Fabrication of carbonate apatite pseudomorph from highly soluble acidic calcium phosphate salts through carbonation

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B-type carbonate apatite (CO₃Ap) has recently emerged as an attractive bone scaffold material because it exhibits significantly higher osteoconductivity than its hydroxyapatite analogue. Highly soluble acidic calcium phosphate salts, such as calcium hydrogen phosphate dihydrate (DCPD) and monocalcium phosphate monophosphate (MCPM), are typically used as bone cement and easily produce large calcium phosphate crystals. However, few studies have focused on these salts as precursors despite extensive investigations on the production of CO₃Ap. In this study, DCPD and MCPM were converted into CO₃Ap by hydrothermal treatment in the presence of at least 0.5 mol/L NaHCO₃, which acted as a neutralizer and CO₃²⁻ releaser. The obtained material maintained precursor morphologies and was therefore regarded as a pseudomorph of DCPD and MCPM mesocrystal structures. This synthetic approach is expected to facilitate the fabrication of shape-controlled CO₃Ap compacts from DCPD and MCPM.

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1. Introduction

B-type carbonate apatite [CO₃Ap: Ca_{10-a}(PO₄)_{6-b}(CO₃)_c(OH)_{2-d}] is a calcium phosphate mineral in which carbonate ions (CO₃²⁻) partially replace the phosphate ions (PO₄³⁻) of hydroxyapatite [HAp: Ca₁₀(PO₄)₆(OH)₂]. This main bone component is transformed into new bone through bone remodeling,^{1)–6)} making its scaffolds highly attractive candidates as bone substitutes in bone reconstruction applications.¹⁾

Unlike other ceramics, CO₃Ap compacts cannot be obtained by conventional sintering processes because it decomposes at sintering temperature.^{7)–9)} Instead, it is fabricated by phase conversion using calcium salt precursors, such as CaCO₃, Ca(OH)₂, alpha-tricalcium phosphate, and gypsum.^{10)–18)} This synthesis relies on a hydrothermal or solution-mediated dissolution–precipitation process, in which the salt precursor phase initially dissolves and releases Ca²⁺ ions into the surrounding solution. The Ca²⁺ concentration in the surrounding solution increases as the precursor phase continues to dissolve. When the precursor gradually releases PO₄³⁻ and CO₃²⁻ in addition to Ca²⁺, the concentrations of these ions begin to exceed the solubility of CO₃Ap. This causes CO₃Ap to precipitate from the saturated solution:

The rate of this reaction depends on the precursor solubility. Calcium hydrogen phosphate dihydrate [DCPD: CaHPO₄·2H₂O] and monocalcium phosphate monohydrate [MCPM: Ca(H₂PO₄)₂·H₂O] present high solubility in calcium precursor salts.^{2),3)} In addition, these calcium phosphate minerals readily give rise to large crystals that can be interlocked.³⁾ These physicochemical properties, which are suitable for the development of binding

materials, have led to the application of these minerals as main components of brushite cement. Moreover, DCPD and MCPM bind other CO₃Ap precursors, such as calcites, to set.¹⁹⁾ These properties are especially appealing for the quick and shape-controlled fabrication of CO₃Ap compacts.⁴⁾

To achieve such a fabrication, a method that directly converted DCPD and MCPM into CO₃Ap was developed in this study. Upon dissolution, these precursors release H⁺ along with Ca²⁺ and PO₄³⁻ because of their intrinsic acidity. However, OH⁻ plays an essential role in the formation of CO₃Ap, which limits reaction media to basic solutions. Therefore, the synthesis was conducted in the presence of NaHCO₃, which simultaneously produced CO₃²⁻ and neutralized the released H⁺.

2. Experimental methods

2.1 Fabrication of CO₃Ap from precursor

DCPD and NaHCO₃ were purchased from Wako Pure Inc., Japan. MCPM was acquired from Sigma-Aldrich Co., USA.

DCPD (1.72 g, 10 mmol) or MCPM (2.52 g, 10 mmol) was immersed in H₂O (0.015 L) in the presence of NaHCO₃ (0–6.3 g, 0–5 mol/L) and hydrothermally treated at 200°C for 24 h. The specimen was consecutively washed with distilled water at least ten times and 99.5% ethanol before slow drying at room temperature.

The specimens for crystalline and chemical analysis were milled using agate mortar and pestle. The milled specimens were additionally washed using distilled water to completely remove residual NaHCO₃.

2.2 Material characterization method

Sample surfaces were examined by scanning electron microscopy (SEM) at an acceleration voltage of 5 kV using an S-3400N instrument (Hitachi Co., Japan). Fine structures of treated materials deposited on copper microgrids covered with hollow carbon films were observed by transmission electron microscopy (TEM)

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using a JEM-1400Plus apparatus (JEOL Co., Japan) operating at an acceleration voltage of 100 kV.

The pH of the reaction medium after hydrothermal treatment of DCPD and MCPM in the presence of various NaHCO_3 concentrations was measured using a pH electrode (LAQUA 9525-10D, Horiba Co., Japan) connected to a pH meter (LAQUAact D-71, Horiba Co., Japan) at room temperature.

Crystal phases were determined by X-ray diffraction (XRD) using a D08 ADVANCE instrument (Cu $K\alpha$ radiation, Bruker Co., Germany) operating at a voltage of 40 kV and current of 40 mA in a continuous scanning mode for 2θ values ranging between 3 and 70° . These 2θ values were calibrated using the auto calibration mode before measurements. The scanning rate amounted to $150^\circ/\text{s}$. Lattice parameters for a - and c -axes were calculated using the (002) and (300) peaks of apatite observed at 2θ values of 21.78° and 32.94° , respectively, and the lattice parameter of the hexagonal system. Specifically,

$$\frac{1}{d_{hkl}^2} = \frac{4}{3} \left(\frac{h^2 + hk + k^2}{a^2} \right) + \frac{l^2}{c^2} \quad (2)$$

where h , k , and l are the Miller indices denoted as (hkl) , d_{hkl} is the d -spacing of (hkl) , a is the a -axis length of the unit lattice, and c is the c -axis length of the unit lattice. For peak shift measurement, the peak position was calibrated by α -alumina powder which mixed in specimens. The (012), (104) and (113) peaks of α -alumina (Katayama Chemical Industries Co., Ltd., Japan) observed at 25.59° , 35.17° and 43.38° , respectively, were used for calibration.

Chemical changes were evaluated by Fourier transfer infrared spectroscopy (FT-IR) in attenuated total reflection mode using a FT/IR-6200 apparatus (Jasco Co., Japan). Measurements were conducted over 32 scans at a resolution of 2 cm^{-1} using ambient air as the background. The carbon content (C) was determined by carbon–hydrogen–nitrogen (CHN) analysis using a MT-6 instrument (Yanaco Co., Japan) and Ar as a carrier gas. Samples were dried in a P_2O_5 desiccator before analysis.

3. Results

Figures 1(a) and **1(b)** show SEM micrographs of typical DCPD crystals. These crystals presented elongated, hexagonal plate-like shapes averaging $200\mu\text{m}$ in length. They exhibited smooth surfaces devoid of hillock-like structures. **Figures 1(c)–1(h)** show DCPD treated with 0.1 (c, d), 0.5 (e, f), and 5 mol/L NaHCO_3 (g, h). All treated samples retained the morphology of the initial phases, albeit with textured surfaces, except for DCPD treated with 0.1 mol/L NaHCO_3 . Samples exposed to 0.1 mol/L NaHCO_3 mainly consisted of a complex compact of ribbon-like crystals approximating $10\mu\text{m}$ in length and $2\mu\text{m}$ in width [Figs. 1(c) and 1(d)]. In addition, a small amount of block-like crystals measuring $20\mu\text{m}$ in length and $5\mu\text{m}$ in width was detected. Moreover, residual NaHCO_3 was observed on samples treated at a concentration of 5 M [Figs. 1(g) and 1(h)].

Figures 2(a) and **2(b)** show SEM micrographs of typical MCPM crystals. These crystals exhibited slightly elongated diamond plate-like shapes averaging $700\mu\text{m}$ in length. Their surfaces were smooth and free of hillock structures. **Figures 2(c)–2(h)** show SEM micrographs of MCPM treated with 0.1 (c, d), 1 (e, f), and 5 mol/L NaHCO_3 (g, h). The residual NaHCO_3 was also observed on samples treated at a concentration of 5 mol/L [Figs. 2(g) and 2(h)]. MCPM treated with 0.1 and 0.5 mol/L NaHCO_3 mainly consisted of $2\mu\text{m}$ in width in addition to a few block-like crystals measuring $20\mu\text{m}$ in length and $5\mu\text{m}$ in width. In contrast, samples treated NaHCO_3 solutions exceeding

Fig. 1. SEM micrographs of DCPD (a, b) before and (c, d) after hydrothermal treatment with 0.1, (e, f) 0.5, and (g, h) 5 mol/L NaHCO_3 .

1 mol/L maintained the morphology of initial phases overall. Moreover, they presented textured surfaces similar to the treated DCPD equivalents.

Figures 3(a) and **3(b)** show XRD patterns of treated DCPD and MCPM crystals, respectively. These patterns revealed that DCPD treated with 0.1 mol/L NaHCO_3 dicalcium comprised hydrogen phosphate anhydrate (DCPA) as the main phase and a small amount of HAp (CO_3Ap). A similar composition was observed for MCPM treated with NaHCO_3 concentrations at or below 0.5 mol/L. In contrast, hydrothermal treatment with NaHCO_3 solutions at or above 0.5 mol/L and 1 M yielded samples exhibiting typical HAp (CO_3Ap) patterns for DCPD and MCPM, respectively.

Table 1 shows the pH of the reaction medium after hydrothermal treatment of DCPD and MCPM at various NaHCO_3 concentrations. The pH increased with increasing NaHCO_3 concentration. It remained below 5 for reaction media resulting from DCPD and MCPM treated at or below 0.1 and 0.5 mol/L NaHCO_3 , respectively. Media from treated DCPD crystals displayed higher pH values than from treated MCPM crystals for all NaHCO_3 concentrations.

Figures 4(a) and **4(b)** show the FT-IR spectra of treated DCPD and MCPM crystals, respectively. The hydrothermal treatment of DCPD and MCPM in the presence of 0.5 and 1 mol/L NaHCO_3 , respectively, gave rise to similar spectra. Specifically, typical PO_4 vibration bands of HAp were detected at 1021 cm^{-1} along with

Fig. 2. SEM micrographs of MCPM (a, b) before and (c, d) after hydrothermal treatment with 0.1, (e, f) 1, and (g, h) 5 mol/L NaHCO_3 .

CO_3 stretching bands at 1420 and 1460 cm^{-1} , indicating the formation of B-type apatite by the substitution of PO_4^{3-} in HAp by CO_3^{2-} . However, low intensity CO_3 stretching bands were observed at 1525 and 1540 cm^{-1} , consistent with the replacement of OH^- by CO_3^{2-} in HAp to give A-type apatite.²⁰⁾ Furthermore, a band was observed at 866 cm^{-1} for DCPD and MCPM treated above 1 and 5 mol/L NaHCO_3 , indicating that CO_3^{2-} adsorbed onto the apatitic structure.^{21),22)} Consequently, DCPD and MCPM crystals were completely converted into CO_3Ap while retaining their initial morphologies when treated with NaHCO_3 solutions at and above 0.5 and 1 mol/L, respectively.

The CO_3 incorporation into the apatitic unit lattice was estimated using XRD peak shifts. **Figure 5** plots the a - and c -axes lattice parameters of apatite as a function of the NaHCO_3 concentration. The lattice parameters of apatite were taken from calibrated XRD patterns by α -alumina peak positions (Figure S1). The c -axis lattice parameter did not show any significant changes, irrespective of the NaHCO_3 solution concentration. In contrast, the a -axis lattice parameter decreased on increasing the NaHCO_3 solution concentration.

The CO_3 content was evaluated by CHN analysis. **Figure 6** shows the CO_3 content as a function of NaHCO_3 concentration. Treated DCPD crystals presented higher CO_3 content than MCPM crystals regardless of NaHCO_3 concentration. The CO_3 content of DCPD and MCPM crystals treated with 5 mol/L NaHCO_3 amounted to 10.5 and 7.1 mass %, respectively.

Figure 7 shows TEM images of treated DCPD (a, b) and MCPM (c, d). The treated DCPD fine structure mainly consisted

Table 1. pH values of distilled water after treatment with DCPD or MCPM in various concentration of NaHCO_3 at 200°C for 24 h

NaHCO_3 concentration (mol/L)	DCPD	MCPM
0	4.28	1.75
0.1	4.41	1.91
0.5	6.62	2.57
1	7.05	5.20
5	9.72	9.59

Fig. 3. XRD patterns of (a) DCPD and (b) MCPM after hydrothermal treatment at various NaHCO_3 concentrations.

Fig. 4. FT-IR bands of (a) DCPD and (b) MCPM after hydrothermal treatment at various NaHCO_3 concentrations.

Fig. 5. Lattice constants calculated from observed (002) and (300) peaks as a function of NaHCO_3 concentration. Closed circles and closed triangles correspond to DCPD and MCPM, respectively. (a) a -axis; (b) c -axis. $1 \text{ \AA} = 0.1 \text{ nm}$.

of hexagonal plate-like crystals approximating 20 nm. These crystals generated so-called mesocrystal structures through a complex assembly process.^{23–26} High resolution TEM (HR-TEM) observations showed unidirectional lattice fringes, suggesting that CO_3Ap formed in the individual plate-like crystals. Treated MCPM displayed essentially the same as fine structure and components as its DCPD counterpart.

Fig. 6. CO_3 content after hydrothermal treatment as a function of NaHCO_3 concentration. Closed circles and closed triangles correspond to DCPD and MCPM, respectively.

4. Discussion

Hydrothermal treatment of DCPD and MCPM crystals at 200°C and above 0.5 and 1 mol/L NaHCO_3 , respectively, lead to their complete conversion into CO_3Ap after 24 h. In addition, this treatment did not alter the macroscopic morphology of the crystals.

However, CO_3Ap formation was limited to basic conditions in the presence of sufficient CO_3^{2-} .^{2,3)} The dissolution of DCPD and MCPM freed H^+ as described by

Therefore, NaHCO_3 acted as a CO_3^{2-} source and a pH buffer under weakly basic conditions:

Fig. 7. (a, c) TEM micrographs of treated crystals showing a mesoscale structure and (b, d) high-resolution TEM micrographs of single hexagonal plate-like crystals presenting a mesoscale structure. (a, b) DCPD treated with 5 mol/L NaHCO_3 . (c, d) MCPM treated with 5 mol/L NaHCO_3 . Insets: corresponding low-magnification TEM micrographs.

The released H^+ was absorbed and the pH was titrated by NaHCO_3 buffering. However, below 0.5 and 1 mol/L NaHCO_3 for DCPD and MCPM, respectively, the released H^+ yielded to NaHCO_3 buffering, causing the pH to decrease. Consequently, only acidic calcium phosphate materials, such as DCPA and octacalcium phosphate (OCP), were formed without apatitic phases. The MCPM-derived H^+ displayed a greater activity than its DCPD equivalent because of the higher H^+ ratio in MCPM than in DCPD. Therefore, the MCPM system required higher amounts of NaHCO_3 than its DCPD analogue for pH titration.

This study assumed that the ribbon-like OCP formed under acidic conditions was converted into HAP while maintaining its morphology. Several previous works indicate that OCP readily transformed into HAP with a pseudomorphic relationship in a hydrothermal environment.^{2),27)–30)}

The pH of the solution was titrated by NaHCO_3 buffering under weakly basic conditions. The dissolution of DCPD and MCPM produced Ca^{2+} and PO_4^{3-} , which built up to concentrations exceeding the solubility of CO_3Ap . Under weakly basic conditions, CO_3Ap was in a stable phase, which promoted its formation [Eq. (1)].

According to the heterogeneous nucleation theory, CO_3Ap crystal seeds randomly formed on DCPD and MCPM surfaces.³¹⁾ Therefore, the resulting crystals covered the precursors, maintaining their original morphologies. These CO_3Ap crystals began to grow when DCPD and MCPM crystals dissolved, gradually replacing the precursors.

Large amounts of CO_3^{2-} inhibited CO_3Ap formation. FT-IR spectra and peak shift estimates showed that CO_3^{2-} was incorporated into the apatitic unit lattice and adsorbed onto CO_3Ap crystallites. In addition to the CO_3 stretching bands detected at 1420 and 1460 cm^{-1} for CO_3Ap , the band at 866 cm^{-1} was consistent attributable to CO_3^{2-} adsorbed onto the apatitic structure,^{21),22)} which inhibited CO_3Ap growth.^{10),21),22)} As a result, the growth process generated a plethora of small CO_3Ap crystals. This phenomenon may be utilized to maintain fine structures by analogy

to the number of pixels, which greatly improves the resolution of a picture when increasing.

5. Conclusion

In this study, CO_3Ap was obtained by hydrothermal treatment of DCPD and MCPM in the presence of NaHCO_3 . The product retained the morphologies of the precursors. In addition to an easy crystallization, these acidic calcium phosphate salts benefit from greater water solubility than other CO_3Ap precursors, which strongly affects the phase conversion ratio. Therefore, the newly developed CO_3Ap synthesis may lead to rapid fabrication methods for shape-controlled CO_3Ap compacts.

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References

- 1) K. Ishikawa, *Mater.*, **3**, 1138–1155 (2010).
- 2) L. Wang and G. H. Nancollas, *Chem. Rev.*, **108**, 4628–4669 (2008).
- 3) J. C. Elliott, “Studies in Inorganic Chemistry 18, Structure and Chemistry of the Apatites and Other Calcium Orthophosphates”, Elsevier Co., the Netherlands (1994).
- 4) B. D. Ratner, A. S. Hoffman, F. J. Schoen and J. E. Lemons, “Biomaterial Science: An Introduction to Materials in Medicine”, 3rd ed., Academic Press, Elsevier Co., the Netherlands (2013).
- 5) Y. Sugiura, K. Onuma, Y. Kimura, H. Miura and K. Tsukamoto, *J. Cryst. Growth*, **332**, 58–67 (2011).
- 6) R. M. Wilson, S. E. P. Dowker and J. C. Elliott, *Biomater.*, **27**, 4682–4692 (2006).
- 7) R. Z. LeGeros, *Nature*, **206**, 403–404 (1965).
- 8) S. Raynaud, E. Champion, D. Bemache-Assollant and P. Thomas, *Biomater.*, **23**, 1065–1072 (2002).
- 9) A. A. Baig, J. I. Fox, R. A. Young, Z. Wang, J. Hsu, W. L. Higuichi, A. Chhetry, H. Zhuang and M. Otsuka, *Calcif. Tissue Int.*, **64**, 437–449 (1999).
- 10) Y. Sugiura, K. Tsuru and K. Ishikawa, *Ceram. Int.*, **42**, 204–210 (2016).
- 11) H. Wakae, A. Takeuchi, K. Udoh, S. Matsuya, M. Munar, R. Z. LeGeros, A. Nakashima and K. Ishikawa, *J. Biomed. Mater. Res., Part A*, **87**, 957–963 (2008).
- 12) K. Sunouchi, K. Tsuru, M. Maruta, G. Kawachi, S. Matsuya, Y. Terada and K. Ishikawa, *Dent. Mater. J.*, **31**, 549–557 (2012).
- 13) S. Nomura, K. Tsuru, M. Maruta, S. Matsuya, I. Takahashi and K. Ishikawa, *Dent. Mater. J.*, **33**, 166–172 (2014).
- 14) F. Daitou, M. Maruta, G. Kawachi, K. Tsuru, S. Matsuya, Y. Terada and K. Ishikawa, *Dent. Mater. J.*, **29**, 303–308 (2010).
- 15) A. Takeuchi, M. L. Munar, H. Wakae, M. Maruta, S. Matsuya, K. Tsuru and K. Ishikawa, *Biomed. Mater. Eng.*, **19**, 205–211 (2009).
- 16) C. T. Zaman, A. Takeuchi, S. Matsuya, Q. H. M. S. Zaman and K. Ishikawa, *Dent. Mater. J.*, **27**, 710–715 (2008).
- 17) N. X. T. Tram, M. Maruta, K. Tsuru, S. Matsuya and K. Ishikawa, *Adv. Biocera. Porous Cera.*, **4**, 59–65 (2014).
- 18) Y. Lee, Y. M. Hahm, S. Matsuya, M. Nakagawa and K. Ishikawa, *J. Mater. Sci.*, **42**, 7843–7849 (2007).
- 19) N. Koga, K. Ishikawa, K. Tsuru and I. Takahashi, *Ceram. Int.*, in press.
- 20) R. Z. LeGeros, O. R. Trautz, E. Klein and J. P. LeGeros, *Exp. A*, **25**, 14–16 (1969).
- 21) A. Kflak, A. Slosarczyk and W. Kolodziejewski, *J. Mol. Struct.*,

- 997, 7–14 (2011).
- 22) C. Rey, C. Combes, C. Drouet and M. J. Glimcher, *Osteoporos. Int.*, **20**, 1013–1021 (2009).
- 23) H. Imai, *J. Ceram. Soc. Japan*, **122**, 737–747 (2014).
- 24) Y. Nakagawa, H. Kageyama, Y. Oaki and H. Imai, *J. Am. Chem. Soc.*, **136**, 3716–3719 (2014).
- 25) F. C. Meldrum and H. Colfen, *Chem. Rev.*, **108**, 4332–4432 (2008).
- 26) H. Colfen and M. Antonietti, “Mesocrystals and Nonclassical Crystallization”, Wiley & Sons Ltd, USA (2008).
- 27) W. E. Brown, L. W. Schroeder and J. S. Ferris, *J. Phys. Chem.*, **83**, 1385–1388 (1979).
- 28) M. Kamitakahara, N. Ito, S. Murakami, N. Watanabe and K. Ioku, *J. Ceram. Soc. Japan*, **117**, 385–387 (2009).
- 29) D. G. A. Nelson and G. H. McLean, *Calcif. Tissue Int.*, **36**, 219–232 (1984).
- 30) Y.-H. Tseng, C.-Y. Mou and J. C. C. Chan, *J. Am. Chem. Soc.*, **128**, 6909–6918 (2006).
- 31) I. Sunagawa, “Crystals Growth, Morphology and Perfection”, Cambridge University Press, UK. (2005).