperatures [18].

Table 3
Intensity ratios of the β-TCP/HA and α-TCP/HA calculated from XRD analyses of the porous CaP scaffolds sintered at various temperatures (1200 °C, 1250 °C, 1300 °C, and 1350 °C).

Sintering temperature [°C]	1200	1250	1300	1350
β-TCP/HA [%]	76	36	21	17
α-TCP/HA [%]	0	30	57	60

The effect of the sintering temperature on the phase transformation of β-TCP to α-TCP was roughly examined by estimating the ratio of the relative intensities of the peaks corresponding to the (0210) of β-TCP and (170) of α-TCP to that of the peak corresponding to the (211) of HA. The intensity ratio of the α-TCP/HA increased remarkably from 0 to 35% with increasing sintering temperature from 1200 to 1350 °C, while that of the intensity ratio of the β-TCP/HA decreased considerably from 43 to 6%, as summarized in Table 3. These finding suggest that the phase transformation of the β-TCP to α-TCP phases could occur when sintered at temperatures ≥ 1250 °C [18,19,34], which allowed for the production of triphasic CaP scaffolds with the controlled contents of the HA, β-TCP, and α-TCP phases.

The compressive strengths of the porous CaP scaffolds after sintering at various temperatures (1200, 1250, 1300 and 1350 °C) were examined for evaluating their structural integrity. The compressive strength of the samples decreased from 1.9 ± 0.3 to 1.3 ± 0.25 MPa with increasing sintering temperature from 1200 °C to 1350 °C, as shown in Fig. 4. This decrease was attributed to increases in grain size and α-TCP content.

The in vitro bioactivity of the porous CaP scaffolds after sintering at various temperatures (1200, 1250, 1300, and 1350 °C) was evaluated by immersion in SBF up to 7 days at 37 °C. After 3 days of immersion in SBF, the sample sintered at 1200 °C showed negligible change in morphology, as shown in Fig. 5(A). On the other hand, the samples sintered at relatively high temperatures ≥1250 °C showed obvious signs of the dissolution of grains and precipitation of apatite crystals in some grains, indicated by arrows (Fig. 5(B)–(D)). These grains are believed to be α-TCP from the viewpoint of its higher biodegradability than β-TCP and HA [35,36].

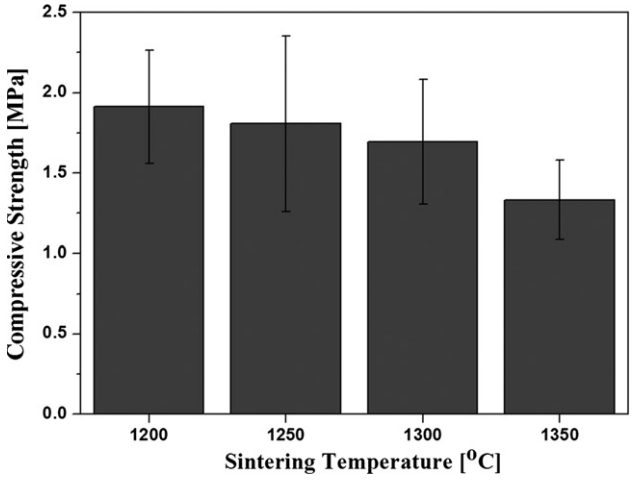

Fig. 4. Compressive strength of the porous CaP ceramics sintered at various temperatures (1200 °C, 1250 °C, 1300 °C, and 1350 °C).

After 7 days of immersion in the SBF, the surfaces of the samples sintered at temperatures ≥1250 °C were entirely covered with flake-like apatite crystals (Fig. 6(A)–(C)), whereas the sample sintered at 1200 °C still showed negligible precipitation of apatite crystals (Fig. 6(A)). These findings suggest that the presence of the α-TCP phase with the HA and β-TCP phases considerably enhance the in vitro bioactivity of the porous triphasic CaP scaffolds. In other words, the α-TCP grains can preferentially dissolve when immersed in SBF and release Ca^{2+} and PO_4^{3-} ions, which can stimulate precipitation of apatite crystals [36–38]. However, it should be noted that the contents of HA, β-TCP, and α-TCP phases should be controlled to provide excellent bioactivity.

The crystalline structure of the porous CaP scaffolds sintered at various temperatures (1250, 1300, and 1350 °C) after 7 days of immersion in SBF was examined by XRD. Basically, all the samples showed similar trends in XRD patterns after the SBF tests, as shown in Fig. 7(A)–(D). The relative intensities of the peaks corresponding to the (211) plane of HA remarkably increased, while those of the peaks corresponding to the (170) plane of α-TCP considerably decreased. This finding suggests that the hydroxyapatite crystals were vigorously formed onto the surfaces of the porous triphasic CaP scaffolds owing to the highly soluble α-TCP phase with the HA and β-TCP phases.

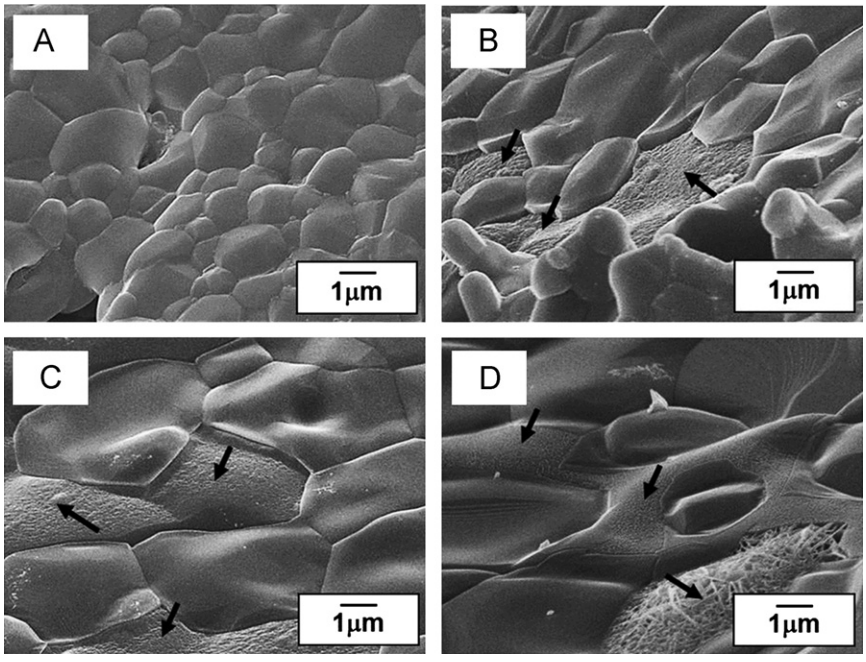

Fig. 5. SEM images showing the precipitation of apatite crystals on the surfaces of the porous CaP scaffolds sintered at various temperatures of (A) 1200 °C, (B) 1250 °C, (C) 1300 °C and (D) 1350 °C after 3 days of immersion in SBF.

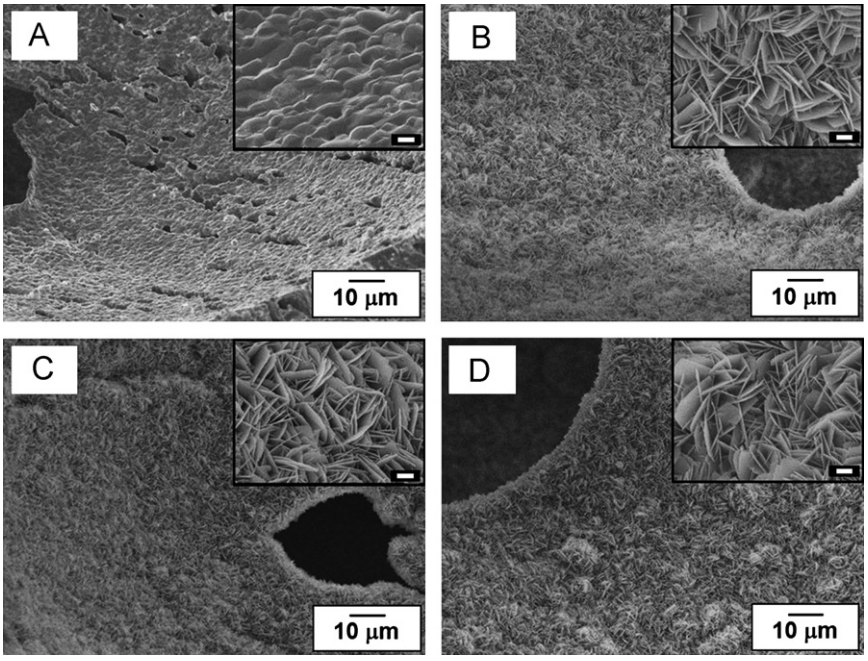

Fig. 6. SEM images showing the precipitation of apatite crystals on the surfaces of the porous CaP scaffolds sintered at various temperatures of (A) 1200 °C, (B) 1250 °C, (C) 1300 °C, and (D) 1350 °C after 7 days of immersion in SBF. Insets show the morphology of the apatite crystals (scale = 1 μm).

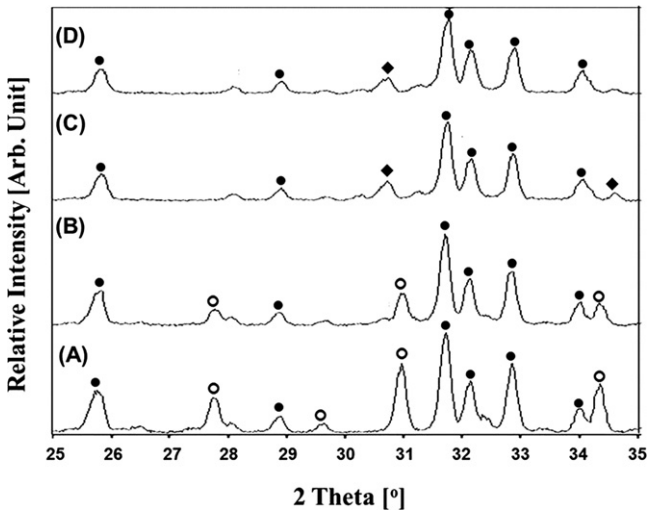

Fig. 7. Typical XRD patterns of the porous structures of the porous CaP scaffolds sintered at various temperatures of (A) 1200 °C, (B) 1250 °C, (C) 1300 °C, and (D) 1350 °C after 7 days of immersion in SBF. (●: HA, ○: β-TCP, ♦: α-TCP).

The porous triphasic CaP scaffolds, comprised of HA, β-TCP, and α-TCP, could have a highly porous structure (high porosity, large pores, good interconnections between the pores) with reasonably high compressive strength and excellent in vitro bioactivity, demonstrating their potential applications in bone tissue engineering. In addition, it should be also noted that the α-TCP grains can preferentially dissolve when implanted, which can create pores in the CaP walls, according stimulating new bone formation and ingrowth into large pores. However, a further in vivo study should be carried out for ensuring the potential of the porous triphasic CaP scaffolds as a bone scaffold.

4. Conclusions

Porous triphasic CaP scaffolds, comprised of HA, β-TCP, and α-TCP phases, were successfully produced using VFC technique coupled with sintering at relatively high temperatures ≥ 1250 °C. All the produced samples showed high porosity, large pore size, and large interconnection size, as well as reasonable compressive strength and excellent in vitro bioactivity. The intensity ratio of α-TCP/HA considerably increased from 0 to 35% with increasing sintering temperature from 1200 °C to 1350 °C, while the average grain size increased from 1.1 μm to 3.8 μm. This led to a slight decrease in compressive strength from 1.9 ± 0.3 to 1.3 ± 0.25 MPa. On the other hand, the samples sintered at relatively high temperatures ≥ 1250 °C showed a vigorous precipitation of flake-like apatite crystals after 7 days of immersion in SBF owing to the presence of the highly soluble α-TCP phase with the HA and β-TCP phases.

Acknowledgments

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