FABRICATION OF POROUS CARBONATE APATITE BASED ON THE DISSOLUTION-PRECIPITATION OF CALCIUM SULFATE HEMIHYDRATE

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ABSTRACT

Background: Carbonate apatite (C-Ap) has been used as a bone replacement material because it has osteoconductive properties. The pores contained in C-Ap are useful so that cells can diffuse during new bone formation. Calcium sulfate hemihydrate ($CaSO_4.1/2H_2O$) precursor has been used as it possesses Ca^{2+} ions, while polymethylmetacrylate (PMMA) is chosen because it is easily burned out at low temperatures. Objectives: To fabricate porous carbonate apatite based on a calcium sulfate hemihydrate precursor using the dissolution-precipitation method and identify its phases by X-ray diffraction. Methods: The pores were made by crushing polymethyl methacrylate (PMMA) granules and sieving them to sizes of 300–500 μm. Specimens of porous carbonate apatite were prepared from a mixed calcium sulfate hemihydrate precursor with different quantities of porogen: 0 wt% PMMA (n = 60), 25 wt% PMMA (n = 60), and 50 wt% PMMA (n = 60). Calcium sulfate hemihydrate containing PMMA was mixed with distilled water at a water: powder ratio of 0.5 ml: 1g. Calcium sulfate dihydrate specimens were placed in an oven at 700°C for 4 h to burn out the PMMA, resulting in porous calcium sulfate anhydrate. This was then immersed in phosphate mixed solution (Na_3PO_4) and carbonate solution (Na_2CO_3) for 1, 3, and 5 days to obtain carbonate apatite through a precipitation-dissolution process. An X-ray diffraction technique (X-Ray Diffractometer, PANalytical XPert PRO) was used to analyze each group of specimens to identify the phase formation of porous carbonate apatite. Results: The X-ray diffraction pattern showed peaks of carbonate apatite in all specimens with 0, 25, and 50 wt% PMMA in 1, 3, and 5 days of immersion, except in the 0 wt% specimens at 1 day. The carbonate apatite diffraction peaks were at 2θ = 25.9°, 29.2°, 32°, and 34°. The earliest complete formation of the carbonate apatite phase was identified from the preparation of porous anhydrous $CaSO_4$ with porogen of 50 wt% PMMA immersed for 1 day. Conclusion: Porous carbonate apatite can be produced from precursors of calcium sulfate hemihydrate and porogen of PMMA via a precipitation-dissolution method.

Keywords: carbonate apatite, calcium sulphate hemihydrate, dissolution, precipitation

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INTRODUCTION

Carbonate is a bioceramic material that has biocompatibility and osteoconductive properties that can be used for bone reinforcement. The inorganic component of bone is apatite carbonate $[Ca_{10}(PO_4)_2(CO_3)_4(OH)_2]$ containing 4-9% carbonate in its apatite crystal structure [1-3]. Calcium sulfate is used as a precursor as it is easily formed and hardens at room temperature[1]. In the present study, calcium sulfate hemihydrate ($CaSO_4.1/2H_2O$, Wako Pure Chemical Industries Ltd., Osaka, Japan) was used for conversion into $CaSO_4$ and subsequent tracing excavation. Ca^{2+} ions in $CaSO_4$ are easily absorbed without causing an inflammatory reaction in bone tissue.[4,5] This

resorption process allows time for bone cells to migrate to bone regions, which can accelerate bone processes without being inhibited by the process of fibrous tissue formation. [4,6]

The production of porous solid carbonate from $CaSO_4$ precursors via a dissolution method from non-porous solid apatite carbonate can be achieved following immersion in a mixture of Na_2CO_3 and Na_3PO_4 at 100^0C for 48 h.[6] Apatite carbonate is produced from the substitution of a carbonate ion (CO_3^{2-}) in the hydroxyapatite phosphate (PO_4^{2-}) group [HAp: $Ca_{10}(PO_4)_6(OH)_2]^{5,7}$. The solid apatite carbonate structure produced by Kunio et al. exhibited a diffraction pattern that corresponded to crystal

carbonate apatite with the three highest peaks at $2\theta = 32^o$, $33°$, and $34,10°$.[1,6,7]

The porous structure of apatite carbonates facilitates development compared with solid or non-porous structures. The pores in this bone replacement material function as a site for cells to migrate to each other and diffuse with other cells in order to develop and attach to the bone surface to accelerate the process of new bone formation.[5,8,9] A porogen that can be used to produce pores is polymethyl methacrylate (PMMA). This material is easy to obtain and has a low melting point of between 220˚C and 240˚C, therefore, it can be easily burned to leave pores in anhydrous $CaSO_4$.[10] The pore size present in a bone replacement material is adjusted to the average human bone cell size of 223 μm and capillary size of 100-200 μm.[5,11] The ideal pore size in a bone replacement material is in the range of 300-600 μm.[11]

The purpose of the present study was to fabricate porous apatite carbonates with precursors of calcium sulfate hemihydrate and porogen PMMA through the precipitation-dissolution processes and characterize their phases through X-ray diffraction.

MATERIAL AND METHODS
Manufacture of porous calcium sulfate anhydrous ($CaSO_4$)

The preparation of porous $CaSO_4$ anhydrous specimens was performed by adding PMMA granules measuring 300-500 μm in size by 0% of weight (n = 60; Group Sp1), 25% of weight (n = 60; Group Sp2), and 50% of weight (n = 60; Group Sp3) into $CaSO_4.1/2H_2O$ powder (Wako Pure Chemical Industries Ltd., Osaka, Japan). PMMA granules at a size of 300-500 μm were produced by grinding PMMA (Wako Pure Chemical Industries Ltd., Osaka, Japan) and then filtering to obtain granule sizes of 300-500 μm. The $CaSO_4.1/2H_2O$, which has been added to the PMMA granules (Sp1, Sp2, and Sp3) was then mixed with distilled water at a water: powder ratio of 0.5 ml: 1 g to form a calcium sulfate dihydrate-PMMA dough. The calcium sulfate dihydrate-PMMA mixture was then placed into a stainless steel split mold (diameter 6 mm; thickness 3 mm) and stored at 23 ± 4˚C for 24 h. Following hardening, the calcium sulfate dihydrate-PMMA specimens were burned in an oven at a temperature of 700^oC for 4 h to remove the PMMA and then allowed to cool in the oven to obtain a porous anhydrous $CaSO_4$ specimen.

Production of porous apatite carbonate

Production of the apatite carbonate was performed using the method of precipitation-dissolution. The porous anhydrous calcium sulfate specimens were immersed in a solution of 0.5 mol/L Na_3PO_4 and 0.5 mol/L $NaCO_3$ (Wako Pure Chemical Industries Ltd., Osaka, Japan) at a ratio of 1: 1. The specimens were soaked for 1, 3, and 5 days at 100˚C. When the immersion was complete, the specimens were washed with distilled water and left to dry for 24 h in an incubator at a temperature of 37˚C.

X-ray diffraction analysis.

Phase analysis of the porous apatite carbonate specimens was performed using an X-Ray Diffractometer (PANalytical XPert PRO, Malvern Panalytical Ltd, Malvern, UK) with CuKα radiation at 40 kV and 40 mA. The diffraction pattern was obtained at the peak range of $2 = 10°$–$36°$ with a scanning rate of 0.01°/10 s. The resulting XRD data were processed using High Score Plus and Kleidograph software.
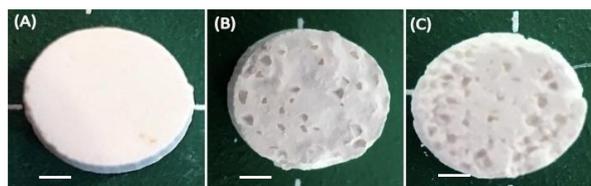
RESULTS

In the manufacture of apatite carbonate, granular porogen PMMA measuring 300-500 μm was used (Figure 1) to obtain the pore shape, as shown in Figure 2.

**Figure 1.** PMMA porogen granules with a size of 300–500 μm.

The resulting porous type B C-Ap made with different quantities of porogen PMMA (0%, 25%, and 50% by weight) can be seen in Figure 2A-C.
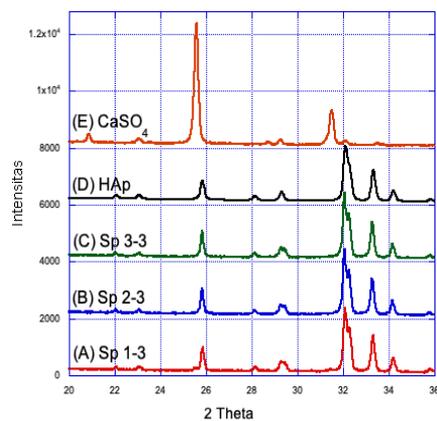

Figure 2. Apatite carbonate specimens.
(A) Apatite carbonate specimens without pores, with PMMA at 0% of weight (Sp1).
(B) Apatite carbonate specimens without pores, with PMMA at 25% of weight (Sp2).
(C) Apatite carbonate specimens without pores, with PMMA at 50% of weight (Sp3)

The results of the analysis using X-ray diffraction in the group of specimens immersed in a mixture of solutions of 0.5 mol/L Na_2CO_3 and 0.5 mol/L Na_3PO_4 at a temperature of 100˚C for 1, 3, and 5 days are shown in Figure 3, 4, and 5, respectively.
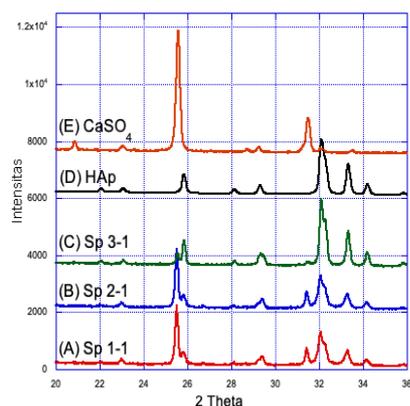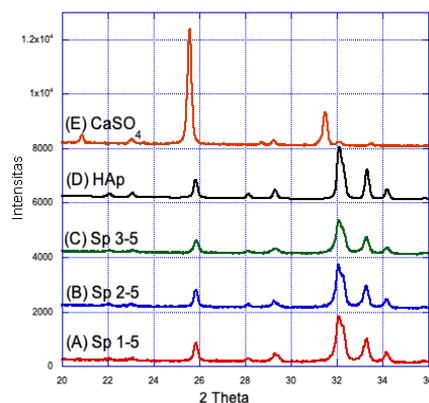
Figure 3. X-ray diffraction patterns from apatite carbonate specimens obtained after 1 day of immersion in a mixture of solutions of 0.5 mol/L NA_2CO_3 and 0.5 mol/L Na_3PO_4 at 100˚C.
(A) Apatite carbonate specimens without pores, with PMMA (0% of weight; Sp1-1).
(B) Apatite carbonate specimens without pores, with PMMA (25% of weight; Sp2-1).
(C) Apatite carbonate specimens without pores, with PMMA (50% of weight; Sp3-1).
(D) Hydroxyapatite (HAp).
(E) Anhydrous calcium sulfate ($CaSO_4$).

Figure 4. X-ray diffraction patterns from apatite carbonate specimens obtained after 3 days of immersion in a mixture of 0.5 mol/L Na_2CO_3 and 0.5 mol/L Na_3PO_4 solutions at 100˚C.
(A) Apatite carbonate specimens without pores with PMMA (0% of weight; Sp1-3).
(B) Apatite carbonate specimens without pores with PMMA (25% of weight; Sp2-3).
(C) Apatite carbonate specimens without pores with PMMA (50% of weight; Sp3-3).
(D) Hydroxyapatite (HAp).
(E) Calcium sulfate anhydrous ($CaSO_4$).

Figure 5. X-ray diffraction patterns from apatite carbonate specimens after 5 days of immersion in a mixture of solutions of 0.5 mol/L Na_2CO_3 and 0.5 mol/L Na_3PO_4 at 100˚C.
(A) Apatite carbonate specimens without pores with PMMA (0% of weight; Sp1 5).
(B) Apatite carbonate specimens without pores with PMMA (25% of weight; Sp2-5).
(C) Apatite carbonate specimens without pores with PMMA (50% of weight; Sp3).
(D) Hydroxyapatite (HAp).
(E) Calcium sulfate anhydrous ($CaSO_4$).

DISCUSSION

After combustion at 700˚C, the PMMA porogen, which has a melting point of between 220˚C and 240˚C was burned out and left no residue. Calcium sulfate dihydrate was changed to anhydrous $CaSO_4$ and, after precipitation-dissolution, porous apatite carbonate was obtained, as shown in Figure 2B and 2C. In calcium sulfate dihydrate with a porogen 0% weight PMMA (without pores), solid apatite carbonate was formed, as shown in Figure 2A, as it was produced without the addition of PMMA granules.

It appears that the porous apatite carbonate (Sp3) specimens had more pores than the Sp2 specimens. Therefore, the number of pores increased with the increasing number of PMMA granules added to the precursors of calcium sulfate hemihydrate.

The results of the analysis using X-ray diffraction in the group of specimens which were immersed in a mixture of 0.5 mol/L Na_2CO_3 and 0.5 mol/L Na_3PO_4 at a temperature of 100˚C for 1 day revealed that the Sp1-1 and Sp2-1 diffraction patterns still showed peaks of anhydrous $CaSO_4$ at the same $2\theta = 25.5°$ and $31.4°$ (Figures 3A and 3B). The characteristic diffraction pattern of anhydrous $CaSO_4$ crystals is shown in Figure 3E and Table 1. Therefore, after 1 day of immersion, pore-free apatite carbonate (0% weight PMMA) and porous apatite carbonate with 25% weight porogen PMMA still contain the composition of calcium sulfate anhydrous, and diffraction peaks of hydroxyapatite were apparent at $2\theta = 25.5°$, $29.2°$,

32°, 33°, and 34° even though the intensity was low. This phase is in accordance with ICDD 96-500-0041, that the diffraction peak at $2\theta = 25.5°$ and $31.4°$ is a diffraction pattern of anhydrous $CaSO_4$.

In the Sp3-1 diffraction pattern (PMMA 50% weight, 1 day immersion) there are visible peaks of $2\theta = 25.8°, 29.2°, 32°, 33°,$ and $34.1°$ with the highest intensity at $2\theta = 32°$, which corresponds to ICDD 96-901-1095 for Hydroxyapatite, Sp3-1 specimens do not have peak $2\theta = 31.4°$ from the peak of anhydrous $CaSO_4$ crystals, as found in Sp1-1 and Sp2-1. The Sp3-1 specimens also exhibited diffraction patterns like those of apatite carbonate which is at 2 theta = 25.9°, 29.2°, 32°, and 34°, as with ICDD Carbonate apatite: 12-0529 .[12,13] From the above conditions, it is clear that the higher the proportion of pores (50% weight porogen PMMA), the change in porous anhydrous $CaSO_4$ into hydroxyapatite crystals or apatite carbonate is reached after 1 day of immersion.

In the groups immersed for 3 and 5 days, the diffraction patterns possessed by Sp1, Sp2, and Sp3 indicated the formation of crystalline carbonate apatite with peaks of $2\theta = 25.8°, 29.2°, 32°, 33°,$ and $34.1°$ (Table 1), with the highest intensity at $2\theta = 32°$ (Figure 4 and Figure 5A-C). The phase formed is in accordance with the apatite carbonate phase.

Peak 2θ									Peak 2θ	
1 day immersion			3 days immersion			5 days immersion				
Sp1-1	Sp2-1	Sp3-1	Sp1-3	Sp2-3	Sp3-3	Sp1-5	Sp2-5	Sp3-5	Anhydrous CaSO₄	HAp
25.5°	25.5°	25.8°	25.8°	25.8°	25.8°	25.8°	25.8°	25.8°	25.5°	25.9°
29.2°	29.2°	29.2°	29.2°	29.2°	29.2°	29.2⁰	29.2°	29.2°		29.2°
31.4°	31.4°								31.4°	
32°	32°	32°	32°	32°	32°	32°	32°	32°		32.5°
33°	33°	33°	33°	33°	33°	33°	33°	33°		33°
34°	34°	34.1°	34.1°	34.1°	34.1°	34.1°	34.1°	34.1°		34.2°

Note: Ref Code ICDD CaSO₄ anhydrate: 96-500-0041, Ref Code ICDD HAp: 96-901-1095

Table 1. Diffraction peak of 2θ from Sp1, Sp2, Sp3 after precipitation and 1, 3 and 5 of days dissolution.

The visible peaks at $2\theta = 31.4°$ in Sp1-1 and Sp 2-1 were a result of the need for time to replace SO_4^{2-} ions into PO_4^{3-} ions when the process of dissolution of precipitation occurs. Thermodynamically, SO_4^{2-} dissolves completely with the duration of immersion and is followed by the presence of PO_4^{3-} ion deposits .[1,5,6] The production of apatite carbonate cannot be achieved using the sintering method as temperatures >400°C lead apatite carbonate to decompose to CO_2. Another method used for producing apatite carbonate is the precipitation-dissolution method using a low temperature of <400°C.[6,14] The present study used a precipitation-dissolution method with an immersion temperature of 100°C.

Anhydrous $CaSO_4$, when is immersed in a mixture of Na_2CO_3 and Na_3PO_4 solution, will decompose into Ca^{2+} and SO_4^{2-} ions, as the solution contains PO_4^{3-} and CO_3^{2-} ions and these deposits of PO_4^{3}, CO_3^{2-}, and Ca^{2+} ions form apatite carbonate $[Ca_{10}(PO_4)_2(CO_3)_4(OH)_2]$. Although SO_4^{2-} ions are present in the solution when they reach equilibrium, only Ca^{2+} can be deposited as a final product, whereas SO_4^{2-} will dissolve completely.[1,4,6] The heating temperature also affects the occurrence of apatite carbonates; the higher the temperature during precipitation-dissolution, the higher the number of carbonate ions deposited, and the soaking time facilitates the precipitation-dissolution process to form carbonate ions.

In the Sp3-1 specimens, it was observed that the increase in pores accelerated the ion transfer process; this accelerated the occurrence of PO_4^{3-} ion deposits to replace SO_4^{2-} ions. In this study, the number of pores strongly influenced the formation of apatite carbonate crystals. The more pores, the more easily apatite carbonate crystals formed (Sp3 specimens), and this was achieved with 1 day of soaking (24 h) compared with specimens that had few or no pores (specimens Sp2 and Sp1). Ishikawa et al. succeeded in producing apatite carbonate without pores in 48 h. The formation of carbonate apatite crystals is strongly influenced by the temperature and immersion time.[1] In this study, the pores affected the formation of apatite carbonates, as shown by the porous calcium sulfate anhydrous specimens with 50% porogen PMMA which turned into apatite carbonate within 1 day. In porous specimens, the ions can diffuse more readily from all directions toward pore crevices during the precipitation-dissolution process.[5,6,15]

From the results of this study, there is potential for apatite carbonate formation in accordance with detected X-ray diffraction patterns. Hydroxyapatite phase or apatite

carbonate with a diffraction pattern of $2\theta = 25.8°$, $29.2°$, $32°$, $33°$, and $34.1°$ (Sp3-1 in Table 1) was formed on porous calcium sulfate anhydrous specimens produced using porogene 50% by weight PMMA and immersion in a mixture of Na_2CO_3 and Na_3PO_4 solutions at 100˚C for 1 day. Therefore, apatite carbonate can be formed more quickly in specimens that have a higher number of pores than those that have no pores or few pores. To further elucidate the function of carbonate ions formed in the apatite structure, continuation of this study is required with characterization using the Fourier Transform Infrared Spectroscopy (FT-IR) tool.

The conclusion are porous carbonate apatite can be made from PMMA porogen precursor $CaSO_4$ using the dissolution-precipitation method. Based on the results of X-ray diffraction, porous apatite carbonate was formed following 1 day of immersion in a mixture of Na_2CO_3 and Na_3PO_4 solutions at a temperature of 100˚C with the use of porogene 50% by weight PMMA.

ACKNOWLEDGMENTS

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