

## Case Report

# Drug Release from a Three-Dimensionally Perforated Porous Apatite/Collagen Composite Cement

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**Abstract** Artificial bone (PC, containing 3% indomethacin) with biocompatibility, inter-connective pore structure, and drug delivery ability was obtained from self-setting apatite/collagen composite cement. X-ray powder diffraction and FT-IR spectroscopy suggested that PC consisted of carbonated apatite and had a structure similar to that of natural rat bone. The IMC release rates from a PC block containing the drug were measured in simulated body fluid. The rate of release increased with the number of macropore, that from planar surface matrix systems followed the Higuchi equation.

**Keywords** drug delivery system; apatite/collagen composite device; inter-connective pore structure; cell scaffold

## 1 Introduction

Inter-connective pores in coral-like-materials are therapeutically effective for generating bone in the body by introducing bone cells and blood vessels [2]. Since inter-connective porous biomaterials are useful as a scaffold for bone cell cultures for implant, apatitic biomaterials with inter-connective pores as three-dimensionally perforated porous  $\beta$ -TCP ceramics, and demonstrated their biocompatibility. In the present study, to improve biocompatibility, the inter-connective pore structure and drug delivery into self-setting apatite/collagen composite cement (ACC) without a compression process, a three-dimensionally perforated porous apatite/collagen composite cement (PC) was obtained by mechanical engineering. Since the drug release rate from the device could be controlled by various geometrical factors, indomethacin (IMC), an anti-inflammation drug was loaded into the composite cement as a model. The relationship between the in vitro IMC-release rate and geometrical structure of PC was investigated by dissolution testing.

## 2 Materials and methods

### 2.1 Preparation of PC

The apatite cement bulk powder consisting of an equimolar mixture of Tetracalcium phosphate (TECP,  $\text{Ca}_4(\text{PO}_4)_2\text{O}$ ) and dicalcium phosphate dihydrate (DCPD,  $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ), was prepared by grinding at 90 strokes per second for 10 min in an agate vibration mixer mill (Retsch Co. Ltd., Germany, 20 mL volume chamber in a ball 10-mm in diameter) according to a procedure described previously [1,5]. The ACC bulk powder was an apatite cement bulk powder with 0 and 20% type I bovine collagen (lot 31K7026, Sigma Co., USA) ground at 90 strokes per second for 20 min. ACC bulk powder (0.750 g) containing indomethacin (IMC) bulk powder (22.5 mg) was mixed homogeneously with 0.60 mL of 11 mM phosphoric acid for 1 min to form a paste. Stainless steel needle-like male dies 0.5 mm in diameter were arranged in a layer parallel to each other at intervals of 1.5 mm in a specific stainless-steel cubic mold,  $10.0 \times 10.0 \times 7.5$  mm. Other needle-like male dies of the same size were arranged on the needlelike die layer with a needle axis angle of  $90^\circ$  [4]. The final paste was poured into the cubic mold in which were arranged multi-crosses with 20, 40, and 60 stainless needlelike male dies, and stored and hardened at  $37^\circ\text{C}$  and 100% relative humidity for 24 h.

### 2.2 Material characterization

The cement powder samples were characterized by a powder X-ray diffraction analysis (Rint Ultima, XD-3A, Shimadzu Co., Japan, Cu radiation, 14 mA, 30 kV, scan speed,  $4^\circ/\text{min}$ ) and FT-infrared spectrophotometer (type Spectrum One, Perkin Elmer Co., Yokohama, Japan).

### 2.3 In vitro drug release test

Drug release test of sample cement pellets were tested in 25 mL of simulated body fluid (SBF) [6] at  $37.0 \pm 0.1^\circ\text{C}$  and

shaken at 90 rpm. All drug concentrations were determined at 320 nm by UV spectrometer (160 type, Shimadzu Co., Kyoto, Japan).

### 3 Results and discussion

#### 3.1 Characterization of PC

After removal of the stress-pins, the PC block had 60 cross macro-pores  $600\ \mu\text{m}$  in diameter, a weight of 750 mg, and a macro-pore porosity was 0.234. The cement block (Figure 1) consisted of macro- and micro-pores in the cement matrices. The results of X-ray powder diffraction analysis and FT-IR spectra suggested that the composite cement transformed into a carbonated apatite and was similar in structure to natural rat bone.

#### 3.2 Effect of geometrical structure on drug release behaviors of PC

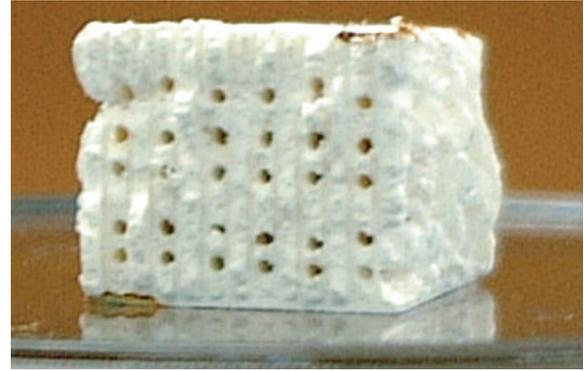
The *in vitro* release of IMC from the 3% IMC-loaded PC block with various numbers of inter-connective macro-pores in SBF indicated that the release rate increased with the number of macro-pores. Since the rate-limiting step in the release from an unerosible-homogeneous drug-loaded matrix system is basically the drug diffusion process in the micro-pores of the matrix, the release from planar surface matrix systems follows the Higuchi equation (1) [3]:

$$M_t = A \sqrt{C_s \frac{D_i \varepsilon}{\tau} (2C_d - \varepsilon C_s) t}, \quad (1)$$

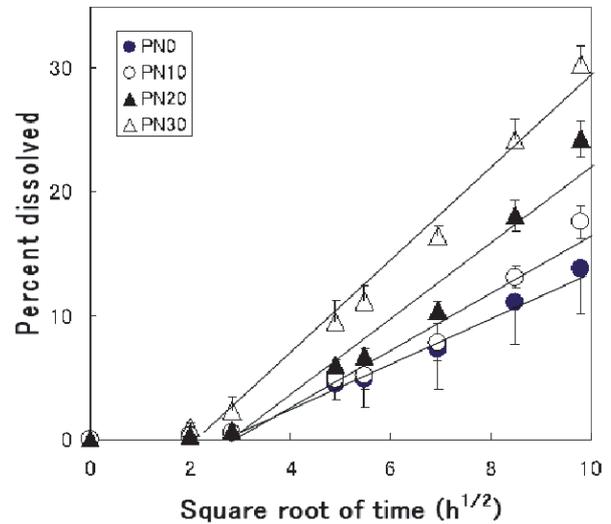
where  $M_t$  is the amount of drug released after time  $t$ ,  $A$  is the surface area of the device,  $D$  is the diffusion coefficient of the drug,  $C_s$  is solubility,  $C_d$  is the concentration of drug in the matrix,  $\tau$  is tortuosity and  $\varepsilon$  is porosity.

The *in vitro* IMC release data for PC with various numbers of macro-pores were applied to plots of release against the square root of time, as shown in Figure 2. The *in vitro* IMC release profiles from all PC were linear at the initial stage in the Higuchi plot. The release rate constants (Higuchi's release rate constant, HC) were therefore evaluated from the initial slope of the plots, less than 30% dissolution, by the least-squares method. The *in vitro* IMC release profiles of PC are linear only in the initial stage on the Higuchi plot. Because, drug release in the present system was from whole tablets, not planar tablets, and the elute was SBF saturated with calcium and phosphate ions, hydroxyapatite crystals precipitated out from SBF [6]. Therefore, it is clear that the rate-determining step of IMC release is diffusion in micro-pores in the matrices. The initial slope of the Higuchi plot was calculated as Higuchi constant (HC) by the least-squares method.

Since the formulations of PC were the same, the geometrical micro-structure (micro-pore structure) of all devices were almost the same, and the diffusion parameters of all PCs, such as porosity, drug concentration in the matrices and



**Figure 1:** Photography of the PC block ( $10.0 \times 10.0 \times 7.5\ \text{mm}$ ) used in the present study.



**Figure 2:** Higuchi-type plot for *in vitro* IMC release from PC in SBF. Closed cycle; 0 macro-pores, open cycle; 20 macro-pores, closed triangle; 40 macro-pores, open triangle; 60 macro-pores.

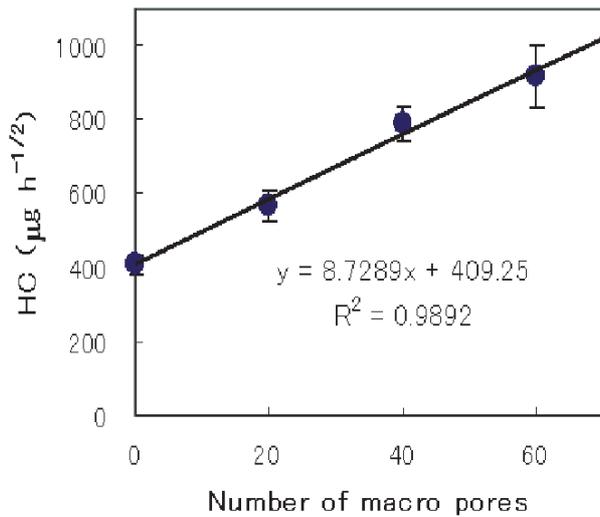
pore tortuosity were almost constant, respectively, except for surface area related to the geometrical macro-structure of PC.

Figure 3 shows the relationship between HC and number of macro pores of PCs. The relationship had a straight line with slope, 8.729,  $y$ -intercept, 409.25, and coefficient of determination, 0.9892.

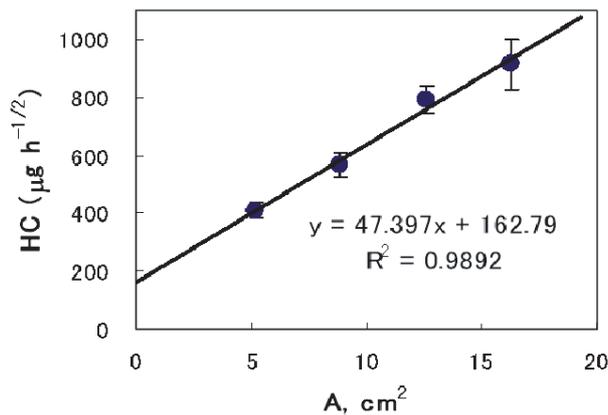
The Higuchi equation (1) is simplified to (2), the surface area of macro-pores is accounted for as the surface area ( $A$ ) of the device, and the drug release rate of PC could be controlled by the value  $A$ :

$$M_t = A \times K \times \sqrt{t}, \quad (2)$$

$$K = \sqrt{C_s \frac{D_i \varepsilon}{\tau} (2C_d - \varepsilon C_s)}. \quad (3)$$



**Figure 3:** Relationship between  $HC$  and number of macro pore of PC.



**Figure 4:** Relationship between  $HC$  and  $A$  of PC.

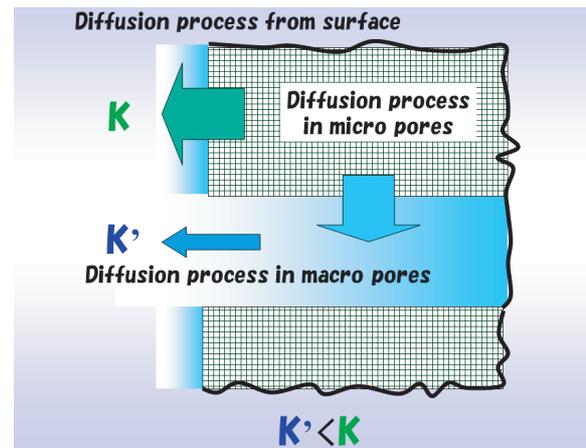
Figure 3 shows the relationship between  $HC$  and  $A$  of PCs. The relationship had a straight line with  $y$ -intercept, 162.79. This result indicated that  $K$  from outer surface of the block was not equal to that from surface of macro pores. Equation (2) could be converted to (4):

$$M_t = (A_p \times N \times K' + (A_0 - 2A_{sp} \times N) \times K) \times \sqrt{t}, \quad (4)$$

$$HC = A_p \times N \times K' + (A_0 - 2A_{sp} \times N) \times K, \quad (5)$$

where  $A_p$  is surface area of a micro pore,  $A_0$  is surface area of the block surface,  $N$  is number of macro pores,  $K$  and  $K'$  are dissolution constants from outer and macro pore surfaces.

The linear relationship between  $HC$  and  $A$  of PC (Figure 4) suggested adequateness of (4). Therefore, this result indicated that drug release rate from the PC device could be controlled by the number of macro-pores for culturing bone cells as shown in a diffusion model (Figure 5).



**Figure 5:** Diffusion model for PC device.

#### 4 Conclusion

The results of physicochemical properties suggested that PC consisted of a carbonated apatite/collagen composite with a chemical structure similar to that of natural bone. The PC block contained inter-connective macro-pores  $600 \mu\text{m}$  in diameter for the culture of bone cells and micro-pores less than  $3 \mu\text{m}$  in radius in biodegradation carbonated apatite/collagen composite cement matrices. In vitro drug release kinetics from PC followed the Higuchi equation, and could be controlled by the number of macro-pores for culturing bone cells. Therefore, PC might be a useful cell scaffold in regenerative bone medicine.

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