Preparation of β -Tricalcium Phosphate by Chelate Reaction of Calcium Ion with Phosphonic Acid

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Beta-tricalcium phosphate (β -TCP) precursors were prepared by chelation of calcium ions with phosphonic acid using calcium nitrate tetrahydrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$) and 2-phosphonobutane-1,2,4-tricarboxylic acid ($\text{C}_7\text{H}_{11}\text{PO}_9$; PBTA) in distilled water in the temperature range of 60–130 °C. β -TCP powder was then synthesized by heating the precursors at various temperatures. β -TCP precursors were gelled in three reactions. First, calcium ions were chelated with carboxyl groups in PBTA. Second, chelation of calcium ions with phosphonic acid and/or the dehydrated condensation of carboxyl groups with phosphonic acid, finally, dehydrated condensation of carboxyl groups occurred in the range of 100–130 °C. Powder obtained after heating the β -TCP precursor prepared at 130 °C had a single crystalline α -TCP phase at 600 °C, following by a single β -TCP phase at 800 and 1000 °C. In addition, the crystallinity of the powder improved with an increase in the preparation temperature of β -TCP precursors, which accelerated gelation. The powder obtained at 600 °C was formed layers with submicrometer pores among particles, and the powder obtained after heating at and above 800 °C, the particles formed a specific three-dimensional network. The particle sizes increased with an increase in the heating temperatures.

Key words: Beta-tricalcium phosphate, Precursor, Polymerized complex method, Chelation, Three-dimensional network structure

1. INTRODUCTION

Calcium phosphate ceramics have used as implant bioceramics for bone repair because of their excellent biocompatibilities and compositional similarities to natural bone [1-2]. β -tricalcium phosphate (β - $\text{Ca}_3(\text{PO}_4)_2$, β -TCP), which has been studied as a bioresorbable material among calcium phosphate bioceramics, is applied for orthopedic and maxillofacial implant surgery [1-4]. β -TCP powder is commonly prepared by solid-phase method, which is one of the synthesis methods obtained the product with stoichiometric composition, because β -TCP forms only at Ca/P

molar ratio = 1.50 [5-8].

On the other hand, in case of liquid-phase method, rigorous controls of synthesis condition, such as pH, temperature, ionic concentration, Ca/P molar ratio, rate of stirring and digestion time, are necessary for β -TCP preparation because of a similar reason [5-6]. Therefore, as simpler liquid-phase method for β -TCP, we have paid attention to polymerized complex method, which is a liquid phase method involving the thermal treatment of a polymer gel with metal ions and is also an excellent method for preparing highly pure and complex compositional ceramic powder [9-11]. As

synthesis of calcium phosphate by polymerized complex method, Takahashi et al. have synthesized HAp powder with a stoichiometric composition using calcium nitrate hydrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$), citric acid, ethylene glycol, and phosphonoacetic acid^[12]. However, this method has been a problem involving the use of large amounts of organic substance and a high temperature for decomposing the stable calcium-citric acid complex in the precursor.

In this study, using $\text{Ca}(\text{NO}_3)_2$ and 2-phosphonobutane-1,2,4-tricarboxylic acid ($\text{C}_7\text{H}_{11}\text{PO}_6$; PBTA), which is a water-soluble chelating agent that directly binds calcium ions and has three carboxyl groups as shown in Fig. 1, β -TCP precursors were synthesized in distilled water by chelating calcium ions with phosphonic acid and dehydration condensing of phosphonic acid and carboxyl group. In this method, there is no need to use excess organic materials such as citric acid and ethylene glycol as complexing agents and polymerization agent, respectively; in addition, a stable calcium-citric complex does not form. Further, the gel mechanism for β -TCP precursors was investigated, and the powder obtained by heating β -TCP precursors was characterized.

Fig. 1 Chemical formula of 2-phosphonobutane-1,2,4-tricarboxylic acid (PBTA).

2. MATERIALS AND METHODS

In preparation of β -TCP precursors, $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (Kishida Chemical, Osaka, Japan) and 50 mass% PBTA (K-I Chemical Industry, Shizuoka, Japan) were used as starting materials. First, 60.0 mmol $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and 40.0 mmol PBTA were dissolved in distilled water (50 ml) to be a Ca/P molar ratio of 1.50, which is the stoichiometric composition of β -TCP. The mixture was stirred with heating at 60, 80, 100, and 130 °C for 3 h to prepare the β -TCP precursors, which hereinafter referred to as preTCP-60, preTCP-80, preTCP-100, and preTCP-130, respectively. These precursors were then heat-treated at 200, 400, 600, 800, and 1000 °C for 5 h in air.

The crystalline phases of the β -TCP precursors and the powder obtained by heating the β -TCP precursors were characterized using an X-ray diffractometer (XRD: RAD-2C, Rigaku, Tokyo, Japan). Qualitative analysis of PBTA, β -TCP precursors and the powder was performed using a Fourier transform infrared

spectrophotometer (FT-IR; FT/IR-230, JASCO, Tokyo, Japan) by the diffuse reflectance method using KBr (Kishida Chemical).

^{31}P nuclear magnetic resonance (^{31}P -NMR) was measured using a nuclear magnetic resonance spectrophotometer (AVANCE 400, Bruker Biospin, Rheinstetten, Germany) operated at 25.9 MHz and 256 scans using 85% phosphoric acid (Kishida Chemical) as the external standard in order to clarify the detailed reaction mechanism for β -TCP precursor, in particular the reaction of phosphonic acid in PBTA. The measurement samples were confected by dissolving the precursor (0.05 mg) in a mixture solution of distilled water (0.3 ml) and deuterium oxide (0.3 ml, D_2O , MERCK, Darmstadt, Germany).

The thermal behavior of the prepared precursors was investigated by a thermogravimetric and differential thermal analysis (TG-DTA: TG-DTA 2000S, Bruker AXS, Rheinstetten, Germany).

The morphology of the powder obtained by heating preTCP-130 was observed using a field emission scanning electron microscope (FE-SEM: S-4700, Hitachi High Technologies, Tokyo, Japan) operating at 10 kV. The observed specimens were sputter-coated with Au before FE-SEM observations.

3. RESULTS AND DISCUSSION

3.1 Formation mechanism of β -TCP precursors

Fig. 2 shows FT-IR spectra of PBTA and β -TCP precursors. The bands at 1735 cm^{-1} , which were attributed to carboxyl groups, were split by reacting $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and PBTA. In addition, in FT-IR spectra of the precursors prepared in the range of 100–130 °C (preTCP-100 and preTCP-130), bands were observed at 1855 and 1776 cm^{-1} , and they were attributed to the formation of a chain acid anhydride ($-\text{C}(\text{O})\text{O}(\text{C})\text{O}-$). These results indicate that calcium ions of $\text{Ca}(\text{NO}_3)_2$ were chelated with the carboxyl groups in PBTA, which is a chelating agent, and the acid anhydrides were also formed via dehydrated condensation of carboxyl groups in PBTA in the range of 100–130 °C.

^{31}P -NMR spectra of the precursors were shown in Fig. 3. The peak attributed to P-O-H groups appeared at 4 ppm in the ^{31}P -NMR spectra of preTCP-60, preTCP-80, preTCP-100, whereas the peak did not appear in preTCP-130. This result suggests that in the range of 100–130 °C, a Ca-phosphonic acid was formed via the chelation of Ca^{2+} ions from $\text{Ca}(\text{NO}_3)_2$ with the phosphonic acid in PBTA and/or via the dehydrated condensation of phosphonic acids with carboxyl groups in PBTA.

From these results, it can be considered that in the $\text{Ca}(\text{NO}_3)_2$ -PBTA- H_2O system, β -TCP precursors were gelled via three

Fig. 2 FT-IR spectra of PBTA and β -TCP precursors prepared by reacting $\text{Ca}(\text{NO}_3)_2$ and PBTA in H_2O at various temperatures. (a) PBTA, (b) 60, (c) 80, (d) 100, and (e) 130 $^\circ\text{C}$.

Fig. 3 ^{31}P -NMR spectra of β -TCP precursors prepared by reacting $\text{Ca}(\text{NO}_3)_2$ and PBTA in H_2O at various temperatures. (a) 60, (b) 80, (c) 100, and (d) 130 $^\circ\text{C}$.

reactions as follows:

- (1) First, a calcium ion was chelated with two carboxyl groups in PBTA.
 - (2) Second, at 130 $^\circ\text{C}$, the chelation of calcium ions with phosphonic acid groups in PBTA and/or the dehydrated condensation of phosphonic acid groups with carboxyl groups in PBTA occurred.
 - (3) Finally, in the range of 100–130 $^\circ\text{C}$, an acid anhydride was formed by the dehydrated condensation of carboxyl groups.
- Furthermore, the results of XRD measurement indicated that the β -TCP precursors formed via this mechanism at and above 80 $^\circ\text{C}$ were amorphous substance.

3.2 Characterization of the powder synthesized from β -TCP precursor

Fig. 4 shows the XRD patterns of the powder prepared from

Fig. 4 XRD patterns of the powders after heating β -TCP precursors (preTCP-130) at various temperatures. (a) precursor, (b) 200, (c) 400, (d) 600, (e) 800, and (f) 1000 $^\circ\text{C}$

preTCP-130. At 600 $^\circ\text{C}$, metastable alpha-TCP (α -TCP) crystallized from amorphous substance. Subsequently, β -TCP was transformed from α -TCP at and above 800 $^\circ\text{C}$, and the crystallinities of the powder synthesized from preTCP-130 increased with the heating temperature. These heating behaviors were as same as amorphous calcium phosphate. In addition, preTCP-130 had excellent homogeneity and its Ca/P molar ratio was 1.50, which is the stoichiometric composition of β -TCP, because any impurities, e.g. calcium pyrophosphate, hydroxyapatite and calcium oxide, could not be observed.

TG-DTA measurement was performed to investigate the thermal behavior of β -TCP precursor, and the result shows in Fig. 5. TG-DTA curves support the result of XRD measurement as shown in Fig. 4; exothermic peaks at 220, 680, and 750 $^\circ\text{C}$ were observed and would be attributed to the removal of NO_x , the phase transition from α to β -phase, and the complete removal of organic substance (CO_2), respectively. Additionally, the weight loss and gradual exothermic curve in the range of 240 and 600 $^\circ\text{C}$ could show the thermal decomposition of organic substance in the precursor.

FE-SEM micrographs of the powder are shown in Fig. 6. PreTCP-130 was an amorphous aggregate; the powder obtained at 600 $^\circ\text{C}$ was agglutinated with submicron sized particles (200–500 nm) and formed layers with approximately 0.5–3.0 μm pores among particles. In the case of the powder obtained after heating at and above 800 $^\circ\text{C}$, the particles formed a three-dimensional network. In addition, each particle size increased with an increase in the heating temperature; the particle sizes were 0.2–0.5, 0.4–1.0, and 1.0–2.0 μm at 600, 800 and 1000 $^\circ\text{C}$, respectively. These results indicated that the powder had a cross-linked structure with micropores, which was formed by the chelation of calcium ions

Fig. 5 TG-DTA curve of β -TCP precursors prepared by reacting $\text{Ca}(\text{NO}_3)_2$ and PBTA in H_2O at 130°C .

Fig. 6 FE-SEM images of the powders after heating β -TCP precursors (preTCP-130) at various temperatures. (a) 400°C , (b) 600°C , (c) 800°C , (d) 1000°C

with phosphonic acid groups and carboxylic acid groups and by the formation of an acid anhydride via the dehydrated condensation of the phosphonic acid groups and carboxyl groups.

4. CONCLUSIONS

As a new synthetic liquid phase method for β -TCP, the polymerized complex method, which is to prepare the β -TCP precursors using $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and $\text{C}_7\text{H}_{11}\text{PO}_9$ (PBTA) and synthesize the powder by heating the precursors at various temperatures, was employed. In addition, the gel formation mechanism for β -TCP precursor was investigated, and the powder obtained by heating the precursor at various temperatures was characterized.

β -TCP precursors were prepared via three reactions. First, calcium ions chelated with carboxyl groups in PBTA. Then, the chelation of calcium ions with phosphonic acids and/or dehydrated

condensation between phosphonic acids and carboxyl groups proceeded, finally, the dehydrated condensation of carboxyl groups occurred in the range of 100 – 130°C .

Metastable α -TCP crystallized from preTCP-130 after heating at 600°C , and following by forming β -TCP from α -TCP at and above 800°C . The powder also consisted of a single phase because any impurities could not be observed, indicating that Ca/P molar ratio of the precursor would be 1.50.

The powder after heating at and above 800°C formed a three-dimensional network. It is possible to synthesize them by a simple process with a smaller amount of organic substance than that required for other polymerized complex methods. Therefore, they have wide applications, for example, in scaffold preparation for tissue engineering.

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