TECHNIQUES FOR PREPARING PURE β -TRICALCIUM PHOSPHATE GRANULES COMPOSED OF ROD-SHAPED PARTICLES

Masanobu Kamitakahara*, Ryosuke Fujii, Koji Ioku

(*Corresponding author: kamitaka@mail.kankyo.tohoku.ac.jp)

Graduate School of Environmental Studies, Tohoku University,
6-6-20 Aoba, Aramaki, Aoba-ku, Sendai, 980-8579 Japan.

Keywords: β -Tricalcium phosphate, Granule, Hydrothermal, Phosphate

Abstract: Spherical β -tricalcium phosphate (β -TCP) granules composed of rod-shaped particles are expected to be useful as bioresorbable bone substitutes. The β -TCP granules can be prepared by the heat treatment of Ca-deficient hydroxyapatite (HA) granules that are synthesized by hydrothermal treatment of α -TCP granules. However, when the Ca/P molar ratio of Ca-deficient HA becomes higher than 1.50, depending on the hydrothermal conditions, not only the β -TCP but also the HA phase appears following heat treatment. To resolve this problem, phosphate ions were incorporated into the Ca-deficient HA granules or monetite was added to decrease the Ca/P molar ratio to less than 1.50 in the starting composition. We revealed that these processes were effective in obtaining the pure β -TCP granules composed of rod-shaped particles and thus preventing the formation of HA.

(Received February 11, 2012; Accepted March 13, 2012)

INTRODUCTION

Calcium phosphate ceramics have been used in biomedical materials as bone substitutes¹⁻³. Among these ceramics, β -tricalcium phosphate (β -Ca₃(PO₄)₂: β -TCP) ceramics are used as bioresorbable bone substitutes^{4,5}. The difference in microstructure plays an important role in the reaction of β -TCP in the bone. We reported that β -TCP ceramics composed of rod-shaped particles demonstrate better performance than conventional sintered β -TCP ceramics composed of globular-shaped particles in applications where porous cylinders of β -TCP were implanted in the rabbit femur⁶. Specifically, the bioresorption and subsequent formation of bone occurs more readily with rod-shaped particles. Therefore, β -TCP ceramics composed of rod-shaped particles are expected to be useful as bioresorbable bone substitutes that can regulate bone regeneration.

The Ca-deficient hydroxyapatite (HA) whose molar ratio is 1.50 decomposes to β -TCP by an adequate heat treatment. β -TCP ceramics composed of rod-shaped particles can be prepared by the decomposition of Ca-deficient HA ceramics composed of rod-shaped particles which are prepared by the hydrothermal treatment of α -TCP⁷. However, the Ca/P molar ratio of Ca-deficient HA usually approaches to 1.67 and becomes greater than 1.50 because stoichiometric HA (Ca₁₀(PO₄)₆(OH)₂) whose Ca/P molar ratio is 1.67 is the stable phase, depending on the synthesis conditions. When the

Ca/P molar ratio of Ca-deficient HA becomes greater than 1.50, not only the β -TCP but also the HA phase appears after the heat treatment and it is difficult to obtain pure β -TCP. In such case, the addition of phosphate ions is an effective way to adjust the Ca/P molar ratio to 1.50 and increase the purity of β -TCP granules. In the present study, the preparation of pure β -TCP granules that are composed of rod-shaped particles was attempted by the addition of phosphate ions to Ca-deficient HA and by the addition of monetite (CaHPO₄) to starting material α -TCP.

MATERIALS AND METHODS

1) Synthesis by immersion in phosphate solution

In an effort to adjust the Ca/P molar ratio to 1.50, phosphate ions were incorporated into Ca-deficient HA granules.

Commercial powder of α -tricalcium phosphate (α -Ca₃(PO₄)₂: α -TCP) (Taihei Chemical Ind., Japan) and gelatin (Wako Pure Chemical Ind., Japan) were used as starting materials. One gram of α -TCP powder was mixed with 5.0 g of aqueous gelatin solution (10 % by mass). The slurry mixture was dropped into the stirred vegetable oil at 70 °C, and the oil was stirred for 10 min. The oil was then cooled at 4 °C with continuous stirring, as performed in a previous study⁸. The granules were collected, washed with ethanol, and then dried at 4 °C. To remove the gelatin and maintain the crystal phase of α -TCP, the granules were heated at 1200 °C for 15 min. The

SEM image of the α -TCP granules is shown in Figure 1.

The granules were exposed to water vapor at 160 °C under saturated vapor pressure for 20 h (Figure 2). After the hydrothermal treatment, the granules were immersed in a 0.1 mol·dm⁻³ (NH₄)₂HPO₄ solution and degassed by vacuum aspiration. The granules were then kept in the (NH₄)₂HPO₄ solution at room temperature for 2 h. Granules without (NH₄)₂HPO₄ immersion were also prepared. The samples with and without (NH₄)₂HPO₄ immersion were named TCP+P and TCP-NP, respectively. After the granules were removed from the (NH₄)₂HPO₄ solution, they were heated at 900 °C for 3 h in air.

2) Synthesis by addition of monetite

Another method for controlling the Ca/P molar ratio was explored. In this method, the Ca/P molar ratio of the starting composition is decreased to less than 1.50 to ultimately obtain Ca-deficient granules composed of rod-shaped particles that possess a Ca/P molar ratio of 1.50. In order to decrease the Ca/P molar ratio in the starting composition less than 1.50, monetite (CaHPO₄) whose molar ratio is 1.0 was added to α -TCP in the starting composition. We consider that monetite is more suitable than brushite (CaHPO₄·2H₂O) because brushite has crystal water and the loss of this crystal water might induce the large structural change during the heat treatment.

An α -TCP and monetite (Taihei Chemical Ind., Japan) powders were mixed at the molar ratio of 7:3, 8:2 or 9:1. The samples were named TM73, TM82 and TM91, respectively. One gram of the mixed powder was mixed with 5.0 g of aqueous gelatin solution (10 % by mass). The slurry mixture was dropped into the stirred vegetable oil at 70 °C and the oil was stirred for 10 min. The oil was then cooled down at 4 °C with continuous stirring. The granules were collected, washed with ethanol, and dried at 4 °C. The granules were heated at 1200 °C for 15 min, exposed to water vapor at 160 °C under saturated vapor pressure for 20 h, and finally heated at 900 °C for 3 h in air.

FIGURE 1 SEM image of α -TCP granules.

FIGURE 2 Schematic of hydrothermal treatment.

3) Characterization of materials

The crystalline phases were identified by powder X-ray diffraction (XRD; RINT-2200VL; Rigaku, Japan) with CuK α radiation. The microstructure of specimens was observed by scanning electron microscopy (SEM, S-4100; Hitachi, Japan).

RESULTS AND DISCUSSION

1) Immersion in phosphate solution

Figure 3 shows the XRD patterns of granules with and without immersion in the phosphate solution after heat treatment at 900 °C for 3 h. Although the main phase of the TCP-NP granules, which were prepared without immersion in the phosphate solution, was β -TCP, HA phase was also detected. The Ca/P molar ratio was 1.55, which was determined based on a previously reported method⁹. This indicates that the Ca/P molar ratio of the Ca-deficient HA formed after the hydrothermal treatment was 1.55 and pure β -TCP was not obtained after the heat treatment at 900 °C. Conversely, the TCP+P granules which were prepared with the immersion in the phosphate solution were comprised of pure β -TCP. This indicates that the Ca/P molar ratio of the granules became 1.50 by the immersion in the phosphate solution and pure β -TCP was obtained after the heat treatment at 900 °C.

The spherical shape of the granules was maintained even after the final heat treatment. Figure 4 shows an SEM image of the surface of the TCP+P granule. Rod-shaped particles were observed even after the heat treatment at 900 °C for 3 h. These results indicate that higher purity β -TCP granules composed of rod-shaped particles can be obtained by the additional process of immersion in the phosphate solution.

The Ca/P molar ratio of stoichiometric HA is 1.67, while that of TCP is 1.50. It is speculated that when α -TCP is reacted with water to form HA, Ca-deficient HA is formed first, and then the composition shifts towards that of stoichiometric

HA¹⁰. During the transition from Ca-deficient HA to stoichiometric HA, phosphate ions are released and the Ca/P molar ratio increases from 1.50 to 1.67. However, to obtain pure β -TCP, the Ca/P molar ratio of the Ca-deficient HA must be 1.50 or phosphate ions must remain in the specimen. Depending on the hydrothermal conditions, the Ca/P molar ratio of the Ca-deficient HA is speculated to vary from 1.50 to 1.67. When the release of phosphate ions from the specimen occurs, the Ca/P molar ratio becomes greater than 1.50. In such cases, this method which involves which can adjust the composition by providing phosphate ions to Ca-deficient HA is a suitable method to obtain pure β -TCP granules composed of rod-shaped particles.

FIGURE 3 XRD patterns of granules with and without immersion in the phosphate solution after heat treatment at 900 °C for 3 h.

FIGURE 4 SEM image of surface of TCP+P granule after heat treatment at 900 °C for 3 h.

2) Addition of monetite

Figure 5 shows the XRD patterns after heat treatment at 900 °C for 3 h of granules derived from the starting materials with monetite added. The XRD pattern of the granules that were generated in absence

of monetite is the same as the TCP-NP sample. With the addition of monetite in the starting material, the intensity of the HA diffraction line decreased. However, when the addition of monetite increased to a ratio of 8:2 or greater, the $\text{Ca}_2\text{P}_2\text{O}_7$ phase was detected in addition to β -TCP. These results indicate that the Ca/P molar ratios of the products after the hydrothermal treatment decreased with increase in the amount of the added monetite, because the Ca/P molar ratios of HA, β -TCP and $\text{Ca}_2\text{P}_2\text{O}_7$ are 1.67, 1.50 and 1.00, respectively. The Ca/P molar ratios of TM91 and TM82 were almost 1.50 after the hydrothermal treatment, and the crystal phase was relatively pure β -TCP.

The $\text{Ca}_2\text{P}_2\text{O}_7$ phase was also observed when the granules of α -TCP/monetite/gelatin were heated at 1200 °C for 15 min prior to hydrothermal treatment. Even after the hydrothermal treatment and the subsequent heat treatment, part of the $\text{Ca}_2\text{P}_2\text{O}_7$ phase remained. Although trace amounts of the $\text{Ca}_2\text{P}_2\text{O}_7$ phase were detected, the HA phase disappeared and a relatively pure β -TCP was obtained.

The spherical shape of the granules was maintained even after the final heat treatment. Figure 6 shows the SEM image of the surface of the TM82 granule after heat treatment at 900 °C for 3 h. The rod-shaped particles were observed even after the heat treatment at 900 °C for 3 h.

These results indicate that the present process in which the Ca/P molar ratio is decreased to less than 1.50 in the starting composition by monetite addition is also a suitable method to obtain pure β -TCP granules composed of rod-shaped particles.

FIGURE 5 XRD patterns after heat treatment at 900 °C for 3 h of granules derived from the starting materials with monetite added.

FIGURE 6 SEM image of surface of TM82 granules after heat treatment at 900 °C for 3 h.

Although the aspect ratios of the rod-shaped particles obtained by immersion in phosphate solution and the addition of monetite were different, the diameter of the rod-shaped particles was similar. The larger aspect ratio of the rod-shaped in TM82 might be due to the slow formation of Ca-deficient HA by the addition of monetite during the hydrothermal treatment and this structure was remained.

Both processes investigated in this study were effective in preparing pure β -TCP granules composed of rod-shaped particles. While the concepts applied here are sufficient for obtaining such granules, the amount of phosphate ions or monetite required to achieve the highest purity of β -TCP will depend on the hydrothermal conditions and the sample size or amount.

SUMMARY

Two processes to obtain pure β -TCP granules composed of rod-shaped particles were investigated. Incorporation of phosphate ions into Ca-deficient granules or addition of monetite to reduce the Ca/P molar ratio in the starting composition below 1.50 were both effective in obtaining pure β -TCP granules

composed of rod-shaped particles and preventing the formation of HA.

ACKNOWLEDGEMENTS

The authors would like to thank Prof. Tohji of Tohoku University for experimental assistance. This research was partially supported by JSPS, KAKENHI (21300175).

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