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Fabrication of low crystalline B-type carbonate apatite block from low crystalline calcite block

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Low crystalline B-type carbonate apatite (CO_3Ap) block was fabricated by compositional transformation based on dissolution–precipitation reaction using microporous low crystalline calcite block as a precursor. In other words, microporous low crystalline calcite block prepared by carbonation of $Ca(OH)_2$ compact was phosphoridated by exposing to 1 mol·dm^{-3} Na_2HPO_4 solutions at 60°C for 14 days. XRD, FT-IR and SEM analysis showed that calcite block completely transformed into B-type CO_3Ap keeping its macroscopic morphology. Diametral tensile strength of the low-crystalline B-type CO_3Ap block was approximately 5.4 MPa, and this value was much higher when compared with the low-crystalline calcite block, 0.73 MPa. Low crystalline B-type CO_3Ap block fabricated in this method could be an ideal bone replacement due to its close similarity in its inorganic chemical composition and crystallinity to bone.

Key-words : Carbonate apatite, Calcite, Bone replacement, Bone remodeling, Crystallinity

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1. Introduction

Although the "golden standard" of bone defect reconstruction is the autograft, e.g. from the iliac crest, autograft has serious shortcomings including the operation to healthy site and insufficient availability. Alternative allogenic transplantation also has shortcomings including potential infection risks.[1)–5)] Therefore, synthetic bone substitutes, especially apatites, have been studied as bone substitute due to its excellent tissue response and osteoconductivity.[6)–8)] However, as the bone reconstruction material used most often, sintered hydroxyapatite (HAp) is not fundamentally biodegradable and therefore not likely to be replaced by new bone. Therefore, many kinds of artificial bone substitutes have been studied. Most of these, however, are different from the biological apatites, which are the main components of bone.[9)] Biological apatites are not stoichiometric HAp ($Ca_{10}(PO_4)_6(OH)_2$) but contain 4–8% carbonate ions, CO_3^{2-}, and other trace elements such as Mg^{2+}, Fe^{2+}, Na^+, HPO_4^{2-}, F^-, Cl^-. Consequently, a more appropriate structural formula for the composition of bone is $(Ca,X)_{10}(PO_4,CO_3,Y)_6(OH,Z)_2$ with X substituting cations and Y and Z substituting anions (with the indices 10, 6 and 2 changing according to stoichiometry),[10)–13)] and are therefore referred to as carbonate apatites (CO_3Ap).[14)] Doi et al. investigated the sintering of CO_3Ap powder and found that a loss of carbon dioxide was indispensable even though fabrication of weakly sintered CO_3Ap was possible.[15)] The loss of carbonate from CO_3Ap occurs in two main stages, the greater loss at 600 and 800°C, and a smaller loss between 800 and 950°C, accompanied by changes in the lattice parameters.[15)] Since carbonate is liberated as carbon dioxide gas, thermally decomposed CO_3Ap became to show alkalinity, and thus results in a much lower rate of biodegradation compared to the nanocrystalline bone mineral, and show cytotoxity when the amount of liberated carbon dioxide is high. Since bone apatite is a low crystalline CO_3Ap, new method has been awaited to fabricate pure low crystalline CO_3Ap block without causing carbon dioxide liberation.

Fortunately, CO_3Ap is the most stable phase thermodynamically at physiologic condition. In fact, that is thought to be one of the reasons why inorganic component of bone is CO_3Ap. Therefore, there is a good chance that pure CO_3Ap block can be prepared by compositional transformation based on dissolution-precipitation reaction if suitable precursor can be chosen. On the other hand, inorganic composition of the skeleton for invertebrate animals is aragonite, a modification of calcium carbonate, whereas that of vertebrate animals is CO_3Ap. Since calcium carbonate contains both calcium and carbonate, it could be a good precursor for the fabrication of pure CO_3Ap. In fact, marine coral with aragonite composition is known to convert to CO_3Ap even though the compositional change occurs only on its surface. Although, it is generally categorized as bioresorbable materials, some reported that coralline apatite was basically not bioresobable.[16)–19)] In the present study, therefore, feasibility for the fabrication of pure CO_3Ap block by compositional transformation from calcite based on dissolution–precipitation reaction was evaluated using low-crystalline microporous calcite as a precursor. Also, its bioresorbability was evaluated using osteoclastic cells.

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2. Materials and methods

2.1 CO_3Ap block fabrication

Low crystalline B-type CO_3Ap block was fabricated in two steps. First, microporous low crystalline $CaCO_3$, calcite, block was fabricated by exposing calcium hydroxide compact to carbon dioxide as described previously.[20),21)] In short, approximately 0.2 g of commercially obtained calcium hydroxide ($Ca(OH)_2$; Nacalai Tesque, Kyoto, Japan) was placed in a stainless steel mold and pressed uniaxially with an oil pressure press machine (Riken Power, Riken Seiki, Japan) under 2 MPa pressure. Cylindrical $Ca(OH)_2$ compacts which has 6 mm in diameter and 3 mm in height or 10 mm in diameter and 2 mm in thickness were placed in carbon dioxide reaction vessel for 72 hours at room temperature for carbonation. The reaction vessel—approximately 5 L—was saturated with water vapor, and carbon dioxide gas was supplied at a flowing rate of 0.15–0.20 L/min as entrance flux. Calcite block thus fabricated shows a microporous structure with a porosity of 42%.[21)] The block would not be washed out even when it was immersed in aqueous solution, and thus can be used as a precursor for the fabrication of CO_3Ap block.

At the second step, calcite block was phosphorylated using 1 mol·dm^{-3} Na_2HPO_4 (Wako Pure Chemical Industries, Ltd.) aqueous solutions. Five calcite block were placed in a closed vessel containing 1 mol·dm^{-3} of Na_2HPO_4 solution and heated at 60°C in a drying oven (DO-300; As One Co. Ltd., Osaka, Japan) for various periods up to 14 days. After prescribed periods, the specimens were carefully rinsed, dried at 37°C for 24 hours. Although this is not hydrothermal condition, hydrothermal vessel was used as a closed vessel simply to prevent water evaporation.

2.2 Compositional analysis

For compositional analysis, specimens were ground into fine powders and characterized by powder X-ray diffraction (XRD) analysis. XRD patterns of specimens were recorded with a vertically mounted diffractometer system (RIGAKU RINT 2500V, Tokyo, Japan) using counter-monochromatized Cu Kα radiation generated at 40 kV, 100 mA. The specimens were scanned from 10 to 60° 2θ in a continuous mode at a scanning rate of 2°/min. The contents of CO_3^{2-} in apatitic structure calculated by the method of Featherstone et al. In this method, proportional relationship between the CO_3 content in B-type carbonate apatite and absorbance by CO_3 at 1410 cm^{-1}/absorbance by PO_4 at 575 cm^{-1} was used for the quantitative analysis of CO_3.[22)]

Fourier transformed infrared spectroscopy (FT-IR) analysis was performed with a FT-IR spectrometer (Spectrum 2000LX; Perkin-Elmer Co. Ltd., Massachusetts, USA) and recorded in the wave number range of 370–7800 cm^{-1}.

Morphology changes of the surfaces and fractured surfaces of the specimens was characterized by means of a scanning electron microscope (SEM; JSM 5400LV, JEOL, Tokyo, Japan) at an acceleration voltage of 15 kV after gold coating.

2.3 Mechanical strength evaluation

Mechanical strength of specimens was evaluated in terms of diametral tensile strength (DTS). For the DTS measurement, specimen with 6 mm in diameter and 3 mm in height was employed. After the accurate diameter and height of each specimen were measured with a micrometer (156-101; Mitutoyo Co. Ltd., Kanagawa, Japan), specimens were crushed using a universal testing machine (IS5000; Shimadzu Co., Kyoto, Japan) at a cross-head speed of 1 mm/min. Each DTS value was the average of at least 5 specimens.

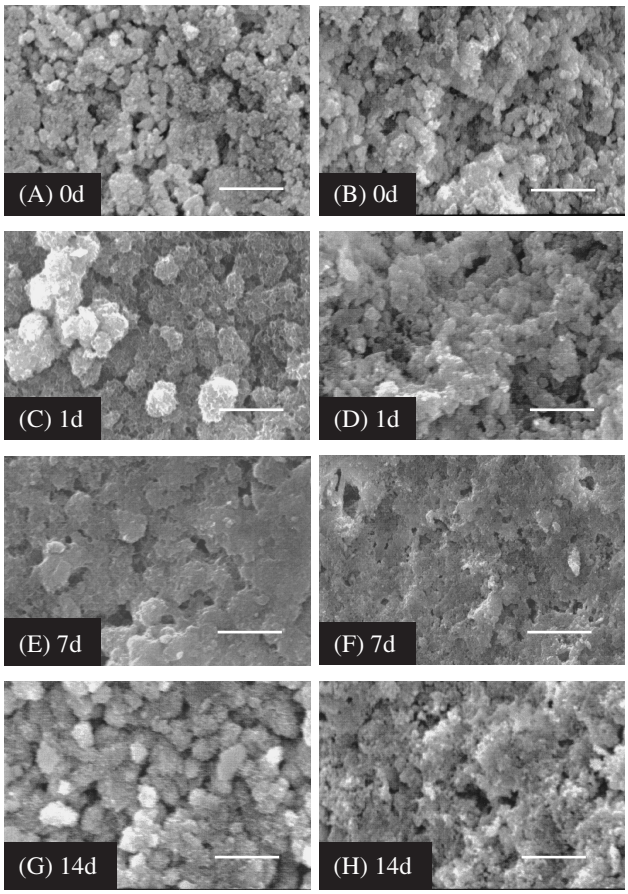

Fig. 1. Scanning electron microscopic observation of the surfaces and fractured surfaces before and after treatment in 1 mol·dm^{-3} Na_2HPO_4 solution at 60°C for 1, 7 and 14 days. (A and B) Before treatment. (C and D) Treated for 1 d. (E and F) Treated for 7 d. (G and H) Treated for 14 d. (A, C, E, G) Surfaces. (B, D, F, H) Fractured surfaces. Bar: 5 µm.

3. Results

Although no macroscopic morphological change was observed before and after the carbonation nor phosphorylation steps, SEM observation revealed that microstructure of the calcite block after treated with 1 mol·dm^{-3} Na_2HPO_4 solution was different from that of before treatment. As shown in **Fig. 1**, small needle like crystal typical for apatite crystals were seen even after 1 day both on the surface and inside the specimen. Larger amount of smaller granular apatite was observed after 7 and 14 days when compared to after 1 day.

Figure 2 shows the powder XRD patterns of calcite block before and after treatment with 1 mol·dm^{-3} Na_2HPO_4 aqueous solutions at 60°C for 1, 2, 4, 7 and 14 days. As shown in the figure, peaks attributed to calcite decreased gradually with time and peaks attributed to apatitic structure appeared after treated for 1 day and their intensities increased with time. At day 4, calcite block transformed to CO_3Ap almost completely. No further reaction was observed even when the reaction time was extended to 14 days.

Figure 3 shows FT-IR spectra of calcite block after treatment in 1 mol·dm^{-3} Na_2HPO_4 aqueous solutions at 60°C for 14 days. Typical peaks of B type CO_3Ap were observed at about 1410, 1455 and 875 cm^{-1}, along with peaks corresponding to phosphate bands at 980–1100 and 560–600 cm^{-1}.[23)] These result indicated that the apatite block formed in the present study was

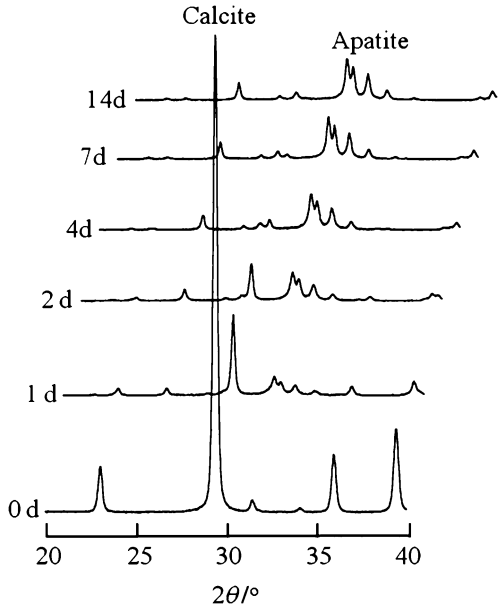

Fig. 2. Changes in XRD patterns after treatment in 1 mol·dm^{-3} Na_2HPO_4 solutions at 60°C for various periods up to 14 days.

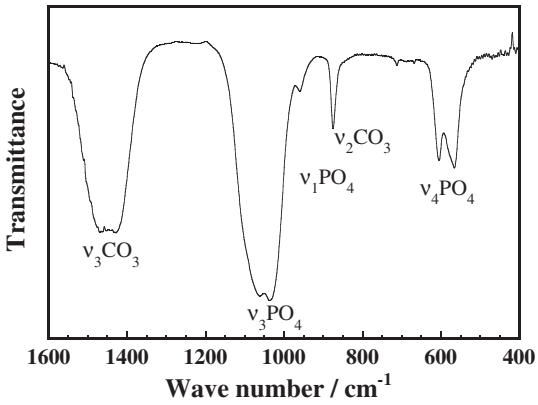

Fig. 3. FT-IR spectra of the apatite phase after treatment in 1 mol·dm^{-3} Na_2HPO_4 at 60°C for 14 days.

B-type CO_3Ap in which PO_4^{3-} lattice site was substituted by CO_3^{2-}, similar to bone mineral apatite. Also, the contents of CO_3^{2-} in apatitic structure calculated by the method of Featherstone et al., was 8.1 ± 0.5 mass%.[22]

Substitution of CO_3 with PO_4 was also confirmed by lattice parameter measurement. Lattice constant of a and c were 0.9376 nm and 0.6916 nm which is 0.9422 nm and 0.6880 nm. Decreased a-axis and increased c-axis are consistent with the result reported for CO_3Ap powder.[24),25)]

Figure 4 summarizes the DTS values of calcite block as a function of immersion time when immersed in 1 mol·dm^{-3} Na_2HPO_4 solutions at 60°C for up to 14 days. As shown, DTS value increased with immersion time and then tapered off at round 8–14 days.

4. Discussion

Results obtained in the present study clearly demonstrated that low crystalline B-type CO_3Ap block can be prepared by exposing low crystalline microporous calcite block to 1 mol·dm^{-3} Na_2HPO_4 aqueous solution. The compositional transformation is thought to occur as a result of dissolution–precipitation reaction. Calcite used as a precursor for the

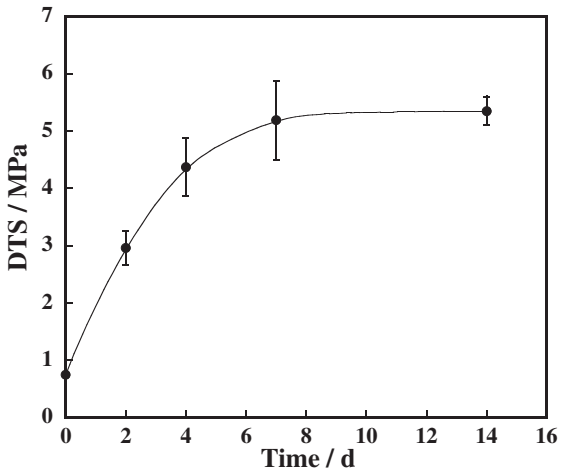

Fig. 4. DTS value of the calcite block when immersed in 1 mol·dm^{-3} Na_2HPO_4 at 60°C as a function of immersion time up to 14 days.

fabrication of CO_3Ap is the unstable phase thermodynamically in Na_2HPO_4 aqueous solution. In other words, Na_2HPO_4 aqueous solution is undersaturated with respect to calcite. Therefore, calcite is partially dissolved to supply Ca^{2+} and CO_3^{2-} into Na_2HPO_4 aqueous solution (Eq. (1)). However, the solution would be supersaturated with respect to CO_3Ap which is the most stable phase thermodynamically at the condition. Therefore, Ca^{2+} and CO_3^{2-} dissolved from calcite would be precipitated as CO_3Ap by taking PO_4^{3-} from the Na_2HPO_4 solution (Eq. (2)).

$$CaCO_3 \rightarrow Ca^{2+} + CO_3^{2-} \quad (1)$$

$$Ca^{2+} + CO_3^{2-} + PO_4^{3-} + OH^- \rightarrow Ca_{10-a}(PO_4)_b(CO_3)_{6-b}(OH)_{2-c} \quad (2)$$

This compositional transformation reaction based on dissolution–precipitation reaction is similar to the setting reaction of gypsum or apatite cements.[26)–28)] When gypsum is mixed with water, calcium sulfate semihydrate which has 0.82 g/100 cm^3 as solubility dissolves to supply Ca^{2+} and SO_4^{2-} since water is undersaturated with respect to calcium sulfate semihydrate (Eq. (3)). However, the solution would be supersaturated with respect to calcium sulfate dihydrate which has 0.20 g/100 cm^3 as solubility before the solution became to be equilibrium with respect to calcium sulfate semihydrate. Therefore, Ca^{2+} and SO_4^{2-} dissolved from calcium sulfate semihydrate would be precipitated as calcium sulfate dihydrate (Eq. (4)).

$$CaSO_4 \cdot 0.5H_2O \rightarrow Ca^{2+} + SO_4^{2-} + 0.5H_2O \quad (3)$$

$$Ca^{2+} + SO_4^{2-} + 2H_2O \rightarrow CaSO_4 \cdot 2H_2O \quad (4)$$

The dissolution–precipitation reaction occured at the surface of calcite, and thus, their compositional change occurred without changing its macroscopic morphology. Of course, microscopic morphology of the calcite block changed based on dissolution-precipitation reaction. As shown in Fig. 1, small needle like crystal typical for apatite crystals were seen even after 1 day immersion of calcite block into 1 mol·dm^{-3} Na_2HPO_4 aqueous solution.

For the complete compositional reaction, one of the requirements may be the use of microporous precursor. It is apparent that dissolution-precipitation reaction would not occur if the precursor would not be exposed to liquid phase. In this study, low crystalline microporous calcite was made by exposing calcium hydroxide compact to carbone dioxide. Therefore, NaH_2PO_4 aqueous solution can penetrate in to the interior of the calcite

block even at 60°C and dissolution-precipitation reaction took place. When marine coral, whose dominant inorganic component is aragonite; the other modification of $CaCO_3$, was exposed to $(NH_4)_2HPO_4$, compositional transformation was observed only on the surface of marine coral. Although no comparative analysis was made in this study, lack of the microstructure and/or the remaining organic component of the marine coral may prevent complete transformation to CO_3Ap.

Increase in the mechanical strength due to the transformation from calcite to CO_3Ap is also an important advantage of the present fabrication method. Although no detailed mechanisms of the increased mechanical strength was studied in the present investigation, stronger interlocking of the CO_3Ap crystals than calcite crystals may due, at least in part, contribute to higher mechanical strength of CO_3Ap block. It should be noted that mechanical strength of CO_3Ap block prepared in the present study is enough for use in non-load bearing area.

Increase in mechanical strength of CO_3Ap may be possible using calcite block with higher mechanical strength. It has been reported that mechanical strength of calcite block depend on the pressure employed for the $Ca(OH)_2$ compact. Higher compacting pressure to $Ca(OH)_2$ and its exposure to CO_2 gas result in calcite block with higher mechanical strength. Unfortunately, such calcite block shows lower porosity. Higher reaction temperature and/or longer reaction time may be required for the compositional transformation from calcite to CO_3Ap.

It should be noted that bone apatite is the low crystalline B-type CO_3Ap containing 6–8% carbonate. And the CO_3Ap prepared in the present method is also B-type carbonate apatite containing 8.1 ± 0.5 mass% carbonate. Although no cell or histological evaluation of the CO_3Ap, similar chemical composition and similar crystallinity indicate the strong possibility of the present CO_3Ap to be a bone substitute that will be replaced to bone based on bone remodeling process. Further investigation is awaited based on the results obtained in this study.

5. Conclusion

Low crystalline B-type CO_3Ap block was fabricated by the compositional transformation based on dissolution-precipitation reaction from microporous calcite block. CO_3Ap block showed enough mechanical strength for use in non-load bearing area, and resorbed by osteoclastic cells. Osteoclastic resorption, similarity to bone apatite and good mechanical strength indicated good possibility of CO_3Ap for in clinical use.

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