Fabrication of carbonate apatite blocks from set gypsum based on dissolution-precipitation reaction in phosphate-carbonate mixed solution

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Carbonate apatite (CO₃Ap), fabricated by dissolution-precipitation reaction based on an appropriate precursor, is expected to be replaced by bone according to bone remodeling cycle. One of the precursor candidates is gypsum because it shows self-setting ability, which then enables it to be shaped and molded. The aim of this study, therefore, was to fabricate CO₃Ap blocks from set gypsum. Set gypsum was immersed in a mixed solution of 0.4 mol/L disodium hydrogen phosphate (Na₂HPO₄) and 0.4 mol/L sodium hydrogen carbonate (NaHCO₃) at 80–200°C for 6–48 h. Powder X-ray diffraction patterns and Fourier transform infrared spectra showed that CO₃Ap block was fabricated by dissolution-precipitation reaction in Na₂HPO₄-NaHCO₃ solution using set gypsum in 48 h when the temperature was 100°C or higher. Conversion rate to CO₃Ap increased with treatment temperature. CO₃Ap block containing a larger amount of carbonate was obtained when treated at lower temperature.

Keywords: Carbonate apatite, Gypsum, Dissolution-precipitation, Phosphatizing

INTRODUCTION

The inorganic component of bone is not hydroxyapatite (HAp: Ca₁₀(PO₄)₆(OH)₂), but carbonate apatite (CO₃Ap: Ca₁₀₋₄(PO₄)₆₋₄(CO₃)₄(OH)₂₋₄) which contains 4–8% of carbonate in its apatitic crystal structure1,2). During the bone remodeling process, osteoclasts produce a weak acidic environment in Howship's lacuna at pH 3–5 to dissolve bone minerals. Solubility of apatite under weak acidic conditions increases with carbonate content in its apatitic structure3). Therefore, CO₃Ap is resorbed by osteoclasts but HAp is not. On this premise, we have been developing CO₃Ap as an effective inorganic scaffold for bone regenerative therapy.

In our previous studies, we have developed different types of methods to fabricate CO₃Ap scaffolds from various precursors to apply in different types of clinical situations. Unfortunately, sintering is not suitable for the fabrication of CO₃Ap blocks due to low thermal stability of CO₃Ap at high temperatures. At 400°C, CO₃Ap begins to decompose and pronounced thermal decomposition occurs at 600–700°C4,5). An alternative method was proposed for the fabrication of apatite blocks based on dissolution-precipitation reaction using gypsum as a precursor. Treatment of set gypsum with diammonium hydrogen phosphate solution resulted in hydroxyapatite6). However, CO₃Ap blocks cannot be prepared using this method since this system is free of carbonate ions. To fabricate CO₃Ap blocks using gypsum, a carbonate source must be added to the system. For example, set gypsum containing calcite was used as a precursor and then treated in phosphate salt solution7). Set gypsum containing calcite was simply prepared by adding calcite to gypsum slurry. Subsequently, unreacted calcite was found after phosphatizing treatment due to increased calcite content. Besides, mechanical strength of the precursor block remarkably decreased with increase of calcite content — since calcite did not set. Decreased mechanical strength resulted in poor handling property and chipping of the precursor during the phosphatizing treatment. Therefore, this method was not suitable for clinical application. Calcite blocks with relatively high mechanical strength and lower crystallinity were prepared via carbonation of Ca(OH)₂ powder compacts in a stream of CO₂ gas8,9). Therefore, Ca(OH)₂ was added to gypsum and the latter was exposed to CO₂ gas, which then resulted in the formation of low-crystalline calcite10). However, a considerable amount of unreacted calcite was still found in CO₃Ap blocks even...
after phosphatizing treatment for 14 days.

In the present study, the objectives were to reduce untreated calcite present in the final product of CO$_3$Ap, improve its chemical and mechanical properties, and maintain its macroscopic structure. A mixture of 0.4 mol/L disodium hydrogen phosphate (Na$_2$HPO$_4$) and 0.4 mol/L disodium hydrogen carbonate (NaHCO$_3$) were used as supply sources of CO$_3^{2−}$ and PO$_4^{3−}$ ions to fabricate CO$_3$Ap blocks. It was hypothesized that the final product of the present method would mimic bone in terms of chemical inorganic composition, and which would have adequate chemical and mechanical properties and a favorable macroscopic structure.

MATERIALS AND METHODS

Preparation of specimens and aqueous solution

Commercially available calcium sulfate hemihydrate (CaSO$_4$ • 1/2H$_2$O, Wako Pure Chemical Industries Ltd., Osaka, Japan) was mixed with distilled water at a water-to-powder ratio of 0.5. Obtained paste was packed into a split stainless steel mold (6 mm diameter and 3 mm thickness). Both sides of the mold were covered with glass plates and kept at room temperature for 24 h to obtain set gypsum. A mixture of 0.4 mol/L disodium hydrogen phosphate (Na$_2$HPO$_4$, Wako) and 0.4 mol/L disodium hydrogen carbonate (NaHCO$_3$, Wako) was prepared for the phosphatizing and carbonation treatments of the set gypsum.

Compositional transformation reaction

Two specimens were immersed in 20 mL of the mixed solution in a hydrothermal vessel (Shikoku Rika Co. Ltd., Kochi, Japan), which consisted of a Teflon® inner vessel and a stainless steel jacket. The vessel was kept at different temperatures (80, 100, 120, 160, or 200°C) for varied periods (6, 12, 24, 48, or 72 h) in a drying oven (DO-450FA, As One Corp., Osaka, Japan). After the treatment, the specimens were washed with distilled water and dried at 60°C for 24 h.

X-ray diffraction analysis and Fourier transform infrared spectroscopy

Specimens were ground to fine powder and characterized by X-ray diffraction (XRD) analysis and Fourier transform infrared spectroscopy (FT-IR) analysis. XRD patterns were recorded using a powder X-ray diffractometer (D8 Advance A25, Bruker AXS GmbH, Germany) with CuKα radiation operated at 40-kV tube voltage and 40-mA tube current. FT-IR spectra were recorded on an FT-IR spectrometer (FT/IR-6200, JASCO Corp., Tokyo, Japan) using the KBr method.

Chemical analysis

Carbonate content in the specimens was analyzed using a carbon, hydrogen, and nitrogen (CHN) coder (Yanako CHN coder, MT-6, Tokyo, Japan); it was calculated from the amount of carbon contained in the specimens. Sulfate content in the specimens was analyzed using an inductively coupled plasma-optical emission spectrometer (ICP-OES; Optima 7300 DV, Perkin Elmer, Inc., Boston, USA); it was calculated from the amount of sulfur in the specimens.

Mechanical strength evaluation and porosity measurement

Mechanical strength of the specimens was evaluated in the form of diametral tensile strength (DTS). After drying, the diameter and height of each specimen were measured using a micrometer (MDC-25MU, Mitutoyo Co. Ltd., Kanagawa, Japan). A load was applied to crush each specimen in a universal testing machine (AGS-J, Shimadzu Corp., Kyoto, Japan) at a crosshead speed of 1 mm/min. Each DTS value obtained was the mean value of at least 10 specimens.

Apparent porosity was calculated using Archimedes’ principle. For each specimen, its dry weight (w1), weight-in-kerosene (w2), and weight-containing-kerosene (w3) were measured and the following formula was used to compute its apparent porosity:

\[
\text{Apparent porosity} (%) = \left( \frac{w3–w1}{w3–w2} \right) \times 100
\]

Scanning electron microscope analysis

Morphological evaluation of the fractured surfaces of specimens was performed using a scanning electron microscope (SEM; S-3400N, Hitachi High-Technologies Co., Tokyo, Japan) at an accelerating voltage of 10 kV after gold-palladium coating.

Statistical analysis

One-way analysis of variance (ANOVA) and Fisher’s PLSD method as a post hoc test were performed using KaleidaGraph 4.0J (Hulinks Co., Tokyo, Japan).

RESULTS

Specimen shape remained the same before and after treatment, regardless of reaction temperature (80–200°C) and reaction duration time (6–48 h). These data are not shown here.

Figures 1 and 2 show the powder XRD patterns of set gypsum before and after treatment at 80–200°C for 6 and 48 h respectively. Powder XRD pattern of standard HAp (HAP-200, Taihei Chemical Industrial Co., Ltd., Osaka, Japan) was also shown for comparison. When treated at 80°C, no compositional change was found at 6 h (Fig. 1(b)). After 48 h, peaks corresponding to apatite were found but with very low peak intensities (Fig. 1(b)). When treated at 100°C or higher, peaks assigned to apatite were detected even at 6 h. When treated at 200°C for 6 h (Fig.1(f)) or at 100°C or higher for 48 h (Figs. 2(c)–(f)), only apatitic peaks were found. Diffraction peaks assigned to CaSO$_4$ • 1/2H$_2$O were detected in specimens treated at 100 and 120°C (Figs. 1(c) and (d)), and diffraction peaks assigned to calcium sulfate anhydrite (CaSO$_4$) were detected in specimen treated at 160°C (Fig. 1(e)) for 6 h.

Figure 3 presents the conversion ratios of set gypsum to apatite as a function of treatment time at different treatment temperatures. Conversion rate to apatite increased with increase in treatment temperature.
Figure 4 shows the FT-IR spectra of set gypsum before and after the hydrothermal treatment at 100–200°C for 48 h. The FT-IR spectrum of HAp was also shown for comparison (Fig. 4(f)). Set gypsum showed absorption bands in three wave number regions, namely, 1700–1600 cm\(^{-1}\), 1200–1100 cm\(^{-1}\), and 700–600 cm\(^{-1}\), which were assigned to bending vibration of H\(_2\)O molecule, stretching vibration and bending vibration of SO\(_4^{2-}\) respectively\(^{19,22}\) (Fig. 4(a)). The FT-IR spectra of set gypsum after treatment were basically the same as that of HAp, except for the presence of additional peaks due to CO\(_3^{2-}\) at around 1550–1350 cm\(^{-1}\) and at 870 cm\(^{-1}\). In addition, OH peak observed at around 630 cm\(^{-1}\) decreased with decrease in treatment temperature.

Figure 5 shows the carbonate content of set gypsum.
after hydrothermal treatment at 100–200°C for 48 h. Carbonate content was calculated from the amount of carbon in the specimens as determined by CHN analysis. Carbonate content decreased with increase in treatment temperature. It was found to be 7.18±0.25, 6.12±0.06, 4.48±0.06, 1.93±0.05 wt% for specimens treated at 100, 120, 160, 200°C respectively.

Figure 6 shows the sulfate content of set gypsum after treatment at 100–200°C. Sulfate content was 4.80±0.19, 5.81±0.23, 7.12±0.13, 0.48±0.02 wt% for specimens treated at 100, 120, 160, 200°C respectively.

Figure 7 shows the apparent porosity values of set gypsum before and after treatment at 100–200°C for 48 h. The apparent porosity of set gypsum after treatment was significantly (p<0.01) higher than that before treatment, regardless of treatment temperature. Apparent porosity of set gypsum after treatment at 200°C was significantly (p<0.01) higher than that treated at 100–160°C.

Figure 8 shows the DTS values of set gypsum before and after treatment at 100–200°C for 48 h. DTS values of set gypsum after treatment were significantly (p<0.01) lower than that of set gypsum before treatment. DTS value of set gypsum after treatment at 200°C was significantly (p<0.01) lower than that treated at 100–160°C.

Figure 9 shows the SEM images of the fractured surfaces of set gypsum before and after treatment at 100–200°C for 48 h. Tight tangles of needle-like crystals were observed in set gypsum, which is the typical morphology of calcium sulfate dihydrate. On the other hand, crystal size of set gypsum became smaller after treatment, and the extent of tangles of crystals decreased.
Fig. 9 SEM images of the fractured surfaces of set gypsum before treatment (a) and after treatment at 100°C (b), 120°C (c), 160°C (d), and 200°C (e) for 48 h.

**DISCUSSION**

Results obtained in the present study clearly demonstrated that CO₃Ap blocks could be fabricated by dissolution-precipitation reaction in a mixed solution of Na₂HPO₄ and NaHCO₃ based on set gypsum as a precursor. Conversion rate of the precursor to apatite was dependent on treatment temperature, as well as treatment time at each reaction temperature. The mechanism of compositional transformation, which maintained the macroscopic structure, might be the same as the proposed mechanism. In short, set gypsum dissolved in the solution and supplied Ca²⁺ and SO₄²⁻. However, the solution containing Ca²⁺, CO₃²⁻, and PO₄³⁻ became supersaturated with respect to CO₃Ap. Therefore, these ions precipitated into CO₃Ap, which is the most stable phase in neutral and basic conditions. One key issue for dissolution-precipitation reactions is the balance between dissolution and precipitation reactions. If the dissolution reaction is too fast compared to precipitation reaction, precursor structure would not be maintained but would be washed out. On the other hand, the dissolution-precipitation reaction would take a long time if the dissolution reaction is too slow.

The solubility of set gypsum was found to be within the suitable range for compositional transformation based on the dissolution-precipitation reaction. However, the solubility of calcite is lower when compared with that of gypsum. Therefore, some calcite remained unreacted when a mixture of gypsum and calcite was used as a precursor. In contrast, pure set gypsum with the same composition was used as a precursor in this study. Therefore, no attention was paid to the dissolution rate of the precursor. During the dissolution-precipitation process at higher temperature, set gypsum (which was CaSO₄•2H₂O) transformed to CaSO₄•1/2H₂O or CaSO₄ — to some extent (Fig. 1). It should be noted that the solubility of CaSO₄•2H₂O, CaSO₄•1/2H₂O, and CaSO₄ were almost the same at higher temperature although they differed at lower temperature.

Based on the dissolution-precipitation reaction of set gypsum in a mixed phosphate-carbonate solution, CO₃Ap was formed. Carbonate content of
fabricated CO$_3$Ap decreased with increase in treatment temperature. Barralet et al. reported that at a lower temperature between 3°C and 70°C, the maximum carbonate substitution in the precipitated apatite phase was higher$^{20}$. Carbonate stability in CO$_3$Ap phase might vary according to the temperature. In the present study, CO$_3$Ap with a smaller carbonate content was more stable than CO$_3$Ap with a higher carbonate content at higher temperature. Set gypsum treated at 100, 120, and 160°C contained approximately 4–8 wt% of carbonate, which is almost the same as that observed in bone$^{1,2}$. In contrast, the DTS of CO$_3$Ap block fabricated using the form of DTS, was approximately 1–2 MPa only. It should be noted that in bone replacement, higher porosity promotes quicker replacement but lower mechanical strength. In the case of CO$_3$Ap blocks, porosity was approximately 23%. When CO$_3$Ap blocks were prepared from gypsum, porosity was approximately 5–6%. Higher porosity could be a reason for the lower carbonate substitution in the precipitated apatite phase was higher$^{20}$. Carbonate stability in CO$_3$Ap phase might vary according to the temperature. In the present study, CO$_3$Ap with a smaller carbonate content was more stable than CO$_3$Ap with a higher carbonate content at higher temperature. Set gypsum treated at 100, 120, and 160°C contained approximately 4–8 wt% of carbonate, which is almost the same as that observed in bone$^{1,2}$.

FT-IR spectra not only revealed the incorporation of CO$_3$ in apatitic structure, but also the location of apatitic structure. A-type CO$_3$Ap, of which A-site or OH site is replaced with CO$_3$, gives peaks at 880,1450 and 1545 cm$^{-1}$, whereas B-type CO$_3$Ap, of which B-site or PO$_4$ site is replaced with CO$_3$, gives peaks at 873, 1412 and 1465 cm$^{-1}$. FT-IR spectra exhibited by the specimens after phosphatizing showed CO$_3$ absorption corresponding to B-type CO$_3$Ap, which is the type of CO$_3$Ap found in bone. Therefore, CO$_3$Ap blocks thus prepared could be used as autograft bone for bone replacement.

Although B-type CO$_3$Ap is more effective than A-type, its mechanical strength, in the form of DTS, was approximately 1–2 MPa only. In contrast, the DTS of CO$_3$Ap block fabricated using calcite block as a precursor was approximately 5–6 MPa$^{9}$. Higher porosity could be a reason for the lower mechanical strength. In the case of CO$_3$Ap blocks prepared from calcite, porosity was approximately 25%. When CO$_3$Ap blocks were prepared from gypsum, porosity was approximately 60–70%. Higher porosity could be caused, at least in part, by the absence of compacting process during the fabrication of CO$_3$Ap blocks in the present study. Unfortunately, it was impossible to apply the compacting process since the advantage of using gypsum was its self-setting ability. The other reason lay in the number of components used to fabricate the CO$_3$Ap blocks. When calcite was used as a precursor, both calcium and carbonate were used to fabricate CO$_3$Ap blocks. When set gypsum was used as a precursor, only calcium was used to fabricate CO$_3$Ap blocks. Therefore, CO$_3$Ap block had a higher porosity than set gypsum even as phosphate and carbonate ions were supplied from the solution for its fabrication. It should be noted that in bone replacement, higher porosity promotes quicker replacement but lower mechanical strength is a disadvantage.

In the present study, the existence of sulfate in CO$_3$Ap block also differed from that fabricated using calcite precursor. Apart from apatitic peaks, XRD revealed no other peaks. This meant that sulfate was mostly replaced by PO$_4$. Compared with HAp, PO$_4$ absorption peaks of specimens treated at 100, 120 and 160°C were broadened toward higher wave numbers. This could be caused by the absorption band of SO$_4$. Effects of sulfate incorporation into CO$_3$Ap structure on tissue response, osteoconductivity and bone replacement have not been studied yet. Therefore, further studies on CO$_3$Ap blocks fabricated from set gypsum as a precursor are needed to demonstrate their efficacy and safety before they can be used to regenerate missing bone.

**CONCLUSION**

The present study demonstrated that CO$_3$Ap block, with its original macroscopic structure intact, could be fabricated by dissolution-precipitation reaction based on set gypsum as a precursor in a mixed solution of Na$_2$HPO$_4$ and NaHCO$_3$. Carbonate content in CO$_3$Ap can be controlled to mimic the inorganic components of living bone, which can then be applicable for bone regenerative therapy. Further investigation is necessary to demonstrate in vivo efficacy and safety.

**REFERENCES**


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