Effect of Sodium Bicarbonate Amount on *In Vitro* Indomethacin Release from Self-Setting Carbonated-Apatite Cement

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Purpose. In the present study, to develop a drug delivery system with higher bioacitivity in hard tissues by using the self-setting bioactive carbonate apatite cement, we have investigated the effects of sodium bicarbonate content on the *in vitro* drug release from a self-setting bioactive carbonate apatite cement containing indomethacin (IMC).

Methods. The cement powder systems constituted an equimolar mixture of tetracalcium phosphate ($Ca_4(PO_4)_2O$) and dicalcium phosphate dihydrate ($CaHPO_4 \cdot 2H_2O$), hydroxyapatite (HAP, $Ca_{10}(PO_4)_6(OH)_2$) seed crystals and sodium bicarbonate. Two types of 2% IMC loaded-cements were prepared as follows, one containing 0% HAP seed crystal and 0–10% sodium bicarbonate, and the other containing 40% HAP seed crystal and 0–10% sodium bicarbonate. The drug release profiles from 2% IMC loaded-cements were measured in simulated body fluid at pH 7.25 and 37.0°C.

Results. The drug release profiles from the cement matrix systems with or without seed crystals were estimated using a moment analysis computer program. The mean drug release time (MDT) and the time required for 50% drug release of the cement containing 0 and 40% seed crystal decreased with an increase of sodium bicarbonate. Furthermore, after the drug release the total pore volume of the cement matrix, as measured by mercury porosimetry, increased with an increase of sodium bicarbonate.

Conclusions. MDT and T_{50}'s were a function of adding the amount of sodium bicarbonate. The results of the relationship between the micropore distribution, total volume of pores after drug release and drug release supported the hypothesis that the variation in drug release from the cements resulting from the addition of sodium bicarbonate was mainly due to an increase in the diffusion of the drug in the micropores of the cement by dissolution or erosion of the cement matrix.

KEY WORDS: drug delivery system; biomaterials; self-setting bioactive carbonate apatite cement; *in vitro* indomethacin release; mercury porosimetry.

INTRODUCTION

When implanting artificial bone materials made of calcium phosphates into natural hard tissues, the bioacitivity and bioaffinity of the materials with natural hard tissues has subsequently been associated with the crystalline structure of the calcium phosphate materials (e.g., hydroxyapatite, tricalcium phosphate, biphasic calcium phosphate) including bone-derived materials (1–2). Recent studies speculate that carbonate apatite crystals, similar to bone apatite (3), are believed to become subsequently incorporated into the mineralization of the collagen matrix during the formation of the new bone at the materials/host bone interface (4,6). Therefore, bioactivity and bioaffinity of implant materials with hard tissues may be related to the formation of carbonate apatite crystals in new bones by bone cells (4–5). Since osteoblasts form new bone from calcium and phosphate ions in body fluid after resorption of bone mineral by osteoclasts (7), *in vivo* bioactivity and bioaffinity of calcium phosphate materials may be related to biodegradation. Therefore, it seems that biodegradation of calcium phosphate materials is related to *in vitro* dissolution behavior, which in turn, relates to the solubility of the materials.

On the other hand, some attempts have been made to prepare to biocompatible self-setting cement (8) using metastable calcium phosphates. Cortze *et al.* (9) applied a similar bone cement containing metastable calcium phosphate to bone defects in human subjects, and reported excellent bone conduction after 3 months. We investigated a drug delivery system for several drugs using a self-setting calcium phosphate cement containing tetracalcium phosphate, a new way to deliver drugs to bone, and successfully controlled the drug release rate from the cement (10–14). In the present study, in order to develop a drug delivery system with higher bioactivity with hard tissue we used the self-setting carbonate apatite to investigate the effect of carbonate content on apatite cement containing the anti-inflammation drug, indomethacin, as a model drug, to illustrate *in vitro* drug release properties.

MATERIALS AND METHODS

Materials

Tetracalcium phosphate (TTCP, $Ca_4(PO_4)_2O$) was obtained from Kyoritsu Ceramic Co., Japan. Dicalcium phosphate dihydrate (DCPD, $CaHPO_4 \cdot 2H_2O$) and sodium bicarbonate powder ($NaHCO_3$) powders were obtained from Nakalai Tesque Co., Japan. Hydroxyapatite (HAP, $Ca_{10}(PO_4)_6(OH)_2$) used as seed crystals was synthesized by a precipitation method at 105°C (15). Bulk indomethacin (IMC) powder JP XII (lot No. 99131598) was obtained from Nihon Bulk Pharm. Co., Japan. All other reagents were of analytical grade.

Procedures for IMC Delivery System Cement Formation

The cement powder consisted of an equimolar mixture of TTCP and DCPD, 0 or 40% HAP seed crystals and various amounts of sodium bicarbonate as summarized in Table 1. The cement powder was mixed with 0.25 ml of 20 mM H_3PO_4 for 1 min, then 2% IMC powder were mixed with the paste. The mixtures were then placed in a mold (16.0 mm in diameter, 2.0 mm in thickness) and stored at 37·C and 100% relative humidity for 24 h. The hardened cement pellet (475±20 mg) was mounted on a dissolution apparatus, so that the entire pellet surface (5.027 cm^2) was exposed. Since IMC recovery from the cement was almost 100% and the sample solutions were identical with no impurities as shown by tin layer clomatography, the amount of IMC degraded during preparation of the cement and the drug release test in SBF was negligible.

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X-ray Diffraction

The X-ray powder diffraction profiles of the cement and drug-loaded cement samples were measured by powder X-ray diffraction analysis (XD-3A, Shimadzu Co., Japan, Cu radiation, 15 mA, 35 kV).

Fourier-Transformed Infrared (FT-IR) Spectra Measurement

The sample powder was dispersed in micronized KBr powder (sample concentration 5%) by pestle and mortar. The mixed sample powder was loaded on a sample cup as a flat loose powder bed with a flat surface formed by a micro spatula. FT-IR spectra were obtained by powder diffuse reflection on an FT-infrared spectrophotometer (type FT-IR 1600, Perkin Elmer Co., Yokohama, Japan); 50 co-added scans were collected at 4 cm^{-1} resolution and corrected using the Kubelka-Munk equation.

Drug Release Test

The drug release profiles from all cement pellets containing the drug were measured as follows: a sample cement was introduced into 25 ml of simulated body fluid (SBF) (16) containing 142 mM of Na^+, 5.0 mM of K^+, 1.5 mM of Mg^{2+}, 147.8 mM of Cl^-, 0.63 mM Ca^{2+}, 4.2 mM of HCO_3^-, 0.5 mM of SO_4^{2-} and 1.0 mM HPO_4^{2-} (pH 7.25) in a 50 ml capped test tube. The tube was fixed on the sample holder in a thermostatically regulated water bath maintained at 37.0 ± 0.1°C and shaken horizontally at 90 strokes/min. During the release test, the entire dissolution medium was replaced with fresh buffer at 3, 6, 24, 28, 48, 72 and 78 h. The concentrations of IMC were measured spectrophotometrically (UV 160A, Shimadzu Co., Kyoto, Japan) at 262 nm. The data represents the average of 3 measurements from the independent experiments.

Micropore Distribution

The micropore distribution of the fixed cements was measured by means of mercury porosimetry (Porosimeter 2000, Carlo Erba Instruments, Japan). Pore size ranged from 6×10^{-3} μm to 50 μm.

Mechanical Strength of the Cement

The crushing strength of the hardened cement was measured along with the diameter of the cement using an accurate compression/tension testing machine (Autograph model IS-5000, Shimadzu Co.) at a compression speed of 15mm/min.

Table 1. The Formulation and Drug Release Rate of IMC Delivery System by Using Self-Setting Carbonated-Apatite Cement

Cement	TTCP (%)	DCPD (%)	HAP (%)	$NaHCO_3$ (%)	IMC (%)	MDT[a] (h)	T50[b] (h)
Carbonate-cement containing 0% seed crystal							
cement A	66.67	31.33	0.00	0.00	2.00	54.3	39.1
cement B	65.31	30.69	0.00	2.00	2.00	44.6	29.2
cement C	63.27	29.73	0.00	5.00	2.00	23.6	13.1
cement D	59.87	28.13	0.00	10.00	2.00	4.80	3.1
Carbonate-cement containing 40% seed crystal							
cement E	40.03	18.81	39.21	0.00	2.00	59.0	45.2
cement F	39.21	18.43	38.36	2.00	2.00	42.9	30.1
cement G	37.99	17.85	37.16	5.00	2.00	7.92	3.2
cement H	35.95	16.89	35.16	10.00	2.00	1.67	1.1

[a] The mean dissolution time obtained by the moment method.
[b] Time required for 50% of the drug to be released.

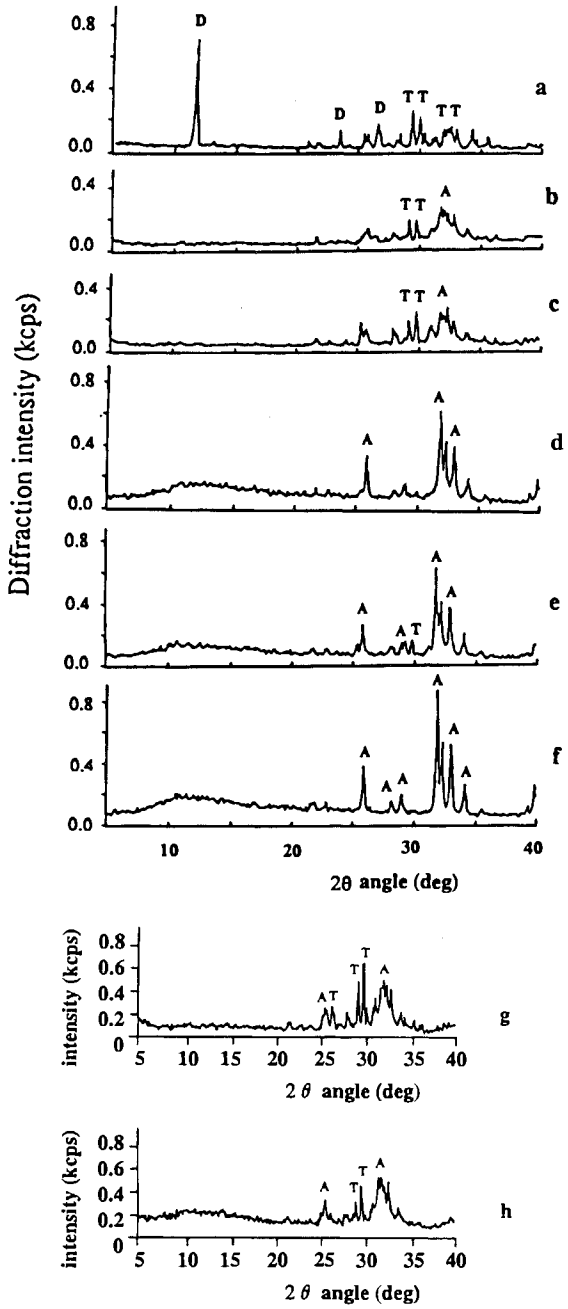

Fig. 1. X-ray powder diffraction profiles of 2% IMC-loaded carbonate apatite cements before and after drug release. (a) the powder mixture of TTCP and DCPD, (b) cement A after drug release, (c) cement D after drug release, (d) cement E after drug release, (e) cement H, (f) synthetic HAP. (g), cement C before, (h), cement C after drug release, (A) apatite, (T) TTCP, (D) DCPD.

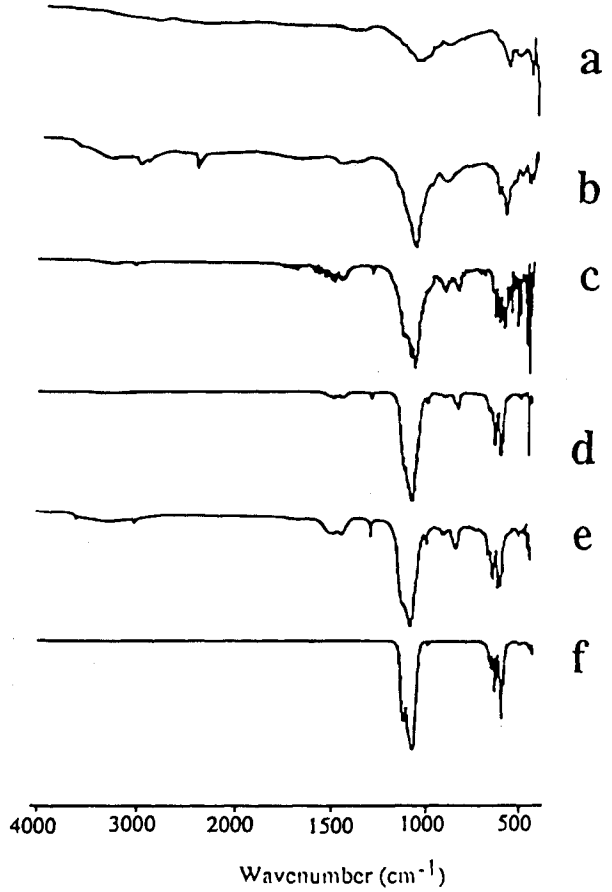

Fig. 2. FT-IR spectra of 2% IMC-loaded carbonate apatite cements after drug release. (a) the powder mixture of TTCP and DCPD, (b) cement A, (c) cement D, (d) cement E, (e) cement H, (f) synthetic HAP.

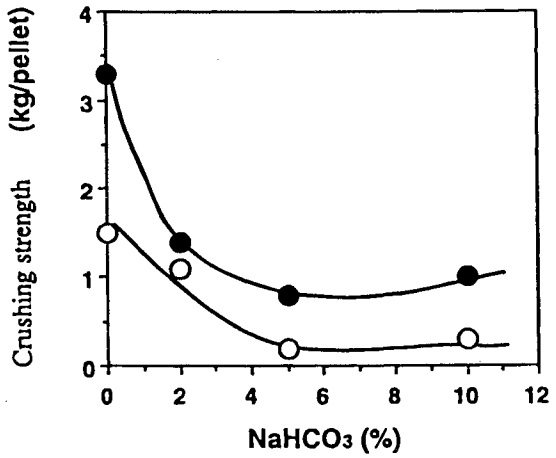

Fig. 3. Effect of sodium bicarbonate amount on the mechanical strength of 2% IMC-loaded carbonate apatite cements before drug release. ○, 40% seeded cement, ●, 0% seeded cement.

RESULTS AND DISCUSSION

Characterization of Carbonate Apatite Cement Containing IMC

Figure 1 shows the X-ray diffraction profiles of 2% IMC loaded, self-setting carbonate apatite cement systems before and after drug release test. Since there was no diffraction peaks due to sodium bicarbonate (2θ = 30.5°) and DCPD (2θ = 11.5°) in diffraction profiles of all cement formulations, both rows of the material transformed into hydroxyapatite during cement setting process. In the cement system containing 40% seed crystal, the cement E, without sodium bicarbonate before and after drug release, transformed into almost 100% hydroxyapatite since the seed crystals accelerated the crystal growth rate, but the cement H, contained 10% sodium bicarbonate before drug release, had small diffraction peaks due to row material (TTCP). This suggested that IMC and sodium bicarbonate did not interfere with the cement setting, but apatite formation was deleted by the presence of sodium bicarbonate.

On the other hand, in X-ray diffraction profiles of cement systems without seed crystal, the cements A and D contained 0 and 10% sodium bicarbonate, before and after drug release, respectively, showed the diffraction peaks due to TTCP, and the peaks at 2θ = 31–34° due to hydroxyapatite. Since their diffraction peaks due to hydroxyapatite were much broader than those of synthetic hydroxyapatite, the cement without seed crystals transformed into apatite with a low-crystallinity during cement preparation, which is reported to have a higher affinity for hard tissue than the high-crystallinity apatite (4–5). The diffraction peaks intensity due to TTCP of cement D were larger than those of cement A, indicating that the apatite formation during cement setting did not interfere, but was deleted by the adding of sodium bicarbonate.

On the other hand, the cement C, containing 5% sodium bicarbonate before drug release, had small diffraction peaks due to TTCP, but the intensity of the diffraction peaks slightly decreased during drug release. This suggested that a remaining part of TTCP gradually transformed into apatite during drug release, but did not completely transform for 2 weeks after drug release. The results suggest that the transformation of apatite during cement preparation and drug release could be controlled by adding sodium bicarbonate and/or seed crystals.

Figure 2 shows the FT-IR spectra of the fixed cement containing 2% IMC after the drug release test. The FT-IR spectrum of the carbonated-apatite shows the bands at 604 and 567 cm^{-1}, and 700–1000 cm^{-1} and 1100 cm^{-1} due to HPO_4^{2-} and PO_4^{3-} group, and at 750–950, 1430 and 1470 cm^{-1} due to carbonate ion (9). All of the cements had a typical FT-IR spectra of carbonate-apatite (9). This suggests that the cements A and E without sodium bicarbonate absorbed carbon dioxide gas from air. These results suggest that most parts of the cement bulk powder transformed into carbonated-apatite.

Mechanical Strength of Carbonate Apatite Cement Containing IMC

Figure 3 shows the effect of sodium bicarbonate on the crushing strength of carbonate apatite cements containing 0 and 40% seed crystal. Since the crushing strength was measured along with the diameter and the cement thickness was thin (2 mm × 16 mm Φ), the absolute values of the crushing strength for all fresh fixed cements were low. The crushing strength of both cements containing 2% IMC decreased with an increase in the amount of sodium bicarbonate, because the pore size of 3–20 μm in the cement increased with increase of sodium bicarbonate as shown below. On the other hand, the crushing

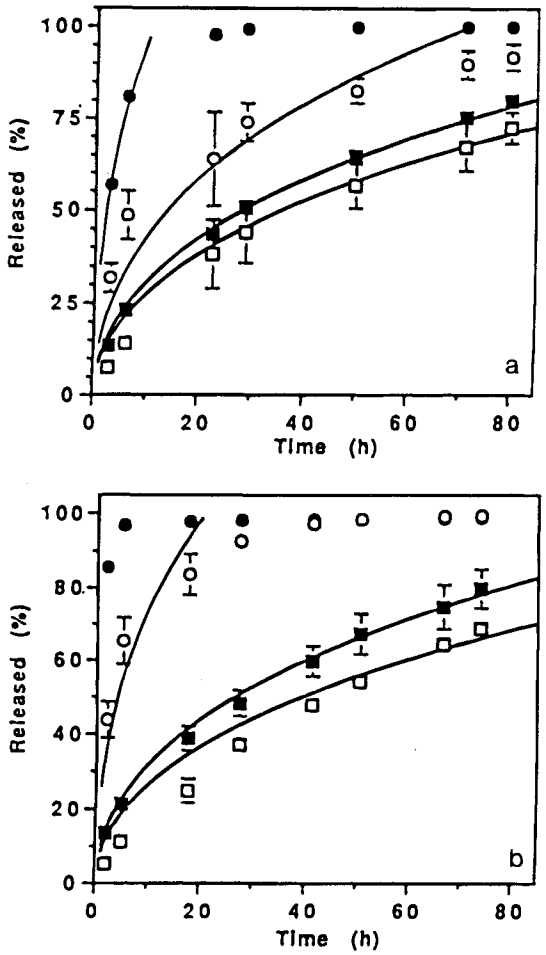

Fig. 4. Effect of amount of sodium bicarbonate on drug release profiles from 2% IMC-loaded carbonate apatite cements. (a) 0% seeded cement, (b) 40% seeded cement, □, 0% sodium bicarbonate, ■, 2%, ○, 5%, ●, 10%. The data and bars represent averages and the standard deviation of 3 measurements from independent experiments.

strength of the cements without seed crystals were higher than those with seed crystals since the cements with seed crystals (cements A and C) had porosity about 2.5 times larger than those without seed crystal (cements E and G). These results indicate that the mechanical strength of the cement might be related to the factors of the cements geometrical structure, such as porosity, particle size, and crystallinity, which were affected by increasing the amount of sodium bicarbonate.

Effect of Amount of Sodium Bicarbonate on Drug Release from Carbonate Apatite Cements

Figure 4 shows the effect of sodium bicarbonate amount on the drug release profiles of the carbonate apatite cement systems containing 0 and 40% seed crystals in pH 7.25 SBF at 37°C. The percent of drug released from the cements changed quite dramatically due to the adding of sodium bicarbonate. As reported previously (9,10), the drug release profiles from the planar surface of homogeneous drug loaded calcium phosphate cement systems follow the Higuchi equation (17), but the geometrical pore structure of this cement system changed during drug release as shown below. Therefore, the IMC release from the carbonated apatite cement did not follow Higuchi equation.

The drug release profiles from the cement matrix systems were analyzed by using the moment analysis method (18) as shown in equation 1.

$$MDT = \frac{\int_0^{X(\infty)} t \cdot dX}{X(\infty)} \quad (1)$$

MDT is the mean drug release time, $X(\infty)$, the total drug content and X is drug released amount at time t. *MDT,* were estimated using a moment analysis computer program (18).

Table 1 shows the effect of the sodium bicarbonate amount on *MDT* and time required for 50% of the drug to be released (T_{50}) from the carbonated apatite cements. T_{50} values were estimated from drug release profiles. The *MDT* and T_{50} for drug release from the carbonate apatite cement exhibited 35 and 50 fold difference between the minimum and the maximum values obtained from the cement containing 0 and 40% seed

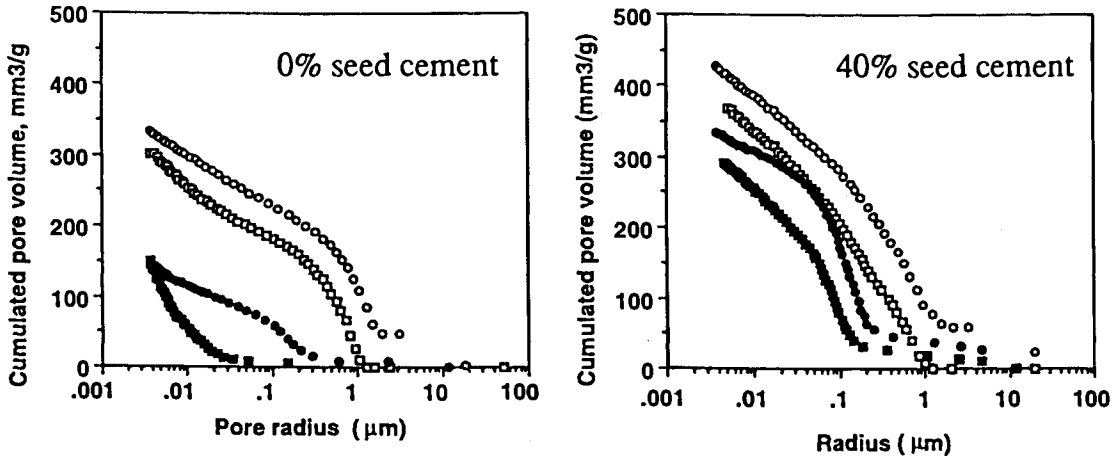

Fig. 5. Micropore distribution of 2% IMC-loaded carbonate apatite cements before and after drug release. □, ■, 0% sodium bicarbonate, ○, ●, 5% sodium bicarbonate. Open and closed symbols represent after and before drug release, respectively.

crystal respectively. For both systems, the values were proportional to the sodium bicarbonate amount, indicating that drug release rate could be controlled by varying the amounts of sodium bicarbonate.

The Micropore Structure of Carbonate Apatite Cement Obtained by Addition of Various Sodium Bicarbonate Amount

Figure 5 shows the micropore distribution of the carbonate apatite cements before and after drug release. In the cement without seed, most of the micropores of cement A (without sodium carbonate) were less than 0.04 μm in pore radius, but the micropores increased in size 0.1–1 μm after drug release. The cement C (5% sodium bicarbonate) increased in pore volume at 0.07–0.3 μm pore radius with increased sodium bicarbonate, and their volume around 150 mm^3/g increased 0.4–10 μm pore radius after drug release.

The cement containing 40% seed, cement E (without sodium carbonate), had much larger pore volume, with pore radius at 0.003–0.1 μm than that at 0.1–1 μm, but the volume of micropores at 0.1–1 μm increased in radius after drug release. The cement G (5% sodium bicarbonate) increased in pore volume through the range of pore radii by increasing sodium bicarbonate, and the pore volume, about 150 mm^3/g, increased at 0.4–10 μm in pore radius after drug release. In all cement systems, the pore volume at larger pore radius increased after drug release, indicating that the cement matrix of remaining raw materials and/or amorphous which were the more soluble part, dissolved and/or eroded.

After the drug release tests of the carbonate apatite cement obtained by adding 0–10% sodium bicarbonate, the micropore distribution of their cements were measured and are shown in Figure 6. The total pore volume of the cements containing 40% seed crystal at various carbonate levels were larger than those of the cements containing 0% seed crystal. The porosity of the cement containing seed crystals was higher than that of cement without seed crystals indicating that the packing of the seed crystals in the cement matrix affected the geometrical structure characteristics, such as total cement porosity.

On the other hand, the total pore volume of both apatite cement systems increased with increasing amounts of sodium bicarbonate. The pore size of 3–20 μm for the cement obtained by increasing sodium carbonate 5–10% was higher than for the others, which means that the micropore distribution of the cement containing more sodium bicarbonate show biphasic profiles below and above pore size at 1 μm. This suggests that the pores with radium more than 1 μm were formed by dissolution of carbonate apatite cement matrix after drug release.

The results support the hypothesis that the variation in drug release from the cements resulting from an increasing in cement porosity was mainly due to the increase of dissolution or erosion of carbonated apatite in the cement matrix by increasing their solubility by adding sodium bicarbonate. This suggests that the drug release rates for the cements were accelerated by increasing the porosity by adding sodium bicarbonate, indicating that it is possible to control drug release from the cement by increasing the amount of sodium bicarbonate.

Morphological Characterization by SEM Observation of Carbonate Apatite Cement Containing IMC After Drug Release

Figure 7 shows SEM photographs of 2% IMC loaded-carbonate apatite cements after drug release. Surface morphological differences of the cements were evident before and after the drug release tests. The fresh cements A and C had jagged surfaces consisting of aggregates fine particles. However, after drug release, both cements had many mushroom-shaped structures on their surfaces. These results suggested that the cements were converted into fine hydroxyapatite crystals during drug release.

CONCLUSIONS

The IMC release rate from carbonate apatite cements increased with increasing carbonate content. The drug release profiles from the cement, MDT and T_{50}'s, were a function of adding to the amount of sodium bicarbonate. The relationship between the micropore distribution, total volume of pores after drug release, and drug release behavior were consistent with

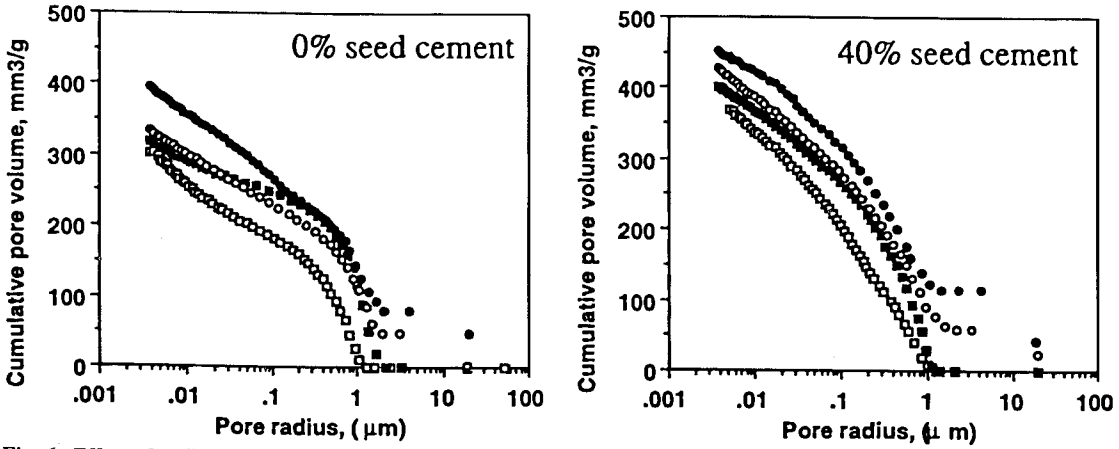

Fig. 6. Effect of sodium bicarbonate amount on micropore distribution of 2% IMC-loaded carbonate apatite cements after drug release. □, 0% sodium bicarbonate, ■, 2%, ○, 5%, ●, 10%.

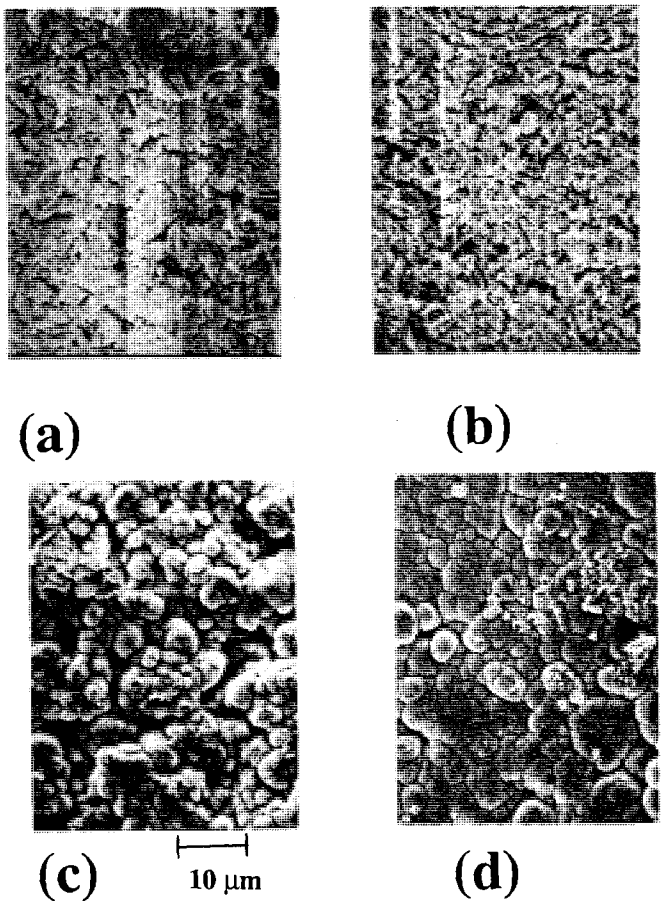

Fig. 7. SEM photographs of 2% IMC-loaded carbonate apatite cements before and after drug release. (a), surface of cement A before drug release, (b), surface of cement C before drug release, (c) surface of cement A after drug release, (d) cement C after drug release.

each other. This consistency supports the hypothesis that the variation in drug release from the cements results from the addition of sodium bicarbonate is mainly due to an increase in the diffusion of the drug in the micropores of the cement by the dissolution or erosion of the cement matrix.

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