Formation of macropores in calcium phosphate cement implants

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A calcium phosphate cement (CPC) was shown to harden at ambient temperatures and form hydroxyapatite as the only end-product. Animal study results showed that CPC resorbed slowly and was replaced by new bone. For some clinical applications, it would be desirable to have macropores built into the CPC implant to obtain a more rapid resorption and concomitant osseointegration of the implant. The present study investigated the feasibility of a new method for producing macropores in CPC. Sucrose granules, $NaHCO_3$, and Na_2HPO_4 were sieved to obtain particle sizes in the range of 125 μm to 250 μm. The following mixtures of CPC powder (an equimolar mixture of tetracalcium phosphate, $Ca_4(PO_4)_2O$, and dicalcium phosphate anhydrous, $CaHPO_4$) and one of the above additive granules were prepared: control–no additive; mixture A–0.25 mass fraction of sucrose; mixture B–0.25 mass fraction of $NaHCO_3$; mixture C–0.25 mass fraction of Na_2HPO_4, and mixture D–0.33 mass fraction of Na_2HPO_4. Cement samples were prepared by mixing 0.3 g of the above mixtures with 0.075 ml of the cement liquid (1 mol/l Na_2HPO_4). After hardening, the specimens were placed in water for 20 h at about 60 °C to completely dissolve the additive crystals. Well-formed macropores in the shapes of the entrapped crystals were observed by scanning electron microscope (SEM). The macroporosities (mean $\pm$ standard deviation; $n = 6$) expressed as volume fraction in % were 0, 18.9 ± 1.7, 26.9 ± 1.6, 38.3 ± 4.4 and 50.3 ± 2.7 for the control, A, B, C and D, respectively. The diametral tensile strengths (mean $\pm$ standard deviation; $n = 3$) expressed in MPa were 10.1 ± 0.7, 3.7 ± 0.3, 2.4 ± 0.2, 1.5 ± 0.5 and 0.4 ± 0.1, respectively, for the five groups. The results showed that macropores can readily be formed in CPC implants with the use of water-soluble crystals. The mechanical strength of CPC decreased with increasing macroporosity.

1. Introduction

Calcium phosphate cements have become a subject of much interest in dental and medical materials research because of their excellent biocompatibility. These cements are self-hardening and form hydroxyapatite (OHAp) or carbonated OHAp as the end product [1–4]. A calcium phosphate cement (CPC) that initially contains an equimolar mixture of tetracalcium phosphate (TTCP) and dicalcium phosphate anhydrous (DCPA) hardens in about 30 min after mixing the powder with water [4]. The cement develops sufficient mechanical strength for repairing hard tissues defects [5–10]. It also has unique *in vivo* properties: slow resorption and replacement by new bone formation with no loss in volume [5, 6, 9].

The cement was found to be highly effective in repair of craniofacial defects where mechanical integrity is important, and a relatively slow resorption and replacement by bone is ideal [11]. However, for certain clinical applications a more rapid resorption and replacement by new bone is desirable. Previous studies have shown that ceramic calcium phosphate implants with macropores (> 100 μm) allowed ingrowth of bone tissue with functional haversian systems and facilitated osseointegration [12–17]. Although the presence of macropores in CPC is not critical to implant resorption and replacement by bone, incorporation of macropores in CPC is likely to promote the process. The present study investigated the feasibility of a new method for producing macropores in CPC implant that may lead to a more rapid resorption and concomitant osseointegration of the implant.

2. Materials and methods

Tetracalcium phosphate (TTCP), $Ca_4(PO_4)_2O$, was prepared by heating a mixture of dicalcium phosphate anhydrous (DCPA), $CaHPO_4$, (Baker Analytical Reagents, J.T. Baker Chemical Co., NJ, USA) and calcium carbonate, $CaCO_3$, (J.T. Baker Chemical Co.) at 1500 °C for 6 h in a furnace, followed by quenching at room temperature in a desiccator. The Ca/P ratio in the

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mixture was 1.9 and the solid phases present in the product, as determined by X-ray diffraction (XRD) analysis, were predominantly TTCP with a trace of α-tricalcium phosphate. The TTCP was first crushed by mortar and pestle, followed by further dry-grinding in a ball mill (Retsch PM4, Brinkman, NY, USA) for 6 min to obtain a median particle size of about 16 μm. Commercial DCPA was ground for 24 h in the ball mill in ethanol (volume fraction of 95%) to obtain a median particle size of about 1 μm. The particle size distributions of the ground TTCP and DCPA were measured in isopropanol by a sedimentation method based on Stokes' law with the use of a centrifugal particle analyzer[1] (SA–CP3, Shimazu, Kyoto, Japan). CPC powder, consisting of an equimolar mixture of TTCP (mass fraction of 72.9%) and DCPA (mass fraction of 27.1%), was prepared by thoroughly mixing the ground TTCP and DCPA in a blender. Sucrose granules (Domino Sugar Corporation, NY, USA) and reagent grade $NaHCO_3$ (Fisher Scientific, NJ, USA) and Na_2HPO_4 (Aldrich Chemical Co. Inc., WI, USA) were sieved to obtain particle sizes in the range of 125 μm to 250 μm. Cement mixtures that contained the CPC powder and up to 0.33 mass fraction of the sucrose, $NaHCO_3$ or Na_2HPO_4 granules were prepared (Table I).

CPC samples were prepared by mixing 0.3 g of the cement mixture and 0.075 ml of cement liquid (1 mol/l Na_2HPO_4) with a spatula for 30 s and placing the paste into a stainless steel mold (6 mm diameter × 3 mm height) with a plunger and a manually applied pressure of 1 MPa to 2 MPa. The samples were stored in a 100% relative humidity box for 4 h at 37 °C and then removed from the molds and placed in distilled water for 20 h at 37 °C. Subsequently, the CPC samples were placed in distilled water for another 24 h at 60 °C to dissolve the additive crystals. The samples were dried and their mass and dimensions were measured for calculating microporosity (MicP) and macroporosity (MacP). The samples were placed again in distilled water for 24 h and the diametral tensile strengths (DTS) of wet samples were determined using a universal testing machine (Instron, United Calibration Corp., Garden Grove, CA, USA) at a displacement rate of 10 mm/min. The set cement products were characterized by XRD (Rigaku, Danvers, MA, USA) to determine the degree of OHAp formation. The estimated standard uncertainty of the 2θ measurement was 0.01° and the minimum mass fraction of a crystal phase to be detected by XRD in the present system was about 3%. The morphology of the set products was examined by scanning electron microscopy (SEM) (JEOL JSM-5300, JEOL USA, Inc., Peabody, MA, USA).

2.1. Calculation of MicP and MacP

Cement samples prepared without the additives contained inherent micropores which are primarily a result of the volume taken up by the cement liquid. The inherent microprosity (I-MicP), expressed as volume fraction in per cent, can be calculated by the equation

$$\text{I-MicP} = ((d_{OHAp} - d_{cpc})/d_{OHAp}) \times 100\% \quad (1)$$

where $d_{OHAp} = 3.14\,\text{g/cm}^3$ is the crystal density of OHAp [18], and d_{cpc} is the density of the dried additive-free sample, which is calculated by the equation

$$d_{cpc} = m_{cpc}/V \quad (2)$$

where m_{cpc} is the mass, $V = \pi r^2 H$ is the volume, and r and H are the radius and height of the dried sample.

Samples that were prepared with an additive can be considered to consist of a CPC phase and macropores formed by dissolution of the additive crystals. The MicP of the CPC phase in the sample should essentially be the same as the I-MicP of the additive-free samples. The overall MicP of the entire sample is lower because the sample contained the macropore phase and, as a result, contained less CPC per unit volume. The MacP of the sample, expressed as volume fraction in per cent, can be calculated by the equation

$$\text{MacP} = (1 - d_{cpc-add}/\text{mean } d_{cpc}) \times 100\% \quad (3)$$

where $d_{cpc-add} = m_{cpc-add}/V$ is the density and $m_{cpc-add}$ is the mass of the water-extracted, dried sample prepared with an additive. The averaged density, mean d_{cpc}, of the additive-free samples calculated from Equation 2 was used in Equation 3. The MicP, expressed in volume fraction in per cent, was estimated by the equation

$$\text{MicP} = (d_{cpc-add}/\text{mean } d_{cpc}) \times \text{mean I-MicP} \quad (4)$$

where mean I-MicP is the average I-MicP of the additive-free samples calculated from Equation 1. Combining Equations 3 and 4 lead to a direct relationship between MicP and MacP as expressed by the equation

$$\text{MicP} = (100 - \text{MacP}) \times \text{mean I-MicP} \quad (5)$$

ANOVA tests and Newman-Keuls multiple comparisons were performed on MicP, MacP and DTS values to

TABLE I Compositions, microporosity (MicP), macroporosity (MacP) and diametral tensile strength (DTS) of the CPCs

Mixture	Composition mass fraction (%)				Density	MicP vol %	MacP vol %	Total porosity vol %	DTS MPa
	CPC	Sucrose	$NaHCO_3$	Na_2HPO_4					
Control	100				1.93(0.01)	38.6(0.5)*	0	38.6(0.5)	10.1(0.7)
A	75	25			1.56(0.03)	31.4(0.6)	18.9(1.7)	50.3(1.0)	3.7(0.3)
B	75		25		1.41(0.03)	28.3(0.6)	26.9(1.6)	55.2(1.0)	2.4(0.2)
C	75			25	1.19(0.09)	23.9(1.6)	38.3(4.4)	62.2(2.7)	1.5(0.5)
D	67			33	0.96(0.06)	19.2(1.0)	50.3(2.7)	69.5(1.7)	0.4(0.1)

*Number in parentheses denote standard deviation ($n = 6$ for MicP and MacP; $n = 3$ for DTS).

determine whether there were significant differences among the groups.

3. Results

The mean MacP value (Table I) of each sample group was significantly different ($p < 0.05$) from the others. MacP produced by a given amount (25%) of additive increased in the order of sucrose $< NaHCO_3 < Na_2HPO_4$. Samples that contained Na_2HPO_4 (mass fraction = 33%) had a higher MacP than samples containing Na_2HPO_4 (mass fraction = 25%). Therefore, the mean MicP values of the various groups were also significantly different ($p < 0.05$) and are inversely proportional to the mean MacP values, as would be expected from Equation 5. The total porosity, i.e. MacP + MicP, increased with increasing additive content. The mean DTS values of the various groups were also significantly different ($p < 0.05$) and are strongly correlated (correlation coefficient = 0.94) with the MacP values; the DTS decreased with increasing MacP.

SEM examinations revealed well-formed macropores in the shapes of the entrapped crystals (Figs 1–4), whereas the CPC implant without additives (control) did not exhibit any macropores (Fig. 5). With higher magnifications, rod and plate-shaped crystalline OHAp can be clearly seen in all specimens except for samples prepared with $NaHCO_3$ where OHAp crystals appear to be much smaller. The SEM also showed that larger OHAp crystals were formed in areas adjacent to the pores.

X-ray patterns showed that OHAp is the only product in the set CPC samples. While no additives were detected, a small amount of unreacted TTCP was found in all of the samples.

4. Discussion

The 1 mol/l Na_2HPO_4 solution used as the cement liquid reduced the setting time from 30 min to 5 min when water was used as the cement liquid [19, 20]. Thus, despite its relatively high solubility (2 g/ml), sucrose was able to produce some macropores because it did not dissolve completely before the cement had hardened. The solubility of the other additives in the phosphate solution is quite limited, and for a given amount of additive (mass fraction = 25%) they produced significantly more macropores than did sucrose (Table I).

X-ray patterns showed that OHAp was the only product formed in the set CPC samples and all of the additives were completely extracted by soaking the

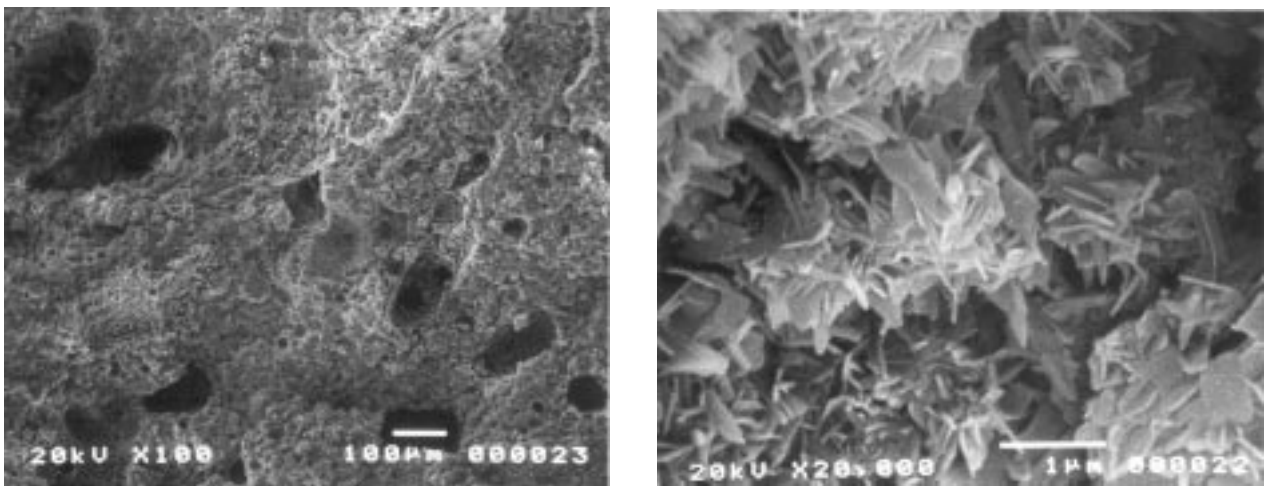

Figure 1 SEM photographs of cement samples prepared from the mixture A (0.25 mass fraction of sucrose). Bar denotes 100 µm in left and 1 µm in right micrographs.

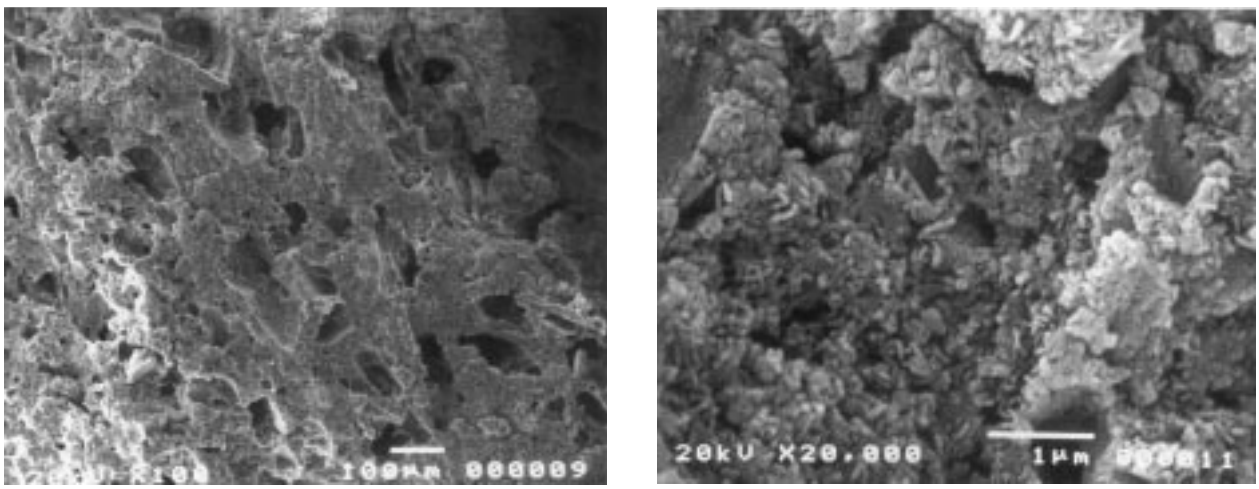

Figure 2 SEM photographs of cement samples prepared from the mixture B (0.25 mass fraction of $NaHCO_3$). Bar denotes 100 µm in left and 1 µm in right micrographs.

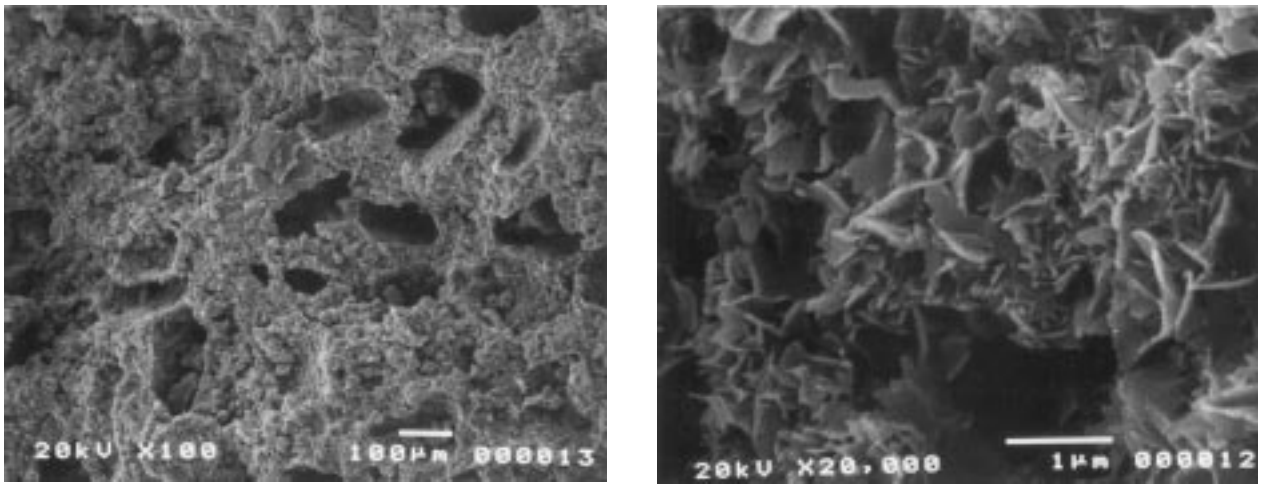

Figure 3 SEM photographs of cement samples prepared from the mixture C (0.25 mass fraction of Na_2HPO_4). Bar denotes 100 µm in left and 1 µm in right micrographs.

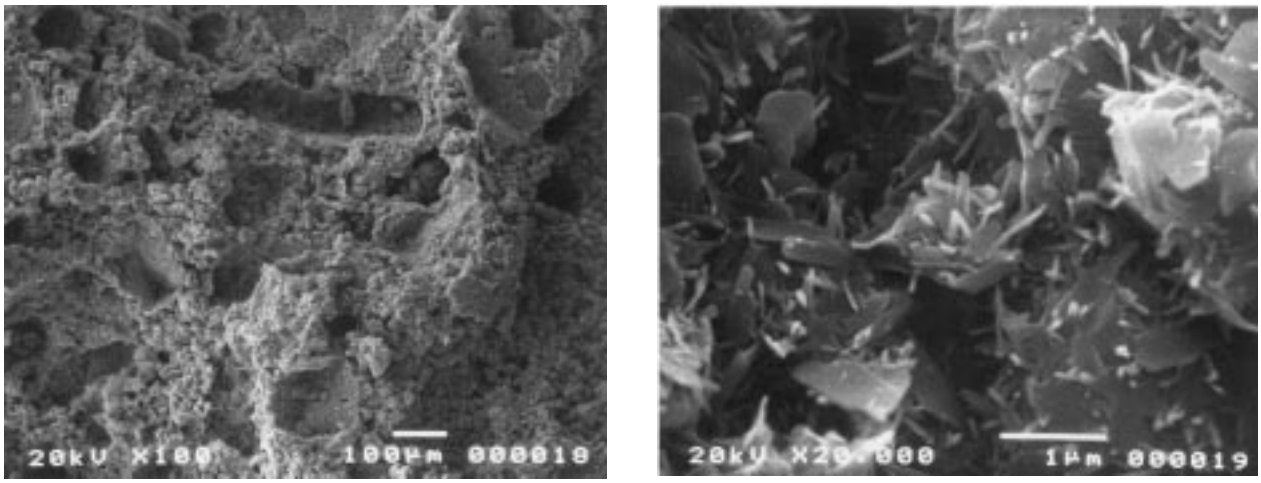

Figure 4 SEM photographs of cement samples prepared from the mixture D (0.33 mass fraction of Na_2HPO_4). Bar denotes 100 µm in left and 1 µm in right micrographs.

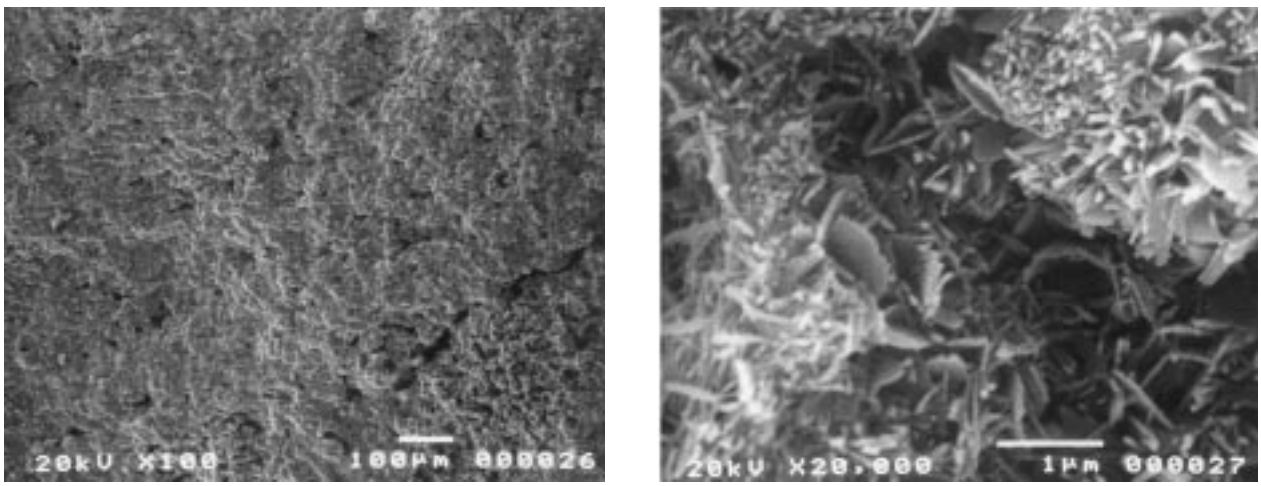

Figure 5 SEM photographs of control cement samples. Bar denotes 100 µm in left and 1 µm in right micrographs.

samples in water for approximately 2 d. The data additionally indicated that none of the additives retarded the OHAp formation in the cement. Therefore, these cements would be expected to have *in vivo* properties similar to those of the control.

The DTS decreased rapidly with increasing macroporosity (Table I) as would be expected. However, the DTS values of 1.5 MPa and 2.4 MPa for CPC samples with 38% and 27% MacP, respectively, are in the range of the DTS of several other calcium phosphate-based cements without macropores [21–25]. Thus the macropore-containing CPCs reported here should be useful for those clinical applications where more rapid resorption rates and remodeling are highly important.

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Notes

1 Certain commercial equipment, instruments, or materials are identified in this paper to foster understanding. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology or the American Dental Association Health Foundation, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

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