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# Processing of porous hydroxyapatite scaffolds containing calcium phosphate glassceramics for bone tissue engineering

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Highly porous hydroxyapatite (HA) scaffolds containing calcium phosphate glass-ceramics with large pore size and improved compressive strength have been prepared using a combined processing route of polymer sponge and freeze/gel-casting. Tertiary-butyl alcohol (TBA) was used as a solvent. The sintered scaffolds were characterized in terms of microstructure, physical and mechanical properties. A reticulated structure with large pore size of 220-300  $\mu$ m was formed on burn-out of polyurethane foam where the struts consisted of a few intergranular small-sized pores (< 2  $\mu$ m) formed by freeze/gel-casting. The addition of glass-ceramics seemed to lead a matrix phase to partial liquid phase sintering and thus the strut structure became more dense with the small size reduction of coarsened pores consisting of reticulated structure. As a consequence, the compressive strength was improved. Also, with the addition of 10 wt.% glass-ceramics the in vitro bioactivity of the scaffolds was further enhanced.

Key words: Hydroxyapatite scaffolds, Calcium phosphate glass ceramics, Tertiary-butyl alcohol, Polymer sponge, Freeze/gel-casting.

#### Introduction

The scaffolding materials require intensive biological affinity with bone tissue. Hydroxyapatite (HA) is well received as a potential scaffolding material for tissue engineering since it has a similar chemical composition,  $Ca_{10}(PO_4)_6(OH)_2$  to the major inorganic component of natural bone and thus excellent chemical and biological affinity with bone tissue [1-3]. Also, the scaffolds must satisfy structural requirements (e.g., 3-D interconnected pore structure and desirable pore size) to allow cell infiltration and host tissue growth in scaffolds [4, 5], mainly determined by the processing route and its conditions applied; in this case, the strategy to design novel pore structure with sufficient mechanical strength to handle scaffolds and to support their skeleton in the initial stage of tissue attachment must be considered.

A variety of processing routes for producing porous ceramic scaffolds have been proposed, which may be classified into replica, sacrificial template, and direct foaming techniques [6]. Because of inherent drawbacks in spite of their merits, none of these techniques can wholly satisfy the structural characteristics required for perfectly acceptable scaffolds, i.e., highly porous, largesized (the order of several hundreds of micrometer), fully interconnected pore structure combined with reasonable mechanical strength. For example, the polymer replica method can give the desirable porous morphology with highly interconnected and large-sized pores. However, the porous scaffolds obtained from this process commonly show insufficient mechanical strength for load-bearing tissue engineering applications [7-9]. Gel casting method is used to produce ceramic scaffolds with high mechanical strength; however, in this case the morphology of pores cannot routinely be controlled [10, 11]. Freeze casting is widely applied to prepare ceramic scaffolds with special porous structures which are directly related to the solvent used and its freezing conditions [12-16]; however, the scaffolds processed by this route generally cannot have sufficiently large pore size and high porosity coincident with desirable mechanical strength. As often as not, a combined processing route is applied to supply weak points appeared in the application of single processing. Porous HA scaffolds with large pore size (200-400 µm) and relatively high compressive yield strength was processed using a gel casting combined with polymer sponge method [17]; however, in this case any exquisite microstructure control of the skeletons of scaffolds could not be obtained. The compressive strength values of HA scaffolds prepared by freeze/gelcasting have been reported to be  $\sim 17 \text{ MPa}$  (24%) porosity) with dendrite-shaped pores [18] and 35.1-2.7 MPa (41.9-79.3% porosity) with unidirectional pore channels [19].

The objective of this paper is to produce highly porous HA scaffolds containing bioactive calcium phosphate glass-ceramics with controlled microstructure and improved compressive strength, which are adequate for bone tissue engineering application. To

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achieve the purpose, a polymer sponge method combined with freeze/gel-casting has been utilized; in this case, tertiary-butyl alcohol (TBA) was selected as the freezing solvent. The resulting scaffolds were characterized in terms of crystalline phase, microstructure, physical and mechanical properties. Especially, the influence of sintering temperature and glass-ceramics addition on such characteristics was investigated and discussed.

# **Experimental Procedure**

Commercially available HA powders  $(Ca_{10}(PO_4)_6(OH)_2)$ (DC Chemical Co., Korea) and synthetic calcium phosphate glass ceramics (Fig. 1) were selected as the bioactive ceramic materials, and reagent-grade TBA (C<sub>4</sub>H<sub>10</sub>O) (Junsei Chemical Co., Japan) as the freezing liquid phase. After heat treatment of calcium phosphate invert glass having the batch composition of 55CaO35P<sub>2</sub>O<sub>5</sub>3TiO<sub>2</sub>7Na<sub>2</sub>O (mol.%) at 850 °C for 1 hr, a glass-ceramics with relatively small-sized crystallite (<~90 nm) was prepared, where the crystallization temperature of the glass was in the range 750-780 °C, determined by DTA curve. The synthetic processing was already reported in detail [20].

TBA-based freeze/gel-casting slurries were prepared using various processing additives including citric acid (Aldrich Chemical Co., USA) dispersant, ethoxylated acetylenic diol (Dynol 604, Air Products and Chemicals, Inc., USA) surfactant, reactive organic monomer gelators, i.e. mono-functional acrylamide (AM, C<sub>2</sub>H<sub>3</sub>CONH<sub>2</sub>, Aldrich Chemical Co., USA) and di-functional N,N'methylenebisacrylamide (MBAM, (C<sub>2</sub>H<sub>3</sub>CONH)<sub>2</sub>CH<sub>2</sub>, Aldrich Chemical Co., USA), and free radical initiator, i.e. ammonium persulphate (APS, (NH<sub>4</sub>)<sub>2</sub>S<sub>2</sub>O<sub>8</sub>, Kanto Chemical Co., Japan).

The freeze/gel-casting slurries were prepared at 10 vol.% solid loading by adding HA powder containing 0, 5, and 10 wt.% glass-ceramics into premixed solution with the weight percent of TBA/AM/MBAM = 90/9.6/ 0.4, and then 1 wt.% citric acid based on HA powder content and 0.25 wt.% Dynol 604 based on monomer solution were added. The prepared slurries were homogenized by ball milling at 40 °C for 24 hrs and then stabilized at 30 °C in a water bath to restrict further polymerization of AM. After de-airing under vacuum for 3 min, an aqueous solution of 20 wt.% initiator,  $(NH_4)_2S_2O_8$ , was slowly dropped into the



**Fig. 1.** SEM micrographs of (a) as-received HA and (b) synthetic calcium phosphate glass-ceramics powders.

slurries which were continuously stirred using a magnetic stirrer.

A flexible polyurethane sponge with the cell size of 75 PPI (pores per linear inch) was cut into a suitable shape and size and placed in a close fit polyethylene mold backed by a heat insulating later in which the bottom face of the mold was tightly capped. The prepared warm ceramic component slurry was then poured into the foam molds. To aid complete impregnation the foams were squeezed slightly five times. Subsequently, the mold was placed on a stainless steel plate which was temperature-controlled at < 0 °C. The frozen samples were carefully removed from the molds. Subsequently, the TBA was sublimated in a freeze drier (Labconco 77540, Western Medics, USA). After preliminary calcination at 600 °C for 1 hr with a heating rate of 1 °C/min to remove organic additives and remnant polyurethane foams, the samples were finally sintered at 1050-1250 °C for 2 hr (heating rate: 3 °C/min) in air.

The crystalline phase, morphology, porosity, and mechanical properties of the fabricated porous scaffolds were investigated. The crystalline phase was examined using X-ray diffractometry (XRD, D/max-Rigaku, Japan). The pore and skeleton IIA, morphologies were observed using a scanning electron microscopy (SEM, JSA-840A, Jeol, Japan). The pore size and strut thickness were determined with 15-20 pores and struts were arbitrarily selected in each specimen. The compressive strength was measured for five sintered specimens with dimensions of  $\sim 5 \times 5 \times 8$ mm using a Universal Test Machine (model 6025, Instron, USA) with a crosshead speed of 0.5 mm/min, and calculated the load at fracture by cross-sectional area. Sintered porosity was measured by the water immersion method based on the Archimedes' principle.

Hanks' balanced salt solution (HBSS), an extracellular solution with an ionic composition similar to human blood plasma was used to test the in vitro bioactivity of the resulting scaffolds. The simulated solution consisted of 8.00 g NaCl, 0.35 g NaHCO<sub>3</sub>, 0.40 g KCl, 0.06 g KH<sub>2</sub>PO<sub>4</sub>, 0.10 g MgCl<sub>2</sub>6H<sub>2</sub>O, 0.14 g CaCl<sub>2</sub>, 0.06 g Na<sub>2</sub>HPO<sub>4</sub>2H<sub>2</sub>O, 0.06 g MgSO<sub>4</sub>7H2O, and 1.00 g glucose in 1000 ml distilled H<sub>2</sub>O and had an initial pH of 7.4. The scaffolds were immersed in 50 ml of HBSS at 37 °C in a Teflon-sealed polystyrene bottle. After finishing immersion for 3 weeks, the scaffolds were washed with double-distilled water to remove residual HBSS, and then dried in a vacuum desiccator at 21 °C. The morphology of the scaffolds was examined using a scanning electron microscope.

### **Results and Discussion**

The XRD patterns of porous scaffolds sintered at 1250 °C with the addition of 0-10 wt.% glass-ceramics are given in Fig. 2. The presence of characteristic peaks corresponding to HA (JCPDS card # 9-432),  $\beta$ -calcium



**Fig. 2.** XRD patterns of porous HA scaffolds sintered at 1250°C with the addition of (a) 0 wt.%, (b) 5 wt.%, and (c) 10 wt.% glass-ceramics.



**Fig. 3.** SEM micrographs of porous HA scaffolds sintered at (a, b) 1050 °C (c, d) 1150 °C, and (e. f) 1250 °C.



**Fig. 4.** SEM micrographs of porous HA scaffolds sintered at  $1150 \,^{\circ}$ C with the addition of (a) 5 wt.% and (b) 10 wt.% glass-ceramics, and at 1250  $\,^{\circ}$ C with (c) 5 wt.% and (d) 10 wt.%.

pyrophosphate (JCPDS card # 9-346) and  $\beta$ -tricalcium phosphate (JCPDS card # 9-169) was confirmed; the peak intensities of  $\beta$ -CPP and  $\beta$ -TCP were very weak compared to those of HA, due to the relatively small addition of glass-ceramics having a poor crystallinity. The diffraction intensity of HA decreased slightly with the addition of 10 wt.% glass-ceramics. However, any loss of (OH)<sup>-</sup> groups in the HA was not observed at a given temperature (Fig. 2(a)), indicating that it is possible to sinter the green scaffolds at  $\leq$  1250 °C with ignorable decomposition of HA.



**Fig. 5.** Pore size and strut thickness of porous HA scaffolds with 10 wt.% glass ceramics addition as a function of sintering temperature.



Fig. 6. SEM micrographs of the struts of porous HA scaffolds sintered at  $1150 \,^{\circ}$ C with the addition of (a, b) 0 wt.%, (c, d) 5 wt.%, and (e, f) 10 wt.% glass-ceramics.

Microstructure, porosity, and mechanical properties of the resulting scaffolds are influenced by processing variables (e.g., solid loading, sintering temperature, and glass-ceramics addition). The effects of sintering temperature (1050-1250 °C) and the addition of glass ceramics (0-10 wt.%) on sintered microstructure of the scaffolds have been examined, and the SEM micrographs are given in Figs. 3 and 4. Consequently, similar reticulated structure was obtained; in this case, the presence of a few microcracks was observed in the reticulated struts, which could be occurred during pyrolysis of the sponge template. With increasing sintering temperature from 1050 to 1250 °C, the average pore size of the HA scaffolds with the addition of 10 wt.% glass-ceramics decreased in the range of 295-225  $\mu$ m where the strut thickness (79-103  $\mu$ m) increased inversely to the pore size (Fig. 5), due to the constant pore cell size and volume, of the polyurethane foam used. Such a pore structure and pore size obtained by this process can allow bone tissue to growth into the scaffolds [21]. At higher magnifications (Fig. 6), the struts of the interconnected pore channels showed a relatively dense structure containing a few intergranular small pores ( $< 2 \mu m$ ). The degree of densification seemed to be further increased with especially 10 wt.% glass-ceramics addition, probably

due to the rearrangement and growth of HA grains during liquid phase sintering in part.

The porosity of porous scaffolds fabricated at different sintering temperatures is shown in Fig. 7. With increasing sintering temperature, the average porosity decreased (82.3-60.4%) due to relatively large firing shrinkage (average values of 12.0-25.0%) and consequently, the enhanced compressive strength would be expected, due to the more dense and thickened struts. If this were not the true, the presence of any other critical inherent flaws rather than porosity would be likely to cause a reduced strength in the porous scaffolds. The addition of glass-ceramics is considered to improve the sintering of HA green body, resulting in higher compressive strength (Fig. 8). It is assumed that this higher compressive strength with glass-ceramics addition is due to the improved densification of the matrix phase by partial liquid phase sintering in the presence of glass component melts. Especially, with 10 wt.% glass-ceramics addition, relatively high compressive strength values of 2.32-4.27 MPa (75.2-60.4% porosity) were obtained, depending on sintering temperature. A few studies on the compressive strength of HA scaffolds fabricated by polymer sponge-based processing route have been reported, however in this case the simple comparison of compressive strength of the scaffolds is unreasonable because of their different porous morphologies. For example, Yang et al. [22] obtained porous HA scaffolds



Fig. 7. Apparent porosity of porous HA scaffolds with different glass-ceramics addition as a function of sintering temperature.



Fig. 8. Compressive strength of porous HA scaffolds with different glass-ceramics addition as a function of sintering temperature.

with large pore size of 180-360 µm by TBA-base freeze/gel-casting/polymer sponge technique. After sintering at 1250-1450 °C with the addition of 5 wt.% magnesia, the porosity and compressive strength were 4.03-6.57 MPa and 68-63%, respectively. Jun et al. [7] prepared highly porous HA scaffolds coated with apatite-wollastonite (A/W) glass-ceramics using a polymeric sponge replication method; after sintering of HA struts and then A/W glass-ceramics coating layer at 1200 and 1050 °C, respectively, the scaffolds had the compressive strength of  $\sim 1 \text{ MPa}$  (93% porosity). Ramay and Zhang [17] obtained interconnected porous HA scaffolds with a pore size of 200-400 µm using a water-based gel-casting combined with polymer sponge method. The compressive yield strength of the scaffolds sintered at 1350 °C was ~ 5 MPa (71% porosity).

For tissue engineering applications, the high mechanical strength of porous scaffolds is desirable simultaneously with high porosity; however, it is very difficult technically since the mechanical strength of materials generally behave in an opposite manner to the porosity. The compressive strength of porous



**Fig. 9.** The relationship between compressive strength and porosity in porous HA scaffolds sintered at 1050-1250 °C with 0-10 wt.% glass-ceramics addition.



**Fig. 10.** SEM micrographs of porous HA scaffolds sintered at  $1150 \text{ }^{\circ}\text{C}$  with the addition of (a, b) 0 wt.%, (c, d) 5 wt.%, and (e, f) 10 wt.% glass-ceramics after immersion in HBSS for 3 weeks.

scaffolds is influenced by many parameters, including porosity, grain size, pore size, wall thickness and wall density, which are mainly determined by processing routes; in this case, it is not easy to consider all the parameters in strength evaluation because they are related to each other and the compressive strength is finally determined by the sintered microstructure governed by all of them. Generally, among these parameters the compressive strength of porous scaffolds depends primarily on the degree of porosity. In this study, therefore, the relationship between porosity and compressive strength was plotted and is given in Fig. 9. As might be expected, low porosity resulted in high compressive strength, possibly due to the high bulk density (shrinkage) of sintered scaffolds. The linear relationship between compressive strength and porosity obtained from this study can be approximately expressed by the equation;

$$y = 11.88 - 0.13x$$
 (1)

where y is the compressive strength (MPa) and x is the porosity (%).

The SEM micrographs shown in Fig. 10 demonstrate the relatively good bioactivity of the HA scaffolds containing glass-ceramics. After immersion in HBSS for 3 weeks, a large number of tiny sphere-shaped particles were precipitated in the preferred nucleation sites on the surface of the scaffolds especially with the addition of 10 wt.% glass-ceramics. The solubility of calcium phosphate minerals greatly depends on the pH of the aqueous solution; it increases in the order of HA <<< \beta-TCP <  $\alpha$ -TCP [23]. Similarly, calcium phosphate glass-ceramics may be preferentially dissolved in HBSS, at which time very fine bone-like apatite particles could be precipitated on the preferred surface sites of the scaffolds by the standard dissolutionprecipitation mechanism [24-26].

#### Conclusions

A tertiary-butyl alcohol-based freeze/gel-casting combined with polymer sponge route has been processed to prepare porous hydroxyapatite scaffolds with the small addition of calcium phosphate glass-ceramics for bone tissue engineering applications. The sintered porosity and pore size generally behaved in an opposite manner to the sintering temperature, i.e., a high sintering temperature gave low porosity and small pore size, and a thickening of the strut cross section, thus leading to higher compressive strengths. As the sintering temperature increased from 1050 to 1250 °C with 10 wt.% glass-ceramics addition, the sintered porosity (82.3-60.4%) and pore size (300-220  $\mu$ m) decreased while the thickness of struts increased in the range 80-102  $\mu$ m. The addition of glass-ceramics more

or less increased the compressive strength together with the bioactivity of the HA scaffolds.

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