Control of β-Tricalcium Phosphate Formation in Macroporous Phosphate Glass-Ceramic Composites

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A macroporous phosphate invert glass-ceramic (PIGC) was derived from the mother glass with a composition of 60CaO-30P₂O₅-3TiO₂-7Na₂O in mol%. The mother glass powders were coated on a polymer sponge skeleton and subsequently sintered at 850°C for 1 h through burning off the sponge, resulting in the preparation of macroporous PIGC. The resulting macroporous PIGC consists predominantly of β-tricalcium phosphate (β-TCP) and β-calcium pyrophosphate and has large-sized pores of 300–1000 μm in diameter and small-sized pores of several tens micrometer in diameter. A new type of composite containing a large amount of β-TCP was also prepared by heating the mixture of the mother glass powders with Ca(OH)₂ at 800°C for 1 h. The amount of β-TCP in the crystalline phase of the composites increased with increasing the Ca(OH)₂ amount. The β-TCP-containing glass-ceramic composites can be prepared by firing at a considerably lower temperature than a conventional β-TCP ceramics. The amount of β-TCP in the composites is controllable by variation of the Ca(OH)₂ amount. The composites showed higher solubility in an acetic acid solution at 37°C with an initial pH value of 5.0 in comparison with PIGC. The solubility of one of the composites was comparable with that of pure β-TCP. The composites are expected to be applicable for biodegradable scaffold for bone tissue engineering.

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1. Introduction

Various types of calcium phosphate materials have been investigated for application to bone substrates and scaffolds for bone tissue engineering. Calcium phosphate materials such as hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) have been reported to have bioactivity and cellular compatibility, and therefore already applied in clinical use. Moreover, cellular compatibility and bone formation were reported to be enhanced in/on porous materials. The enhancement was suggested to attribute to each effect by large-sized pores of >50 μm and small-sized pores of <10 μm. In the present work, these pores are denoted by macropores and micropores, respectively. It has been reported that macropores have a significant impact on osteogenic outcomes and micropores bring out abilities of high bone-inducing protein adsorption, ion exchange and bone-like apatite formation. Moreover, the porosity of materials is regarded as one of the important factors for cellular compatibility. On materials with high porosity, cell proliferation was reported to be activated due to transport of oxygen and nutrients to inside. On materials with low porosity, high alkaline phosphatase activity and osteocalcin of cultured mesenchymal stem cells were reported to be induced. Thus, it is reasonable to apply porous materials as the bone tissue engineering scaffolds. High biodegradability is also required for the scaffolds. The biodegradable scaffolds can implant effective cells cultured on its surface beforehand in vitro, induce bone formation on its surface, and finally replace by newly formed tissue at the same time as resolving. Bioglass® and β-TCP have been well-known as biodegradable materials with high solubility. Bioglass®, however, has a tendency to worry about its fragility and its toxicity, originating from high silicon-releasability. It is difficult to control the biodegradability of β-TCP effectively.

In our earlier work, a phosphate invert glass-ceramic (PIGC) containing no silica was prepared, and its bonelike-apatite-forming ability and its bioactivity were reported with the results of implant tests in the femurs of Japanese rabbits. PIGC, consisting of crystalline phases such as β-TCP and β-calcium pyrophosphate (β-CPP) with residual glassy phase containing sodium and titanium, is prepared by sintering the mother glass of 60CaO-30P₂O₅-3TiO₂-7Na₂O in mol%. Good machinability of PIGC was shown by the result of a drilling test using a conventional carbide tool. β-CPP crystal in the glass-ceramic has a plate-like microstructure and interlocks with another one, and therefore the structure of β-CPP is suggested to be related closely to the cleavability. However, the degradability of PIGC would be influenced by the low solubility of β-CPP. Increasing the amount of β-TCP in PIGC is expected to be effective for application of PIGC to biodegradable scaffolds for bone tissue engineering.

In the present work, porous PIGC was prepared by using urethane sponges, and PIGC composites containing a large amount of β-TCP were prepared by mixing Ca(OH)₂ with the mother glass powders. The thermal decomposition temperature of Ca(OH)₂ is about 450°C, lower than the crystallization temperature of PIGC. Heating the mixture of the mother glass powders with Ca(OH)₂ would induce high calcium concentration in its composite to enhance β-TCP formation in the crystalline phase.

2. Materials and Methods

The mother glass, with a nominal composition of 60CaO-30P₂O₅-3TiO₂-7Na₂O in mol%, was prepared with reagents of CaCO₃, Na₂CO₃, TiO₂ and H₃PO₄ (all reagents were purchased from Kishida Chemical Co., Ltd). The reagents were mixed in distilled water and then dried at 140°C overnight. The mixed reagents were melted in a platinum crucible at 1300°C for 30 min using an electric furnace. The
melt was poured onto a stainless-steel plate and immediately pressed with an iron plate to obtain the thin plate-like glass pieces.

The resulting mother glass was pulverized into < 10 μm in diameter in methanol with a ball mill for 48 h and dispersed in 20 mass% polyvinyl alcohol solution with a mass ratio of glass:solution = 2:1. An urethane sponge block of 10 × 10 × 10 mm³ in size was immersed in the resulting glass slurry, and then dried at 50°C for 20 h. The sponges coated with the glass were heated by elevating temperature at rates of 0.5°C/min to 600°C and 5°C/min from 600°C to 850°C, and holding the maximum temperature for 1 h through burning off the sponge and sintering, resulting in the preparation of porous PIGC. The resulting porous PIGC was observed by optical microscopy and field emission scanning electron microscopy (FE-SEM). The compressive strength of the porous PIGC was measured using the sample of 6 × 6 × 8 mm³ at a crosshead speed of 0.5 mm/min.

The mother glass powders were mixed with a reagent of Ca(OH)₂ (Kishida Chemical Co., Ltd) at various weight ratios. The mixture of the mother glass powders and Ca(OH)₂ was heated at an elevating rate of 5°C/min to 800°C and held at the temperature for 1 h to be sintered. The resulting materials were denoted by PIGC-Ca composites. Crystalline phases in the PIGC-Ca composites were examined by X-ray diffractometry (XRD). Differential thermal analysis (DTA) curves of the mother glass, Ca(OH)₂ and the mixture of PIGC with 7.5 mass% Ca(OH)₂ were measured at an elevating rate of 5°C/min. The porous materials composed of the PIGC-Ca composite skeleton were also prepared by the above-mentioned method using the urethane sponges.

In vitro dissolution behavior of β-TCP, β-CPP, PIGC and PIGC-Ca composites was investigated by soaking their particles in an 10 mM/L acetic acid solution at 37°C with an initial pH 5.0. As control materials, β-TCP and β-CPP ceramics were prepared by sintering the purchased reagents at 1000°C and 1070°C, respectively, for 3 h at a heating rate of 5°C/min. Two hundreds milligrams of these sample, which were pulverized into 53–125 μm in size, were rapidly soaked into 100 mL of the above-mentioned acetic acid solution for various periods. The solution with these sample particles was stirred continuously throughout the experiment, and then separated by filtration. The ion amounts of the resulting solution were analyzed by an inductively coupled plasma atomic emission spectroscopy (ICP-AES). They were evaluated through the use of chemical potential plots. The ion activity was estimated using a Davis’ equation \( \log f_i = -A_z^2 \left( \frac{sq(\mu)}{1 + sq(\mu)} - sq(\mu) \right) \); where \( f_i \): ion activity, \( \mu \): ionic strength, \( z \): electrovalence, \( A \): constant) for understanding the resorbability of β-TCP, β-CPP, PIGC or the PIGC-Ca composites.

3. Results and Discussion

Figure 1 shows the macroporous structure and surface morphology of porous PIGC and PIGC-Ca composites. The structure conscientiously traced that of an urethane sponge. The skeleton was about 100 μm in thickness and observed to consist mainly of squarish particles. Diameters of macro pores and micropores formed in the skeleton were estimated to be about 300–1000 μm and about several tens micrometers, respectively. The porosity and the compressive strength were estimated to be 83% and 160 kPa, respectively. The obtained porous PIGC is believed to have the mechanical strength enough for handling during cell culture and the porosity enough for inducing excellent cell adhesion. There were almost no significant differences in structural morphologies between porous PIGC and the PIGC-Ca composites, but the squarish particle in the PIGC-Ca composites was observed to be smaller than that in PIGC.
due to $\beta$-CPP was observed in the spectra of the PIGC-Ca composites derived from the mixtures of 10 mass% $\text{Ca(OH)}_2$. The content of $\beta$-TCP in the PIGC-Ca composites increased with increasing the $\text{Ca(OH)}_2$ amount. The formation of $\beta$-TCP was enhanced by introducing $\text{CaO}$ derived from the decomposition of the $\text{Ca(OH)}_2$ to PIGC during the partial melting of the crystalline phase and the sintering as shown in Fig. 2. Conventional $\beta$-TCP ceramics are prepared by powder sintering at 1000–1100°C. In comparison with the ceramics, the $\beta$-TCP-containing composites in the present work can be prepared by heating at considerably lower temperature.

Biodegradable ceramics are resorbed and decrease in volume due to phagocytosis by cells such as osteoclast after accomplishment bone bonding in body. The phagocytosis by osteoclast may relate to the reactivity of materials to acid medium. Figure 4 shows the chemical potential plots of the solution composition in the dissolution reactions for $\beta$-TCP, $\beta$-CPP, PIGC and PIGC-Ca composite derived from the mixtures of 7.5 mass% $\text{Ca(OH)}_2$. In the present work, $\text{Na}_2\text{O}$ was disregarded for calculation of the chemical potential because its amount was very small. The two lines indicate solubility lines for HA and octacalcium phosphate (OCP), respectively: they mean supersaturated areas of their components below the lines. OCP was reported to behave as the precursor of HA in the solution containing calcium and phosphorous ions.\(^{15,16}\) The variation of solution compositions of all samples moved with immersing time toward the HA solubility line. In Fig. 4(a), the plots of $\beta$-TCP reach to HA solubility line after 15-min immersion. On the other hand, the plots of $\beta$-CPP reach to HA solubility line after 1400-min immersion. The solubility of $\beta$-TCP was higher than that of $\beta$-CPP. In Fig. 4(b), the plots of the PIGC-Ca composite move with immersing time in a wider range in comparison with that of $\beta$-TCP, reach to HA solubility line after 30-min immersion and subsequently reach to OCP solubility line after 1400-min immersion. On the other hand, the plots of PIGC reach to HA solubility line after 60-min immersion and to OCP solubility line after 1400-min immersion. This result implies that the PIGC-Ca composite containing a large amount of $\beta$-TCP has higher solubility than PIGC. The solubility of the PIGC-Ca composite was comparable with that of $\beta$-TCP. The content of $\beta$-TCP in the crystalline phase of the PIGC-Ca composite was controllable by variation of the $\text{Ca(OH)}_2$ amount as shown in Fig. 3. It is possible to control the reactivity of the PIGC-Ca composite by adjusting the amount of $\text{Ca(OH)}_2$ to provide the suitable biodegradability.

$\beta$-TCP has been investigated widely as the scaffold for bone regeneration because of its bioactivity, biodegradability and cellular compatibility.\(^{11,17}\) Porous $\beta$-TCP was reported to induce osteogenic cells around or within its material after 6-month implantation in sinus floor prior to oral dental implants.\(^{11}\) Moreover, the amount of osteocalcin on $\beta$-TCP was reported to be the highest among five kinds of calcium phosphate ceramics, such as $\beta$-TCP, $\alpha$-TCP, HA, fluorapatite and tetracalcium phosphate, by the results of cell culture tests using normal human osteoblasts.\(^{18}\) This implies that the maturation of the cells on $\beta$-TCP precede and attribute to the amount of extracted calcium and/or phosphate ions. Thus,
the β-TCP-rich surface of the PIGC-Ca composites is expected to induce the differentiation and the maturation of osteoprogenitor cells in comparison with PIGC and other calcium phosphate ceramics. The PIGC-Ca composite containing a large amount of β-TCP showed higher solubility in comparison with PIGC and compared favorably with pure β-TCP in solubility. The PIGC-Ca composite can provide calcium and phosphate ions rapidly, and therefore is expected to show high ability of cellular proliferation, differentiation and maturation. The mechanism of bone formation on β-TCP was reported to relate to the efficiency of cell in-growth and differentiation.11) Macroporous PIGC-Ca composites have high efficiency of cell in-growth via macropores and micropores to inducing cell adhesion, protein adsorption and ion-exchange, and therefore the composite is suggested to have high bone forming-ability.

4. Conclusion

Macroporous PIGC and PIGC-Ca composites, having porosity and strength enough for cell culture, were prepared by using urethan sponges. The PIGC-Ca composites containing a large amount of β-TCP were prepared by mixing Ca(OH)$_2$ with the mother glass powders of PIGC on sintering. The content of β-TCP in PIGC-Ca composites increased with increasing the Ca(OH)$_2$ amount. The PIGC-Ca composites showed higher solubility than PIGC. Macroporous PIGC-Ca composites are expected to be useful as biodegradable substrates and scaffolds for bone tissue engineering.

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REFERENCES


Fig. 4 Chemical potential plots of the solution composition in the dissolution reactions for (a) β-CPP and β-TCP and (b) PIGC and PIGC-Ca composites prepared by mixing 7.5 mass% Ca(OH)$_2$. Numbers near the plots indicate reaction time in minutes. The lines in the figure are solubility lines for hydroxyapatite (HA) and octacalcium phosphate (OCP), respectively.