Fabrication of Hydroxyapatite Block from Gypsum Block Based on (NH$_4$)$_2$HPO$_4$

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Received August 9, 2005/Accepted September 9, 2005

The aim of this study was to evaluate the feasibility of fabricating low-crystalline, porous apatite block using set gypsum as a precursor based on the fact that apatite is thermodynamically more stable than gypsum. When the set gypsum was immersed in 1 mol/L diammonium hydrogen phosphate aqueous solution at 100°C, it transformed to low-crystalline porous apatite retaining its original shape. The transformation reaction caused a release of sulfate ions due to an ion exchange with phosphate ions, thus leading to a decrease in the pH of the solution. Then, due to decreased pH, diammonium phosphate anhydrous – which has similar thermodynamic stability at lower pH – was also produced as a by-product. Apatite formed in the present method was low-crystalline, porous B-type carbonate apatite that contained approximately 0.5 wt% CO$_2$, even though no carbonate sources – except carbon dioxide from air – were added to the reaction system. We concluded therefore that this is a useful bone filler fabrication method since B-type carbonate apatite is the biological apatite contained in bone.

Key words: Hydroxyapatite, Gypsum, Ammonium phosphate.

INTRODUCTION

Hydroxyapatite (HAP, Ca$_{10}$(PO$_4$)$_6$(OH)$_2$) has been used widely in medical and dental fields as a bone substitute material because of its good biocompatibility$^1$. Most of HAP products as bone substitutes are prepared by sintering chemically prepared HAP powder at high temperature. Although sintering of HAP powder provides HAP monolith with good mechanical strength, crystallinity of the products is too high to be absorbed in the body when compared with that of bone HAP$^6$.

To improve this shortcoming, we have previously proposed a new method to fabricate low-crystalline, porous carbonate apatite block based on dissolution-reprecipitation reaction or the so-called phase transformation reaction$^2$. This method consisted of two steps. First, calcium hydroxide compact was exposed to carbon dioxide to fabricate calcite block. The calcite block thus prepared would not be washed out even when the block was immersed in an aqueous solution. Next, the calcite block was immersed in a phosphate salt solution. Since carbonate apatite is thermodynamically more stable than calcite in aqueous phosphate solutions at neutral and basic regions, calcite block is transformed to a low-crystalline carbonate apatite porous block.

The method described above has two advantages when compared with a conventional sintering process. The first advantage relates to the composition of the fabricated apatite block. The sintering process is a conventional way to fabricate HAP blocks. However, sintered HAP has a much higher crystallinity than bone apatite even when HAP powder of low crystallinity is used as a starting material. Sintering of carbonate apatite also results in the liberation of CO$_2$ gas and thus fabrication of CO$_2$-free or CO$_2$-less apatite – thereby leading to poor osteoclastic resorbability of the sintered HAP block$^3$. In other words, sintered HAP will not be replaced with bone. On the other hand, low-temperature phase transformation reaction allows low-crystalline – and thus reactive – carbonate apatite blocks to be fabricated.

The second advantage relates to the size and shape of the fabricated apatite block. When HAP blocks are prepared by the sintering process, it is difficult to regulate their size and shape. In other words, a larger mold has to be made first since HAP powder compact shrinks at the sintering process. On the other hand, size and shape of apatite block fabricated through low-temperature phase transformation reaction are the same as those of calcium hydroxide compact.

Despite the above two advantages, fabrication of HAP blocks by phase transformation reaction has two drawbacks. First, a mold must be available to prepare the calcium hydroxide compact. In addition, calcium hydroxide compact needs to be transformed to calcium carbonate block. This is because when calcium hydroxide compact is exposed to aqueous solution, the compact will be washed out. To circumvent these two problems, it is proposed that calcium- or phosphate-containing block be used as a precursor for HAP block preparation – if it meets the...
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The following prerequisites:
1. It should contain calcium and/or phosphate.
2. It should not be washed out when immersed in calcium- or phosphate-containing solution.
3. It should have moderate solubility in an aqueous solution. The dissolution rate should not be too fast or too slow.
4. It should be thermodynamically less stable than HAP.
5. It should not contain elements that are not suitable for implantation.

Although several candidates may satisfy the above requirements, calcium sulfate or gypsum is in particular singled out. Gypsum (CaSO\(_4\)·2H\(_2\)O) contains calcium ion, and set gypsum would not be washed out when immersed in a phosphate salt-containing solution. Gypsum is slightly soluble in water and is thermodynamically an unstable phase in a phosphate salt-containing solution. Gypsum has been clinically used as a bone substitute material for more than a decade\(^3\)–\(^6\) and is now commercially available as Ossteose\(^{6,13–6}\). Therefore, it is undisputedly safe to be used as a precursor for HAP blocks. In addition, it should be noted that gypsum has setting ability — in other words, CaSO\(_4\)·1/2H\(_2\)O paste can set to form gypsum block. This means that gypsum can be prepared without first making a mold. Fortuitously too, it was reported that gypsum waste likewise transformed to apatite\(^7\)–\(^8\). Although the purpose of these papers was to recycle industrial gypsum waste and thus the detailed reaction mechanism was not investigated, it is clear that gypsum can be used as a source material for HAP fabrication.

In this study, we investigated the transformation process of set gypsum in diammonium hydrogen phosphate solution and the possibility of fabricating low-crystalline HAP porous block as a bone substitute material.

**MATERIALS AND METHODS**

Commercially obtained calcium sulfate hemihydrate (CaSO\(_4\)·1/2H\(_2\)O, Wako Pure Chemical Industries Ltd., Kyoto, Japan) was mixed with distilled water at a water-to-powder mixing ratio of 0.5. The paste was packed into a cylindrical stainless steel mold (6 mm in diameter × 3 mm in height). Both ends of the mold were covered with glass plates and kept at room temperature for 24 hours. For the phosphate solution, 1 mol/L diammonium hydrogen phosphate (NH\(_4\))\(_2\)HPO\(_4\) (Wako Pure Chemical Industries Ltd.) was used.

Ten specimens removed from the mold were immersed in 15 mL (NH\(_4\))\(_2\)HPO\(_4\) aqueous solution at 60°C, 80°C, and 100°C. For reaction at 100°C, the specimens were placed in a vessel for hydrothermal treatment with the phosphate solution to prevent rapid water evaporation, and the vessel was put in a drying oven (DO-300, As One Co. Ltd., Osaka, Japan) maintained at 100°C. After the specimens were immersed in the phosphate solution according to their prescribed periods, they were removed from the solution, washed with distilled water, and dried at 37°C for 24 hours. Solution pH was also measured using a pH meter with a glass electrode (TPX-90, Toko Chemical Lab Co. Ltd., Tokyo, Japan) at room temperature before and after the treatment.

Mechanical strength of the specimens was evaluated in terms of diametral tensile strength (DTS). After the diameter and height of each specimen were measured with a micrometer (156-101, Mitutoyo Co. Ltd., Kanagawa, Japan), the 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 10 specimens.

For product characterization, the specimens were ground to fine powders and analyzed by powder X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FT-IR). The XRD patterns of the specimens were recorded with a vertically mounted diffractometer system (RIGAKU RINT 2500V, Tokyo, Japan) using counter-monochromatized Cu\(\alpha\) radiation generated at 40 kV and 100 mA. FT-IR spectra were measured using a FT-IR spectrometer (SPECTRUM 2000XL, PERKIN ELMER Co. Ltd., Kanagawa, Japan).

To observe the morphological changes, a scanning electron microscope (SEM, JSM 5400LV, JEOL Co. Ltd., Tokyo, Japan) was used at an acceleration voltage of 15 kV after gold coating. Carbonate content was evaluated based on CHN analysis.

**RESULTS**

When set gypsum was treated with 1 mol/L (NH\(_4\))\(_2\)HPO\(_4\) at 60°C, 80°C, and 100°C for 24 hours, no significant morphological changes were observed at macroscopic level before and after treatment (data are not shown). In other words, the shape of the specimens was the same before and after treatment.

Fig.1 shows the XRD patterns of the set gypsum (a) before and after treatment with 1 mol/L (NH\(_4\))\(_2\)HPO\(_4\) solution at (b) 60°C, (c) 80°C, and (d) 100°C for 24 hours. Powder XRD pattern of (e) standard HAP was also shown for comparison. As shown in the figure, no compositional changes were found when set gypsum was treated with 1 mol/L (NH\(_4\))\(_2\)HPO\(_4\) aqueous solution at 60°C for 24 hours (Fig.1(b)). Trace amount of HAP was found when treated at 80°C (Fig.1(c)). Although the amount of HAP increased with time, the amount was small even after five-day treatment at 80°C (data are not shown). In contrast, HAP was the dominant
component when set gypsum was treated at 100°C for 24 hours (Fig.1(d)). Therefore, further investigation was performed at 100°C.

Although no macroscopic morphological changes were observed before and after treatment, a difference in the shape of the crystals was microscopically confirmed by SEM observation in Fig. 2. In this figure, typical SEM photographs are shown for the surface and fractured surface of set gypsum (a, b) before and after treatment (c, d) in (NH₄)₂HPO₄ at 100°C for 24 hours, respectively. In Fig. 2(a), the surface of set gypsum showed a featureless granular morphology. In Fig. 2(b), many needle-like crystals typical of set gypsum were found in the fractured surface. On the other hand, we also found many fine needle-like crystals typical of HAP in the fractured surface of set gypsum treated with (NH₄)₂HPO₄ (Fig. 2(d)).

Table 1 summarizes the pH of the solution when set gypsum (10 specimens, 1.3 g) was immersed in 15 mL of 1 mol/L (NH₄)₂HPO₄ aqueous solution at 100°C for up to two days. As shown in this table, the pH of the solution decreased with immersion time.

Fig. 3 shows the XRD patterns of set gypsum when treated with 1 mol/L (NH₄)₂HPO₄ at 100°C for 6 hours, 12 hours, 24 hours, and 48 hours. Table 2 summarizes the existing phases at each time period. When treated with (NH₄)₂HPO₄ solution for 6 hours, peak intensity of gypsum decreased drastically and CaSO₄·1/2H₂O phase was detected. Apatite phase was also confirmed at 6 hours. CaSO₄·1/2H₂O phase

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Fig. 1 Powder XRD patterns of set gypsum (CaSO₄·2H₂O): (a) before and after treatment in 1 mol/L (NH₄)₂HPO₄ solution at (b) 60°C, (c) 80°C, and (d) 100°C for 24 hours. An XRD pattern of (e) standard HAP is also shown for comparison.

Fig. 2 SEM photographs of the surface (a, c) and fractured surface (b, d) of set gypsum (a, b) before and after treatment (c, d) in (NH₄)₂HPO₄ at 100°C for 24 hours.
Table 1 pH of solution when set gypsum (10 specimens, 1.3 g) was immersed in 15 mL of 1 mol/L (NH₄)₂HPO₄ aqueous solution at 100°C for up to two days.

<table>
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<tr>
<td>1</td>
<td>7.6</td>
</tr>
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Table 2 Qualitative analysis of XRD after treatment of gypsum in (NH₄)₂HPO₄ at 100°C

<table>
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<th>Time (h)</th>
<th>Phase</th>
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<tr>
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HH: CaSO₄·1/2H₂O
DCPA: CaHPO₄
HAP: Hydroxyapatite

Fig. 3 Powder XRD patterns of set gypsum (CaSO₄·2H₂O): (a) before and after treatment in 1 mol/L (NH₄)₂HPO₄ solution at 100°C for (b) 6 hours, (c) 12 hours, (d) 24 hours, and (e) 48 hours. XRD patterns of (f) standard HAP, (g) CaSO₄·1/2H₂O, and (h) CaHPO₄ are also shown for comparison.

Fig. 4 FT-IR spectra of set gypsum (CaSO₄·2H₂O) (a) before and after treatment in 1 mol/L (NH₄)₂HPO₄ solution at 100°C for 24 hours. FT-IR spectrum of (c) standard HAP is also shown for comparison. The arrow shows the additional absorption band assigned to a stretching vibration of CO₃²⁻ of B-type carbonate apatite, in which PO₄³⁻ lattice site is substituted by CO₃.

Disappeared and CaHPO₄ (monetite, DCPA) was formed at 12 hours. Apatite and monetite phases were the main products at 24 hours, and prolonged treatment allowed more formation of monetite as shown in the pattern at 48 hours.

Fig. 4 shows the FT-IR spectrum of (b) set gypsum treated with 1 mol/L (NH₄)₂HPO₄ at 100°C for 24 hours. FT-IR spectra of (a) set gypsum and (c) standard HAP are also shown for comparison. The FT-IR spectrum of set gypsum shows absorption bands in three wave number regions, namely 1700-

1600 cm⁻¹, 1300-1000 cm⁻¹, and 700-600 cm⁻¹ — which were assigned to bending vibration of H₂O molecule, stretching vibration and bending vibration of a sulfate (SO₄²⁻) group, respectively. When set gypsum was treated with (NH₄)₂HPO₄ solution, the doublet at 1685 and 1621 cm⁻¹ (bending vibration of crystal water in gypsum) and that at 669 and 601 cm⁻¹ (bending vibration of SO₄²⁻) disappeared, while the absorption at 1300-1000 cm⁻¹ broadened towards lower wave number (Fig. 4(b)). This was caused by the evolution of the stretching vibration of PO₄³⁻ in monetite and/or apatite. In addition, a new band appeared around 600 cm⁻¹, which was assigned to bending vibration of PO₄³⁻ in an apatite lattice. Basically, the FT-IR spectrum of specimen treated with (NH₄)₂HPO₄ was the same as that of HAP shown in Fig. 4(c) except for additional absorption band at around 1400 cm⁻¹ (as indicated by the
This band was assigned to a stretching vibration of CO$_3^{2-}$ of B-type carbonate apatite, in which PO$_4^{3-}$ lattice site was substituted by CO$_3^{2-}$. Elemental analysis using CHN analyzer showed that the specimen treated with $\langle$NH$_4$$_2$HPO$_4$ solution contained 0.10±0.04 wt% carbon which corresponded to 0.48±0.18 wt% CO$_3$.

Fig. 5 shows the DTS values of set gypsum when treated with 1 mol/L $\langle$NH$_4$$_2$HPO$_4$ at 100°C as a function of reaction time. The DTS values of set gypsum treated with $\langle$NH$_4$$_2$HPO$_4$ was significantly lower ($p<0.01$) when compared with that of set gypsum.

**DISCUSSION**

The results obtained in the present study clearly demonstrated that gypsum block transformed to low-crystalline, porous B-type carbonate apatite block when hydrothermally treated in 1 mol/L diammonium hydrogen phosphate at 100°C. The basic reaction between set gypsum and $\langle$NH$_4$$_2$HPO$_4$ aqueous solution that formed HAP may be expressed as follows:

\[
10\text{CaSO}_4 \cdot 2\text{H}_2\text{O} + 6\text{NH}_4\text{HPO}_4 \rightarrow \\
\text{Ca}_{10}(\text{PO}_4)_{6}(\text{OH})_2 + 6\text{NH}_4\text{SO}_4 + 4\text{H}_2\text{SO}_4 + 18\text{H}_2\text{O}
\]

This equation indicated that pH of the solution would decrease due to sulfuric acid formation as the reaction proceeded. The formation of sulfuric acid would cause a decrease in pH after the treatment, as shown in Table 1. Actually, as shown in Table 2, DCPA was also formed together with HAP after 24 hours and the amount of DCPA seemed to increase after 48 hours.

Fig. 6 shows the solubility isotherms at 25°C for gypsum, DCPA, and HAP related to the reaction process, whereby calcium concentration is plotted against pH. Solubility curves were obtained from the solubility products of those compounds based on ion equilibrium among the various ionic species involved. No corrections were made to any activity coefficient as this was used only for qualitative purpose. Range of pH change during the reaction is indicated by the shaded area in Fig. 6. The solubility of HAP was always lower than that of gypsum between 4 and 12, indicating that HAP was thermodynamically a more stable phase in the pH region obtained in the present study. Solubility difference between HAP and gypsum was about three orders of magnitude at an initial pH of 7.8 in the solution. As already described, transformation from gypsum to HAP caused liberation of sulfuric acid and accordingly the solution pH decreased as the transformation proceeded (Table 1). The decrease in pH resulted in smaller solubility difference between HAP and DCPA. The solution pH continued to decrease during the reaction, thus explaining the formation of DCPA together with HAP. The amount of DCPA was higher after 48 hours than after 24 hours. Though it is not clear whether DCPA was formed directly from calcium sulfate or through HAP, the decrease in pH favored DCPA formation because of the similar solubility of DCPA and HAP around lower pH. As shown in Fig. 6, their thermodynamic stabilities in the aqueous solution were almost the same at pH of 4.8 after 48 hours. Since final pH was governed by several factors including $\langle$NH$_4$$_2$HPO$_4$ concentration and weight ratio of gypsum to solution, the final composition would likewise be governed by such factors.

Calcium sulfate hemihydrate was detected only once at 6 hours and then disappeared afterwards (Fig. 3 and Table 2). The hemihydrate was formed by dehydration of gypsum. It was reported that the transformation temperature from gypsum to hemihydrate was about 80-110°C in terms of

**Fig. 5** DTS values of set gypsum when treated with 1 mol/L $\langle$NH$_4$$_2$HPO$_4$ at 100°C. The bars in the figure denote standard deviation.

**Fig. 6** The solubility isotherms at 25°C for gypsum and three calcium phosphate compounds. Range of pH change during the reaction in $\langle$NH$_4$$_2$HPO$_4$ solution is indicated by the shaded area.
solubility. Since we used 100°C as the treatment temperature in the present study, the transformation of gypsum to hemihydrate occurred simultaneously with that of gypsum to HAP. Most certainly, hemihydrate also transformed to HAP, and thus no hemihydrate was detected after 6 hours—at least not within the detection limits of XRD.

Though macroscopic morphological change was not found on the set gypsum treated in the phosphate solution, many fine needle-like apatite crystals were seen on the fractured surface by SEM observation (Fig.2(d)). The crystals were quite different from the needle-like crystals of gypsum in their morphology and size. This observation indicated that apatite crystals were formed through a dissolution-precipitation process. DTS value of set gypsum decreased drastically with the treatment, and strength of set gypsum was derived from the entangling of needle-like gypsum crystals. After the treatment, it would seem that entanglement of newly formed apatite crystals was expected. However, the newly formed crystals were much smaller than the original gypsum crystals and the entanglement was not enough to develop the strength. This could be a probable cause for the decrease in DTS value with the treatment.

It should be noted that the final specimens contained approximately 0.5 wt% CO₂. The carbonate ion was incorporated into B-type carbonate apatite, substituting PO₄ lattice site of HAP as shown in FT-IR spectrum (Fig.4(b)). Since no carbonates ions were added in the present study, the source was thought to be supplied from CO₂ gas in the air. As a result, carbonate apatite was formed instead of carbonate-free apatite because carbonate apatite is a thermodynamically more stable phase than carbonate-free apatite. When it comes to fabrication of bone fillers, B-type carbonate apatite may be preferred over carbonate-free apatite, since carbonate apatite is comparatively more soluble and more easily resorbed by osteoclasts. To control the carbonate content in the apatite phase, there are several ways of adding carbonate salts to the phosphate solution or to calcium sulfate hemihydrate. These trials are now under progress in search of an optimal condition to fabricate carbonate apatite from set gypsum.

CONCLUSION

We found that set gypsum could be used as a precursor for the fabrication of apatite-type bone fillers. Low-crystalline, porous B-type carbonate apatite was formed alongside DCPO when set gypsum was immersed in 1 mol/L (NH₄)₂HPO₄ solution for 24-48 hours at 100°C. Solution pH and resulting difference in solubility or thermodynamic stability between gypsum and apatite were key factors that governed the transformation reaction. Based on the initial findings of this current study, the feasibility of fabricating low-crystalline, porous B-type carbonate apatite blocks from gypsum could be explored more thoroughly and in greater detail in future experiments.

ACKNOWLEDGEMENTS

This study was supported in part by a Grant-in-aid for Scientific Research from the Ministry of Education, Sports, Culture, Science, and Technology, Japan.

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