

## Transformation of Apatite Cement to B-Type Carbonate Apatite Using Different Atmosphere

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**Abstract.** Apatite cement (AC) is a breakthrough in biomaterials for the reconstruction of the bone defect. However, the replacement of AC to bone up to the present time is still controversial for researchers. Several researchers have reported that AC was replaced by bone while others claimed replacement was limited. The aim of this study is to investigate the transformation mechanism of AC to B-type carbonate apatite (CO<sub>3</sub>Ap) using different atmosphere. An *in vitro* study mimicking the body environment was employed in order to examine the effect of setting atmosphere on the composition of set AC. An equimolar of tetracalcium phosphate (TTCP; Ca<sub>4</sub>(PO<sub>4</sub>)<sub>2</sub>O) and dicalcium phosphate anhydrous (DCPA; CaHPO<sub>4</sub>) mixed with distilled water was enabled to harden at 37°C and 100% of relative humidity under presence of 5% CO<sub>2</sub>, 100% CO<sub>2</sub>, and 100% N<sub>2</sub> atmospheres. XRD and FT-IR analyses revealed that in the presence of 100% CO<sub>2</sub> and 5% CO<sub>2</sub>, B-type CO<sub>3</sub>Ap could be determined and only small amounts of TTCP remained unreacted. On the contrary, in the presence of 100% N<sub>2</sub>, the CO<sub>3</sub><sup>2-</sup> bands could not be detected and larger amount of TTCP remained unreacted compared to 5% CO<sub>2</sub> and 100% CO<sub>2</sub> atmospheres. SEM morphology showed that the microstructure of AC was entangled and locked to each other. In addition, the small needle like crystals appeared in the surface of 100% N<sub>2</sub>, similar to hydroxyapatite. We concluded that the CO<sub>3</sub><sup>2-</sup> ions incorporated in AC during setting reaction may be one of the essential factors for CO<sub>3</sub>Ap formation.

### Introduction

Apatite cement (AC) has been widely used in biomaterials field. Due to its setting reaction, bone defect can be reconstructed with apatite without having a gap between the bone and apatite cement. Minimal invasive surgery techniques also became possible by injecting ACs into the bone defect using a syringe. AC consisting tetracalcium phosphate (TTCP; Ca<sub>4</sub>(PO<sub>4</sub>)<sub>2</sub>O) and dicalcium phosphate anhydrous (DCPA; CaHPO<sub>4</sub>) or dicalcium phosphate dehydrate (DCPD; CaHPO<sub>4</sub>·2H<sub>2</sub>O) was developed [1] and reported to be replaced by bone even though replacement of AC to bone is still a controversial issues [2]. In line as previous studies, another reports also showed that the AC is replaced by new bone when implanted [3, 4]. On the contrary, others reported that AC was minimally replaced by bone [5, 6]. Miyamoto *et al.* found minimal replacement of AC by bone or new bone formation adjacent to the bone, while no replacement of AC was found inside the AC [7]. On the other hand, carbonate apatite (CO<sub>3</sub>Ap) was replaced by bone when implanted into a bony defects [8]. One of the factors of replacement of CO<sub>3</sub>Ap to bone, might be caused by low crystalline CO<sub>3</sub>Ap which has similarity to the bone composition [9, 10]. Therefore, AC may convert to CO<sub>3</sub>Ap in the body environment even if the constituent of AC is free from carbonate since carbonate ions are contained in body fluids and blood.

In this present study, *in vitro* condition mimicking body environment was employed as the effect of setting atmosphere. The composition of set AC during setting reaction was investigated using TTCP and DCPA based on dissolution-precipitation reactions under 5% CO<sub>2</sub> and 100% CO<sub>2</sub>

at 37°C and 100% of relative humidity. As a control, 100% of N<sub>2</sub> atmosphere was employed to make a condition of free from carbonate.

## Materials and Methods

**Preparation of apatite cements powder and sample.** Commercially TTCP powder (Taihei Chemicals Inc., Osaka, Japan) was used as received whereas DCPA powder (J. T. Baker Chemical Co., NJ, USA) was crushed in a planetary mill (Fritsch Pulverisette 5, Idar-Oberstein, Germany) with 95% ethanol for 1 hour followed by drying overnight at 80°C. Laser diffraction particle size analyzer (SALD-300V, Shimadzu, Kyoto, Japan) revealed that the average size of TTCP and DCPA are 8 μm and 1 μm, respectively. As powder phase of AC, an equimolar mixture of TTCP and DCPA was used for further study. The powder phase was mixed with distilled water at a liquid to powder ratio (L/P ratio) = 0.3. The paste obtained was packed into a teflon mold with 10 mm in diameter and 10 mm in height. After packing, one end of the mold was covered by a glass slide and clamped by a metal clip. The mold with the sample paste was then placed into an incubator with 5% CO<sub>2</sub> and 100% CO<sub>2</sub>, respectively, kept at 37°C and 100% of relative humidity for 24 hours. For negative control, the mold with sample paste was placed in 100% N<sub>2</sub> atmosphere at 37°C and 100% of relative humidity for 24 hours. After the treatment, the set AC was immersed in the 99% ethanol for 3 minutes and then dried in an oven at 80°C for 2 hours. One millimeter of sample thickness was cut using a diamond cutter (Isomet, Lake Bluff, Illinois, USA) and investigated further on characterization.

**Characterization of samples.** Sample was crushed into powder and characterized by mean of X-ray diffraction (XRD: D8 Advance, Bruker AXS GmbH., Karlsruhe, Germany) and Fourier transform infrared spectroscopy (FT-IR: FT/IR-6200, JASCO, Tokyo, Japan).

**Mechanical strength measurements.** The diametral tensile strength (DTS) test was performed to evaluate set AC samples for mechanical strength measurement. The cement paste was packed into a split stainless steel mold (6 mm in diameter × 3 mm in height). The mold was placed by storing in an incubator for 24 hours at 37°C and 100% relative humidity with 5% CO<sub>2</sub>, 100% CO<sub>2</sub> or 100% N<sub>2</sub> atmospheres, respectively. After the cement sets, the diameter and height of each sample were measured with a micrometer (MDC-25MU, Mitutoyo Co. Ltd., Kanagawa, Japan). The samples were crushed using a universal testing machine (AGS-J; Shimadzu Corporation, Kyoto, Japan) at a crosshead speed of 1 mm/min. DTS values were taken as average of at least 5 samples.

**Morphological analysis.** At first, the fractured surface samples were coated by gold sputters coating and then morphological analysis of the samples was performed using scanning electron microscope (SEM: S-3400N, Hitachi High-Technologies, Tokyo, Japan) at 10 kV of accelerating voltage.

## Results and Discussion

Figure 1 shows the XRD patterns of powder samples of AC collected before and after 24 hours under 5% CO<sub>2</sub>, 100% CO<sub>2</sub> or 100% N<sub>2</sub> atmosphere. The XRD pattern of commercial hydroxyapatite (HAp) was listed as a reference. Transformation to apatite could be observed after 24 hours regardless of the atmosphere, although TTCP was remained in set AC. This transformation reaction is based on dissolution-precipitation reactions. When TTCP and DCPA exposed to an aqueous solution, they dissolve and supply Ca<sup>2+</sup> and PO<sub>4</sub><sup>3-</sup> ions, respectively. The resultant aqueous solution would be supersaturated with respect to HAp and thus HAp crystals would be precipitated.

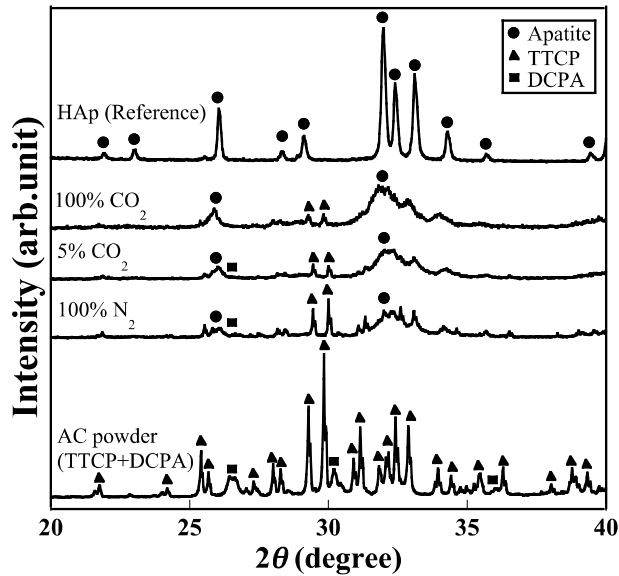


Fig. 1. XRD patterns of powder samples collected from AC before and after 24 hours under 5% CO<sub>2</sub>, 100% CO<sub>2</sub> and 100% N<sub>2</sub> atmospheres. XRD pattern of commercial HAp was listed as reference.

In case of 5% CO<sub>2</sub> and 100% CO<sub>2</sub> atmospheres, almost all AC powder transformed to apatite, although a small amounts of TTCP powder remained. Nevertheless, higher amounts of TTCP powder remained in set AC in case of 100% N<sub>2</sub> compared to 5% CO<sub>2</sub> and 100% CO<sub>2</sub> atmospheres.

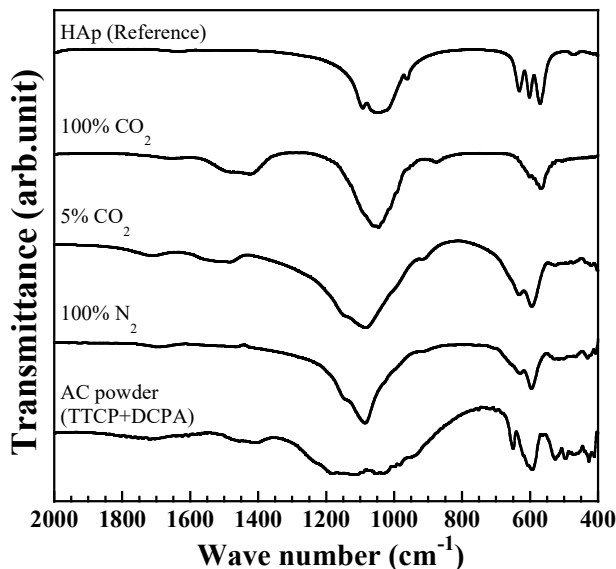


Fig. 2. FT-IR spectra of powder samples collected from AC before and after 24 hours under 5% CO<sub>2</sub>, 100% CO<sub>2</sub> and 100% N<sub>2</sub> atmospheres. FT-IR spectrum of commercial HAp was listed as reference.

Figure 2 presents the FT-IR spectra of the powder samples of AC obtained before and after 24 hours under 5% CO<sub>2</sub>, 100% CO<sub>2</sub> or 100% N<sub>2</sub> atmospheres. FT-IR spectrum of commercial HAp was listed as a reference. In the spectra of all samples, appropriate bands for phosphate were detected at 560-970 and 1010-1100 cm<sup>-1</sup>. These bands were assigned to apatite which was consistent with the XRD results. In 5% CO<sub>2</sub> and 100% CO<sub>2</sub> atmosphere, some additional bands were found at around 873, 1420 and 1470 cm<sup>-1</sup>, assigned to carbonate, whereas the significant band

of carbonate could not be observed in case of 100% N<sub>2</sub> atmosphere. These results indicate that set AC prepared under 5% CO<sub>2</sub> and 100% CO<sub>2</sub> atmosphere contained CO<sub>3</sub><sup>2-</sup> ions in its apatite structure. The obtained carbonate apatite corresponded to B-type CO<sub>3</sub>Ap, determined by band positions of FT-IR spectra.

During the setting reaction of AC, apatite could supply Ca<sup>2+</sup> and PO<sub>4</sub><sup>3-</sup> ions regarding equilibrium of apatite. In the presence of CO<sub>2</sub>, CO<sub>3</sub><sup>2-</sup> ions would partly substitute the PO<sub>4</sub><sup>3-</sup> site. Therefore, the solution would be supersaturated with respect to CO<sub>3</sub>Ap. Since CO<sub>3</sub><sup>2-</sup> ions exist in the body environment, the following carbonation process may occur based on the dissolution-precipitation reactions.

Figure 3 shows the DTS values of set AC cement after treatment at 37°C and 100% relative humidity for 24 hours. DTS values of the set AC cement were 5.5 ± 1.2 MPa for 100% CO<sub>2</sub>, 6.4 ± 2.1 MPa for 5% CO<sub>2</sub>, and 6.7 ± 2.8 MPa for 100% N<sub>2</sub>. There was no statistically significant difference.

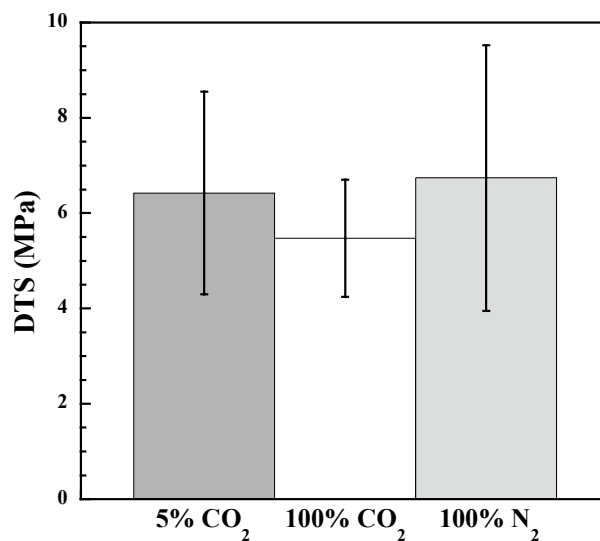


Fig. 3. Mechanical strength values of AC after treated by 5% CO<sub>2</sub>, 100% CO<sub>2</sub> and 100% N<sub>2</sub> for 24 hours, respectively. At least 5 samples were measured for DTS.

Figure 4 shows scanning electron microscopy morphology, the microstructure of AC was entangled and locked to each other. In addition, the small needle like crystals appeared in the surface of 100% N<sub>2</sub> atmosphere was similar to hydroxyapatite.

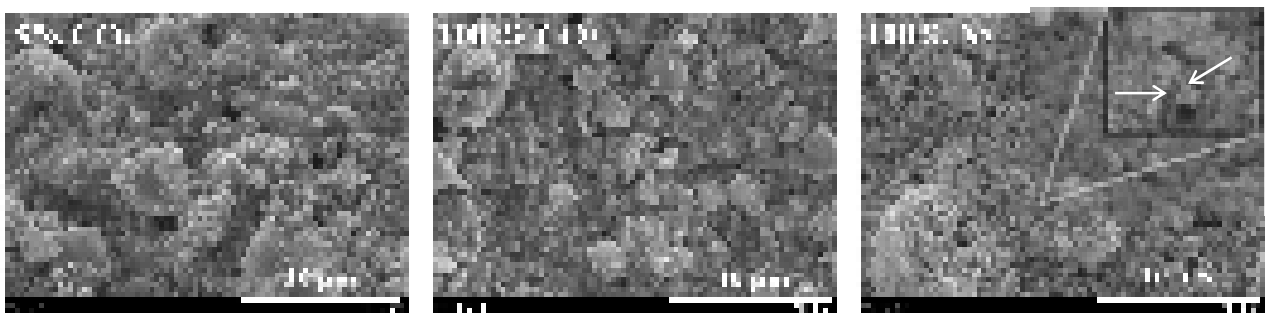


Fig. 4. SEM morphology of AC samples after treated by 5% CO<sub>2</sub>, 100% CO<sub>2</sub> and 100% N<sub>2</sub> atmospheres for 24 hours, respectively.

## Summary

The carbonation process during setting reaction of AC successfully occurred at the surface of the set AC, which converted to low crystalline B-type CO<sub>3</sub>Ap. Therefore, the CO<sub>3</sub><sup>2-</sup> ions incorporated in AC during setting reaction may be one of the essential factors for CO<sub>3</sub>Ap formation.

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