Fabrication of B-type carbonate apatite blocks by the phosphorization of free-molding gypsum-calcite composite

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B-type carbonate apatite (CO3Ap) block may be an ideal artificial bone substitute because it is closer in chemical composition to bone mineral. In the present study, the feasibility to fabricate CO3Ap blocks was investigated using compositional transformation, which was based on the dissolution-precipitation reaction of a gypsum-calcite composite with free-molding behavior. For the compositional change, or phosphorization, gypsum-calcite composites of varying CaCO3 contents were immersed in 1 mol/L (NH4)3PO4 aqueous solution at 100°C for 24 hours. No macroscopic changes were found after the treatment, whereas microscopic change was observed at SEM level. X-ray diffraction, Fourier transform infrared spectroscopy and CHN analysis indicated that the composites were B-type CO3Ap containing approximately 6–7 wt% of CO3, a value similar to that of biological bone apatite. Diametral tensile strength of the CO3Ap block was approximately 1–3 MPa. Based on the results obtained, it was therefore concluded that gypsum-calcite was a good candidate for the fabrication of CO3Ap blocks, coupled with the advantage that the composite can be molded to any shape by virtue of the setting property of gypsum.

Key words: Carbonate apatite, Gypsum, Calcite, Phosphorization

INTRODUCTION

The dominant inorganic component of bone is not stoichiometric hydroxyapatite (HAp: Ca10(PO4)6(OH)2), but carbonate apatite (CO3Ap) that contains 4–8 wt% of carbonate in apatitic structure1,2. The key clinical difference between CO3Ap and HAp lies in the presence versus absence of bone replacement. CO3Ap is resorbed by osteoclasts and replaced with new bone, whereas HAp is not resorbed by osteoclasts and thus would not be replaced by new bone3–5.

In terms of ease of fabrication, the preparation of CO3Ap powder is not difficult. However, the major drawback is that apatite powder invokes an inflammatory response known as crystalline inflammatory response when the powder is exposed to soft tissue. On this ground, CO3Ap has to be used as a block or in the granular form. However, CO3Ap is unstable at high temperature required for the sintering process due to the presence of carbonate. With rise in temperature, CO3Ap liberates carbon dioxide and decomposes to other compounds. On the other hand, CO3Ap is the most stable phase thermodynamically at physiological condition. Therefore, fabrication of CO3Ap should be possible if a suitable precursor is selected.

Previously, we have proposed a novel method to fabricate CO3Ap blocks based on a dissolution-precipitation process using calcite as a precursor6,7. In this method, two-step reaction was employed. First, calcite block was fabricated by exposing calcium hydroxide compact to carbon dioxide. The calcite block thus fabricated would not be washed out even when the block was immersed in a liquid medium. At the second step, the calcite block was phosphorylated based on the dissolution-precipitation reaction by immersing the calcite block in a phosphate salt solution. The apatite formed by this method was low-crystalline, porous B-type CO3Ap. In vitro and in vivo studies have demonstrated that CO3Ap exhibits excellent osteoconductivity as well as resorbability7. While this method seems to be a promising fabrication procedure for the ideal artificial bone replacement material, the process is rather complex and a mold has to be made in order to fabricate bone replacement materials of the desired shapes.

It should be highlighted that the precursor need not be calcite for the fabrication of CO3Ap. We have therefore proposed gypsum as a precursor for the fabrication of HAp due to these inherent benefits: allows free-molding, contains calcium, and has suitable solubility required for the dissolution-precipitation reaction. However, gypsum contains no carbonate. Therefore, HAp block was fabricated when set gypsum was exposed to aqueous phosphate salt solution8.

In the present study, the lack of carbonate content in gypsum is amply compensated by the
abundant carbonate content in calcite. Calcite has a carbonate content of 60 wt%, which is much higher than the required value of 8 wt% carbonate content for the fabrication of CO3Ap. Therefore, the proposed combination of calcite and gypsum seemed like an ideal precursor for the fabrication of CO3Ap blocks. In the present study, the effectiveness of this combination as a precursor of CO3Ap block fabrication was further evaluated based on compositional analysis and mechanical strength evaluation.

MATERIALS AND METHODS
Preparation of gypsum-calcite composite
Commercially available calcium carbonate (CaCO3; Wako Pure Chemical Industries Ltd., Osaka, Japan) and calcium sulfate hemihydrate (CaSO4•½H2O; Wako Pure Chemical Industries Ltd.) were used in this experiment without further purification. Powders of CaCO3 and CaSO4•½H2O were mixed so that the content of CaCO3 became 0-90 wt%. The powder was then mixed with distilled water at a water-to-powder ratio of 0.5 to make a paste, which was then packed into a 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 hours to obtain set gypsum-calcite composite blocks. These composite blocks contained 0, 20, 40, 60, 80, or 90 wt% of CaCO3 and were denoted as G100C0, G80C20, G60C40, G40C60, G20C80, and G10C90 respectively.

Compositional transformation reaction
The gypsum-calcite composite blocks were exposed to 1 mol/L (NH4)3PO4•3H2O (Wako Pure Chemical Industries Ltd., Osaka, Japan) for phosphorization, i.e., compositional transformation, based on dissolution-precipitation reaction. Ten pieces of gypsum-calcite composites were immersed in 20 mL of the solution in a Teflon vessel with stainless steel jacket, and it was kept at 100°C in a drying oven (DO 300, AsOne Co. Ltd., Osaka Japan). After 24 hours in the oven, the specimens were removed from the solution. After being washed with distilled water and dried, they were stored at 37°C for another 24 hours.

Characterization
Morphological changes of the specimens were observed under a scanning electron microscope (SEM; JSM-5400LV, JEOL Co. Ltd., Tokyo, Japan) at 15 kV of accelerating voltage after gold sputter coating. The specimens were characterized by powder X-ray diffraction (XRD) and Fourier transform infrared (FTIR) spectroscopy. For XRD analysis, a diffractometer (RINT 2500V, Rigaku, Tokyo, Japan) was used and the specimens were analyzed using counter-monochromatized CuKα radiation generated at 40 kV and 100 mA at a scanning rate of 2°/min. Lattice parameters were refined by the least-squares method using 25 diffraction peaks automatically searched and centered by means of a software (JADE ver. 5.0) installed in the XRD apparatus. For FTIR analysis, a FTIR spectrometer (FTIR; Spectrum 2000LX, Perkin Elmer Co. Ltd., Kanagawa, Japan) was used and spectra were acquired at room temperature using KBr method (typically 9 scans; 4 cm⁻¹ resolution).

Carbon contents in the specimens were determined using an elemental analyzer (Yanako CHN Coder MT-6), and the results were expressed as weight percentages of CO3²⁻.

Mechanical property of the specimens was evaluated in terms of diametral tensile strength (DTS). After the diameter and height were measured with a micrometer (156-101, Mitutoyo Co. Ltd., Kanagawa, Japan), the specimens were crushed using a universal testing machine (SV-301, Imada Manufacturing Co., Aichi, Japan) at a crosshead speed of 1 mm/min. Each DTS value was obtained from at least 10 samples.

RESULTS
No macroscopic changes were observed before and after the phosphorization treatment, regardless of CaCO3 (calcite) content as shown in Fig. 1. However, clear microscopic differences were found at SEM level. Figure 2 shows the SEM photographs of the surfaces and fractured surfaces of G100C0, G40C60, and G10C90 composites before and after the phosphorization treatment. Before treatment, needle-like crystals which are typical of CaSO4•2H2O in set gypsum were observed in G100C0. Square, plate-like crystals were also observed in G40C60 and G10C90 composites and they were embedded in the needle-like crystals. Similar features were also
observed in G80C20, G60C40, and G20C80 composites (data not shown), and the amount of plate-like crystals increased with increase in calcite content. After treatment, needle-like crystals were also observed in G100C0 but the crystal size was smaller than that before treatment. In G40C60 composite, assemblies of spherical particles were observed after treatment. In G10C90, particles of approximately 2 μm in size and finer deposits were found after treatment.

Figure 3 shows the XRD patterns of gypsum-calcite composites before (a) and after (b) the

Fig. 2 SEM photographs of the fractured surfaces of G100C0, G40C60, and G10C90 composites before and after phosphorization with 1 mol/L (NH₄)₃PO₄ solution at 100°C for 24 hours.
phosphorization treatment. The patterns of CaSO₄・½H₂O, CaSO₄・2H₂O, and calcite were also listed for comparison. Only diffraction peaks assigned to CaSO₄・2H₂O were detected in G100C0 before treatment. In the composites, peaks assigned to calcite were also detected. With increase in calcite content in the composites, the intensity of the peaks of CaSO₄・2H₂O decreased whereas that of calcite peaks increased. After the treatment, peaks assigned to apatite were detected in all the specimens. Peaks assigned to CaSO₄・2H₂O and CaCO₃ (calcite) completely disappeared in the specimens except for G10C90 and G20C80. In G10C90 and G20C80 composites, small and trace peaks assigned to calcite were also detected respectively. No calcite nor gypsum peaks were found in the other specimens.

Figure 4 shows the FTIR spectra of G100C0, G80C20, G60C40, G40C60, G20C80, and G10C90 composites after phosphorization treatment. The spectra of CaSO₄・2H₂O, CaCO₃ (Calcite), and HAp were also listed for comparison. For the composites containing calcite, the doublet peaks assigned to the stretching vibration of C-O bond in CO₃²⁻ at 1450 and 1410 cm⁻¹ were observed. They indicated the existence of CO₃²⁻ in B-type CO₃Ap in which some of PO₄³⁻ were substituted with CO₃²⁻ in the apatite lattice.

Table 1 summarizes the carbonate contents in the gypsum-calcite composites after the phosphorization. Although slight increase in carbonate content was observed with initial increase in calcite content, carbonate content was approximately 6–7 wt% except for G100C0 and G10C90.

Table 2 shows the lattice parameters of α- and c-axes in apatitic phases formed after the phosphorization treatment of gypsum-calcite composites. The lattice parameters of HAp were also listed for comparison. Compared with those of HAp, the lattice...
parameter of \( a \)-axis was smaller whereas that of \( c \)-axis was larger.

Figure 5 shows the DTS values of the gypsum-calcite composites before and after the phosphorization treatment. The DTS values of the composites before the treatment decreased with increase in calcite content. The DTS values of the specimens after phosphorization treatment increased with increase in calcite content, except for G\textsubscript{100C0} and G\textsubscript{10C90}.

**DISCUSSION**

The results obtained in the present study clearly demonstrated that CO\textsubscript{3}Ap blocks could be prepared from gypsum-calcite composites by exposing the composites to 1 mol/L (NH\textsubscript{4})\textsubscript{3}PO\textsubscript{4} solution. The results of XRD, FTIR, and CHN analyses indicated that the resultant apatite was B-type CO\textsubscript{3}Ap, i.e., CO\textsubscript{3}-for-PO\textsubscript{4} substitution, containing 6–7 wt% of carbonate. Substitution site was confirmed not only by FTIR, but also by lattice parameter measurement.

For B-type CO\textsubscript{3}Ap, i.e., CO\textsubscript{3}-for-PO\textsubscript{4} substitution, LeGeros has demonstrated that this mode of carbonate substitution in the apatite structure is compatible with a decrease in \( a \)-axis and an increase in \( c \)-axis\textsuperscript{13}. As for A-type CO\textsubscript{3}Ap, i.e., CO\textsubscript{3}-for-OH substitution, Elliott\textsuperscript{14} showed that this mode of carbonate substitution is compatible with an increase in \( a \)-axis and a decrease in \( c \)-axis. In this study therefore, a decreased \( a \)-axis and an increased \( c \)-axis evidently and soundly indicated the formation of B-type CO\textsubscript{3}Ap fabricated by the phosphorization of gypsum-calcite composite at 100°C.

Before the phosphorization treatment, the mechanical strength of the specimens decreased with increase in calcite content. This result was reasonable since the setting reaction was attributed to gypsum only as calcite had no setting ability. Although higher mechanical strength is not fundamentally required for the precursor, some mechanical strength is desirable with respect to the handling.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Carbonate content / wt%</th>
</tr>
</thead>
<tbody>
<tr>
<td>G100C0</td>
<td>0.17±0.03</td>
</tr>
<tr>
<td>G80C20</td>
<td>6.00±0.13</td>
</tr>
<tr>
<td>G60C40</td>
<td>6.10±0.35</td>
</tr>
<tr>
<td>G40C60</td>
<td>6.75±0.54</td>
</tr>
<tr>
<td>G20C80</td>
<td>6.80±0.37</td>
</tr>
<tr>
<td>G10C90</td>
<td>8.55±0.46</td>
</tr>
</tbody>
</table>

**Table 1** Carbonate contents of the specimens formed after the phosphorization of gypsum-calcite composites with 1 mol/L (NH\textsubscript{4})\textsubscript{3}PO\textsubscript{4} solution at 100°C for 24 hours

<table>
<thead>
<tr>
<th>Sample</th>
<th>( a )-axis / Å</th>
<th>( c )-axis / Å</th>
</tr>
</thead>
<tbody>
<tr>
<td>G100C0</td>
<td>9.4378±0.0057</td>
<td>6.8787±0.0027</td>
</tr>
<tr>
<td>G80C20</td>
<td>9.4367±0.0042</td>
<td>6.8857±0.0020</td>
</tr>
<tr>
<td>G60C40</td>
<td>9.4386±0.0025</td>
<td>6.8987±0.0012</td>
</tr>
<tr>
<td>G40C60</td>
<td>9.4341±0.0041</td>
<td>6.8881±0.0020</td>
</tr>
<tr>
<td>G20C80</td>
<td>9.4325±0.0044</td>
<td>6.8892±0.0022</td>
</tr>
<tr>
<td>G10C90</td>
<td>9.4340±0.0038</td>
<td>6.8869±0.0019</td>
</tr>
</tbody>
</table>

**Table 2** Lattice parameters of the apatitic phases formed after the phosphorization of gypsum-calcite composites with 1 mol/L (NH\textsubscript{4})\textsubscript{3}PO\textsubscript{4} solution at 100°C for 24 hours

**Fig. 5** DTS values of G100C0, G80C20, G60C40, G40C60, G20C80, and G10C90 composites before (○) and after (●) phosphorization with 1 mol/L (NH\textsubscript{4})\textsubscript{3}PO\textsubscript{4} solution at 100°C for 24 hours.
property. In view of this, the mechanical strength of G20C80 seemed to be sufficient for easy handling.

After the phosphorization treatment, the mechanical strength increased with increase in calcite content except for G100C0 and G90C10. The composition of G100C0 after phosphorization was almost HAp due to the absence of calcite, with only a limited amount of carbonate supplied from the air. Although the mechanical strength of HAp seemed to be higher when compared to CO3Ap, it will not be able to be used as a bone replacement material because it cannot be resorbed or replaced by new bone formation.

The DTS value of G10C90 was lower than that of G20C80 even when the former contained a larger amount of calcite. This result could be explained by the incomplete transformation of calcite to CO3Ap. A longer phosphorization treatment will probably allow a complete compositional transformation reaction from G10C90 to CO3Ap, such that a higher mechanical strength may be yielded. However, its benefit may be limited since the mechanical strength of the precursor stage of G10C90 is relatively low as stated above.

Although neither cell nor histomorphological studies using experimental animals were performed in this study, osteoclastic resorption and resulting bone replacement are expected to be similar to previous studies on B-type CO3Ap. At present, G20C80 may be the best precursor because of these advantages: allows free-molding, possesses sufficient mechanical strength as a precursor for easy handling, demonstrates compositional transformation to B-type CO3Ap, and resultant apatite yields good mechanical strength. In light of these encouraging findings, further studies that leverage on the results of this study should be carried out.

ACKNOWLEDGEMENTS

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REFERENCES