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## Original Articles

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# Study of a method to prepare novel carbonate-containing apatite porous bodies

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*Bone apatite contains about 5 to 8% carbonate groups. Sintered carbonate-containing apatite has been proposed as a bone substitute material due to its excellent biocompatibility and bioabsorbability. However, sintering increases crystallization, carbonate ions are lost, and absorbability decreases. Therefore, we examined the transition conditions of carbonate-containing apatite in solution at a temperature close to that of the living body to develop a method to obtain unsintered carbonate-containing apatite. Using dibasic calcium phosphate dihydrate as the starting material, carbonate-containing apatite with continuous pores and more carbonic acid groups than the sintered material could be produced.*

Key words: Carbonate-containing apatite, Dibasic calcium phosphate dihydrate, Porous body

### Introduction

Tissue regeneration and restoration of biological functions using biomaterial fillers are the focus of regenerative medicine<sup>1)</sup>. Autogenous bone grafting is the most reliable means of bone regeneration, and it is considered the golden standard. However, the supply of necessary material is limited. As allogeneic bone is difficult to obtain and infectious diseases are of concern, efforts are focusing on the use of artificial bone instead of autologous bone and allogeneic bone<sup>2)</sup>. At present, tricalcium phosphate beta-phosphate ( $\beta$ -TCP), hydroxyapatite (HAP), carbonate-containing apatite, and similar materials are used as scaffold-type bone substitute materials<sup>3)</sup>. HAP is similar to inorganic components of bone and has excellent biocompatibility<sup>4)</sup>. However, HAP is absorbed only minimally in vivo and will remain for a long time. Although  $\beta$ -TCP has osteoconductive ability, it is thought to be phagocytosed by macrophages<sup>5)</sup>.

Clinically, autogenous bone and artificial bone are mixed and used to adjust the absorption rate<sup>6)</sup>. As an ideal scaffold-type bone prosthetic material, it is equivalent to HAP in terms of biocompatibility and requires only a material to be absorbed. Bone apatite is not pure HAP, but there are many calcium ions and phosphoric acid and hydroxyl groups. The most important difference between bone apatite and HAP is the presence and absence of carbonate groups. Bone apatite contains about 5 to 8% carbonate groups. Sintered carbonate-containing apatite has been proposed as a bone substitute material due to its excellent biocompatibility and bioabsorbability. However,

sintering increases crystallization, carbonate ions are lost, and absorbability declines. Therefore, we examined the transition conditions of carbonate-containing apatite in solution at a temperature close to that of the living body to develop a method to obtain unsintered carbonate-containing apatite.

### Materials and methods

#### 1) Preparation of sintered CAP porous bodies (sCA)

CAP was synthesized according to the method of Doi et al<sup>7)</sup>. CAP powder and granular sugar adjusted to a diameter of 500 to 850  $\mu$ m (inner halide sugar, Shin Mitsui Sugar, Tokyo, Japan) were mixed at a weight ratio of 3:7. This was molded into a cylindrical shape and further subjected to 200MPa hydrostatic pressure treatment. The resulting cylindrical porous bodies were immersed in distilled water for 1 h to elute the sugar. After drying overnight, the temperature was raised to 700°C at a heating rate of 5°C/min, and this temperature was maintained for 1h to prepare sintered porous bodies.

#### 2) Preparation of CA porous bodies (nsCA) from dibasic calcium phosphate dihydrate (DCPD)

Using DCPD as a precursor of CA, granular sugar adjusted to a particle size of 500 to 850  $\mu$ m was mixed with DCPD at a weight ratio of 3:7 and then pressed into a mold at 15kg/I under pressure. Secondary pressurization was carried out using a hydrostatic pressure device (200M), producing a compact composed of DCPD/granulated sugar. In order to enable simultaneous

elution of sugar and transfer of DCPD to CA, 1M sodium bicarbonate solution at 60°C was used as an immersion solution. DCPD porous bodies from which the sugar was eluted were immersed in 100 ml of 1 M sodium hydrogen carbonate solution at 60°C for 3, 6, or 9h or 1, 2, or 3days after sugar elution, washed with distilled water, and freeze-dried.

### (3) Evaluation of porous bodies

#### 1) X-ray diffraction analysis

X-ray diffraction analysis was carried out using powder of various porous bodies pulverized with a mortar and pestle.

#### 2) Infrared absorption spectroscopy (FT-IR) analysis

For FT-IR analysis, 400mg of potassium bromide was added uniformly to 1 mg of each sample, and then pellets were prepared and analyzed using an FTIR 4200 spectroscopy analyzer (Shimadzu Corp., Kyoto, Japan). Using the transmission mode, spectra were collected over the waveform range 4000cm<sup>-1</sup> to 600cm<sup>-1</sup>.

#### 3) Measurement of carbonic acid content

The nsCA porous bodies prepared by immersion for 2 days were used as a standard and analyzed according to Conway's microdiffusion method<sup>8</sup>. Also, FT-IR peaks derived from carbonate ions in the linear absorption mode over the range 2000cm<sup>-1</sup> to 1300cm<sup>-1</sup> were collected, and the integrated values were compared with those of the standard sample.

#### 4) Scanning electron microscopy (SEM)

Each sample was subjected to conductivity treatment using an osmium coating apparatus (HPC-1C, vacuum device) and then observed under a scanning electron microscope (S-4500, Hitachi, Tokyo, Japan).

## Results

### 1. Structure of the porous bodies

Figure 1 shows the results of X-ray diffraction analysis of the transition of DCPD to CA. Using DCPD as a starting material with 3h of immersion, broad peaks typical of low-crystallinity apatite and octacalcium phosphate (OCP) near 5° /2θ were detected in the vicinity of 32° /2θ. Although the DCPD and OCP peaks decreased with time, DCPD showed a higher rate of dissipation and was barely detectable after immersion for 6 h. After 9 h of immersion, a slight amount of OCP was detected, but most of this was transferred to apatite. One day after immersion, it was confirmed as apatite single phase.

Figure 2 compares the IR spectra of the samples shown in Figure 1. Two peaks indicating absorption by carbonate groups are observed at 1400 to 1500cm<sup>-1</sup>, confirming that the apatite formed was carbonate containing. Also, up to the first day of immersion, more carbonate ions were substituted within the apatite lattice as immersion time increased. Figure 3 shows the change in carbonate content, with values of 9.39, 8.46, 9.39, 9.39, 9.39, and 5.31% after 1, 2, 2, 2, 2, and 3 days and 3, 6, and 9h of immersion. It was 91%.

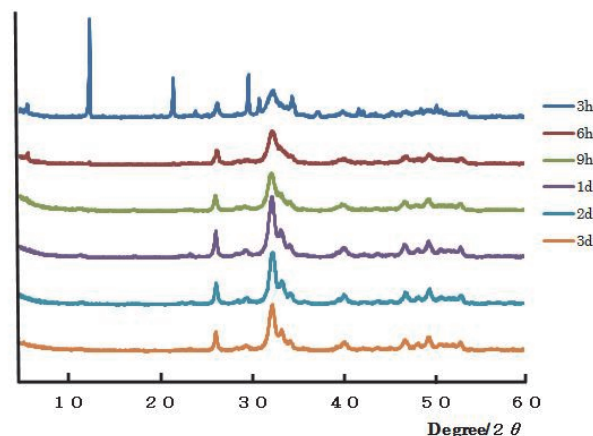


Figure 1. Results of X-ray diffraction analysis of the transition of DCPD to CA.

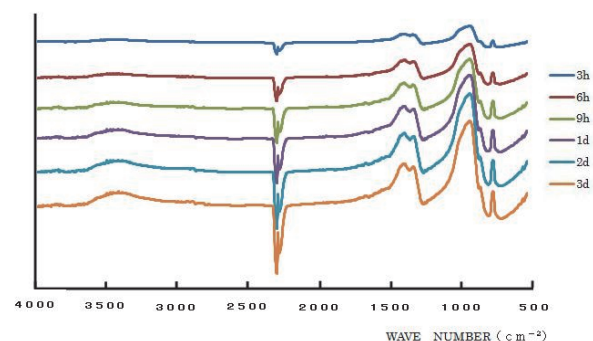


Figure 2. IR spectra of the samples shown in Figure 1.

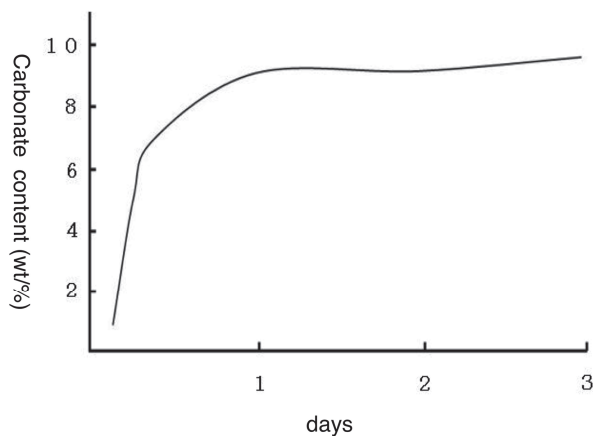


Figure 3. Change in carbonate content with immersion for 1, 2, and 3 days at 3, 6, and 9 h

and 3days and 3, 6, and 9h of immersion. It was 91%.

In the SEM image of a sample immersed for 1 day (Fig. 4), it is apparent from the low magnification (Fig. 4a) that the granulated sugar eluted, forming communicating pores of 100 to 500μm at the elution sites. Observation of a part of the walls constituting the pores at a high magnification (Fig. 4b) revealed numerous fine flake crystals, and it was confirmed that a porous body was formed using this aggregate.

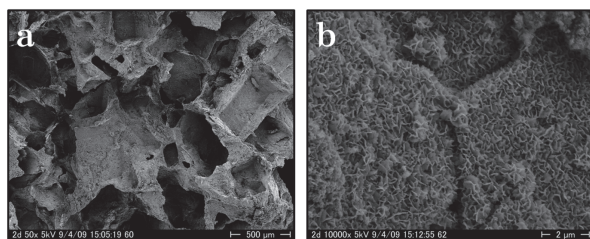


Figure 4. SEM image of a sample immersed for 1 day. (a) Low magnification. (b) High magnification.

## Discussion

Considerable research in the dental field has focused on bone regeneration in tissues such as periodontal bone as well as regeneration to correct loss of bone resulting from cyst removal and morphologic reconstruction after tumor removal. Calcium phosphate bone substitute materials were thought to have no osteoinductive abilities, but in 1990, it was reported that bone formation occurred in 3 months when HAP was implanted in dog muscle<sup>9,10</sup>. It has also been reported that osteoinduction occurs only in materials with a macroporous structure that will induce bone formation by calcium phosphate-type bone substitute materials. In addition, it is often seen in dogs and pigs, although with species-specific differences, but it is said to be hard to detect in rats, mice, and rabbits<sup>11-15</sup>.

Both macro- and micropores are required for osteoinduction with only calcium phosphate porous bodies. The optimal size of macropores is 50 to 400  $\mu\text{m}$ , and because of the need for invasion of blood vessels and collagen fibers into the porous bodies, pore communicability is required<sup>16</sup>. It is also reported that the endogenous BMP concentration increases on the macropore surfaces and eventually leads to differentiation of osteoblasts<sup>16</sup>. In this respect, nsCA prepared using DCPD as a starting material according to the sugar elution method produced consistent SEM findings.

It has also been reported that porous bodies exhibit continuous porosity only when the porosity is 50% or greater. Porosity measured in the present study using  $\mu\text{CT}$  was 56%. This porosity was associated with macropores, which can be discriminated by  $\mu\text{CT}$ ; micropores were not included in that calculation, so the actual porosity would be even greater.

As sugar elution and the transfer reaction proceed at the same time, it became clear that it is possible to prepare porous bodies of arbitrary porosity by adjusting the particle size of the sugar to be used. Previously referred to as bioabsorbable materials,  $\beta$ -TCP and carbonate apatite sintered bodies reportedly exhibit excellent bioabsorbability. The absorption of apatite in vivo is thought to be caused by acid produced by osteoclasts. Substitution of carbonate ions in the apatite lattice distorts the crystal structure and reduces its physicochemical stability, making it more likely to be dissolved in acid. In other words, the degree of crystallization and the carbonate content are thought

to influence the absorption rate. In X-ray diffraction analyses of the nsCA porous bodies, OCP was observed for up to 9h of immersion, but the apatite peak became clearer with time, becoming apatite single phase after 1day. In addition, no sugar residue was observed. FT-IR analyses confirmed the presence of more carbonic acid groups in the apatite. These results demonstrate that the sugar elution method using DCPD as a starting material is suitable for preparing nsCA porous bodies.

## Conclusion

Using DCPD as a starting material enables the production of carbonate-containing apatite bodies containing continuous pores and more carbonic acid groups than are produced using the sintering method.

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