Preparation of a bone-like apatite foam cement

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The preparation of a porous bone-like calcium deficient apatite implant material was investigated. A novel cement system composed of an equimolar mixture of $Ca_4(PO_4)_2O$, $Ca(H_2PO_4)_2 \cdot H_2O$, and $CaCO_3$ was used. At a liquid/powder ratio of 0.83 ml/g low density open framework foam cements were formed due to the rapid evolution of CO_2. The initial product of the reactants was $CaHPO_4 \cdot 2H_2O$ which then reacted with $Ca_4(PO_4)_2O$, forming a calcium deficient carbonated apatite, upon soaking of the cement blocks in SBF. Foam-like cements were composed of a plate-like apatite due to epitaxial overgrowth and conversion of the brushite plate precursor. Cylinders of the foam cement were reinforced with an outer layer of a solid apatite cement to form a material suitable for application as a bone-section implant.

1. Introduction

Calcium phosphate cements have generated much interest because of their application as implant materials in medicine. Many formulations have been reported using combinations drawn from dicalcium phosphate dihydrate ($CaHPO_4 \cdot 2H_2O$, DCPD, brushite), tetracalcium phosphate monoxide ($Ca_4(PO_4)_2O$, TCPM), alpha tricalcium phosphate ($Ca_3(PO_4)_2$, αTCP), beta calcium phosphate ($Ca_3(PO_4)_2$βTCP), and monocalcium phosphate monohydrate ($Ca(H_2PO_4)_2 \cdot H_2O$, MCPM) among others. Brown and Chow [1] reported the preparation of an apatite cement formed from TCPM and DCPD, the setting time of this cement was later reduced by the use of sodium phosphate acclerator solution [2]. A brushite cement has been reported for αTCP and MCPM [3] and MCPM and TCPM systems [4]. More recently a nanoporous carbonated apatite cement has been prepared from a mixture of αTCP, MCPM and calcium carbonate ($CaCO_3$) [5].

Calcium deficient apatites are more similar to bone mineral than stoichiometric Hap and have better osteogenic and osteoconductive properties [6]. Here we report the preparation of a novel carbonated calcium deficient apatite implant cement that has an open foam-like structure similar to trabecular bone which may promote osteoconductivity *in vivo*.

2. Material and methods

TCPM was prepared from an equimolar mixture of DCPD (172 g) and $CaCO_3$ (100 g) by first stirring the mixture in acetone for 1 h. The homogeneous suspension was then filtered and air dried. This was followed by heating at 1500 °C for 5 h, at which time it was removed from the furnace and cooled rapidly to room temperature in a desiccated vessel. X-ray diffraction (XRD) analysis showed the presence of a TCPM phase only.

$CaCO_3$ (as calcite) and DCPD was obtained from Kanto Chemical Co. Inc., and MCPM was obtained from Wako Pure Chemical Industries Ltd.

Two cement systems were prepared, firstly a support jacket formed from a known DCPD/TCPM formulation [1, 2] and secondly a foam-like cement core. For foam cement preparations all reagents were handground only for several minutes in a pestle and mortar before use.

For the support cement the TCPM reagent was ball milled for 90 min to reduce the particle size from 13.9 μm, (σ 8.3 μm, $n = 40$) to 3.4 μm, (σ 1.4 μm, $n = 45$). Commercially available DCPD required ball milling for several days before cements that hardened within a reasonable time-scale could be obtained. Therefore DCPD was prepared by adding 25.2 g of MCPM to 250 ml of distilled water in a large beaker, 10.0 g of $CaCO_3$ was then slowly added and the effervescing mixture stirred for 15 min. The rapidly precipitated DCPD was removed by filtration, washed through with acetone and air-dried. Average particle size as measured by scanning electron microscopy (SEM) was 2.1 μm (σ 1.1 μm, $n = 40$).

XRD measurements of all reagents were taken to determine the phase purity, indexing of peaks was carried out by means of measuring known samples together with reference to cards JCPDS card no. 9–347 for MCPM, 25–1137 for TCPM, 9–77 for DCPD, 26–1056 for OCP 46–905 for calcium deficient apatite and 9–432 for hydroxyapatite (Joint Committee on Powder Diffraction Standards). XRD were recorded as thin films using a Philips PW1729 X-ray diffractometer.

Simulated body fluid (SBF) at a pH of 7.4 stored at 37 °C was used for soaking of prepared cements. Compressive strength measurements on cylinders of

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prepared cement (6 replicates) using a loading speed of 0.5 mm min^{-1} were conducted using a Shimadzu Corp., Kyoto, Japan, strength test machine. Carbonate content of the soaked foam cement was determined to $\pm$ 0.01% using a Leco Corporation, Michigan, USA, CS-444LS carbon and sulfur determinator with coupled HF-400 induction furnace. SEM observation of gold coated samples was conducted on a JEOL JSM 5800 LV SEM. FTIR spectra were recorded on a Perkin-Elmer Spectrum 2000 with a diffuse reflectance attachment as 10% in KBr mixtures.

2.1. Support cement

3.66 g of ball milled TCPM was thoroughly mixed with 3.44 g of precipitated DCPD (1 : 2) TCPM/DCPD molar ratio) and 2.4 ml of an accelerator solution composed of 0.25 mol/l Na_2HPO_4 solution pH adjusted to 7.4 with 0.25 mol/l NaH_2PO_4 solution was added (liquid/powder: 0.34 ml/g). The mixture was then mixed by hand for 1 min to form a paste. Hollow cylinders of cement were prepared by packing the paste into the gap between two different sized hollow cylindrical molds placed concentrically. The central mold was removed after 2 h and the remaining hollow cylinder of cement stored at room temperature at 80% humidity for a further 24 h. Final setting times at room temperature and 37 °C were determined using a Gilmore needle test. Cylindrical samples of the cement of 12 mm height and 6 mm diameter formed from packing thin plastic molds were also prepared. Compressive strength test measurements were conducted on demolded samples (6 replicates) after 24 h at room temperature and after 5 days soaking in SBF (pH 7.4) solution at 37 °C.

2.2. Foam cement

Foam-like cements were prepared by thoroughly mixing 2.52 g of MCPM, 3.66 g TCPM and 1.0 g $CaCO_3$. An accelerator solution of 0.15 mol/l Na_2HPO_4 solution pH adjusted to pH 7.4 with 0.15 mol/l NaH_2PO_4 was added at a w/p ratio of 0.83 ml/g (6 ml). The effervescing mixture was then rapidly mixed by hand for 10 s before being lightly packed into the hollow cylinders of the cement prepared previously. The cement block was stored at 37 °C in air for 24 h, before being soaked for 5 days in SBF solution at 37 °C, the XRD being recorded daily. SEM observation was conducted of samples after standing in air at 37 °C for 24 h and after 5 days soaking in SBF at 37 °C. Infra-red spectra of the cement after 5 days soaking were also taken. The pH of the foam cement forming mixture at room temperature was monitored from the start of the reaction up to 18 h, by encasing the probe in cement in an atmosphere at 80% humidity.

Cylinders of 12 mm height and 6 mm diameter were prepared from a more thoroughly stirred mixture, where the gas bubble size was reduced to below 1 mm, being packed into thin plastic molds. The compressive strength was recorded after storage at 37 °C in air for 24 h and after storage in SBF (pH 7.4) at 37 °C for 5 days. The carbonate content of the soaked sample was also determined.

3. Results

3.1. Foam cement

Addition of accelerator solution to the foam cement mixture resulted in an immediate evolution of CO_2 from the mixture, together with a $\sim$ 10 °C warming. After stirring briefly a self-supporting foam was formed which could be easily packed into the hollow cylinders of DCPD/TCPM cement previously prepared. After storage at 37 °C for 24 h a solid foam-like cement that was very firmly attached to the inner surface of the support cylinder was obtained. As a result of the cement setting around CO_2 bubbles many circular holes of up to several millimeters diameter were present throughout the structure.

The pH of the setting cement rose sharply upon mixing then fell back to pH 7.4 on setting (Fig. 1), which required several hours. The compressive strength measurements of foam cylinders were found to be 11 MPa ($\pm$ 3 MPa) rising to a final strength of 15 MPa ($\pm$ 2 MPa) upon SBF soaking.

XRD analysis of the cement after 24 h in air at 37 °C showed the presence of DCPD and TCPM together with HAp and a trace level of OCP ($Ca_8H_2(PO_4)_6 \cdot 5H_2O$, octacalcium phosphate) (Fig. 2b). Upon soaking in SBF the DCPD and TCPM peaks diminished with a concomitant increase in HAp and OCP (Fig. 2c, d). After 3 days only broad HAp and diminished OCP peaks were visible (Fig. 2e). Subsequent soaking resulted in slight sharpening of HAp and further slight diminution of OCP peaks (data not shown). The FTIR spectra of a 5 day soaked sample shows the material is carbonated, a characteristic ν_2CO_3 band at 870 cm^{-1} indicating B-type substitution of carbonate for phosphate ions [7]. Bands at 870, 1135 and the shoulder at 1210 cm^{-1} due to HPO_4 vibrations indicate the material was a calcium deficient apatite [8–10]. The small bands at 910 and 1280 cm^{-1} are probably due the presence of OCP [11] (Fig. 3). Differentiation of precipitated calcium deficient apatite and hydroxyapatite from XRD is not reliable since very minor differences exist.

The average carbonate content of a 5 day SBF soaked sample was 3.3 wt %, SBF soaked cylindrical samples after drying had an average density of 0.69 g/cm^3.

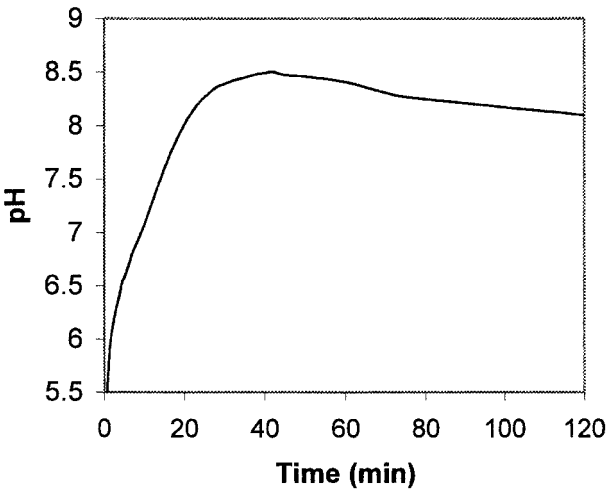


Figure 1 Diagram showing change in pH with time for a foam cement preparation at 20 °C.

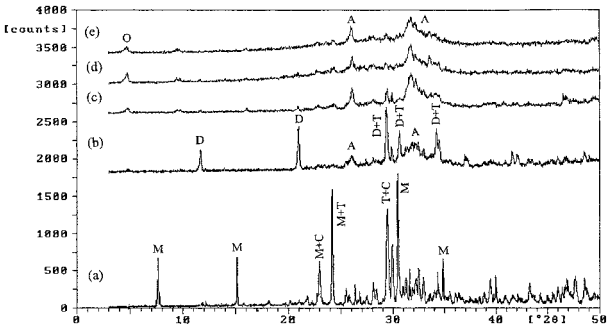


Figure 2 XRD spectra of foam cement reaction sequence, (a) unreacted components, (b) 24 h after mixing, after soaking in SBF at 37 °C for (c) 24 h, (d) 48 h, (e) 72 h. M, MCPM; C, $CaCO_3$; T, TCPM; D, DCPD; O, OCP; A, apatite.

SEM observation of the foam cement before soaking showed the presence of many plates of DCPD of 0.5 to 2 μm in length that covered unreacted TCPM crystals (Fig. 4a). 5 day soaked samples were wholly composed of platelike crystals of similar dimensions to the unsoaked sample, in an interconnected open framework arrangement, TCPM crystals were no longer visible (Fig. 4b).

Samples of foam cement contained in support coat cement, prepared with various dimensions by the use of different sized molds, together with uncoated foam cements are shown in Fig. 5.

3.2. Support cement

DCPD and TCPM at a 2 : 1 molar ratio formed a cement with a final setting time of 19 min at 20 °C and 11 min at 37 °C. XRD measurement of the product after standing for 24 h at room temperature showed the presence of broad HAp peaks together with trace amounts of residual TCPM only. The compressive strength of standard 12 × 6 mm cylinders stored at room temperature was 24 MPa (±4 MPa) rising to 27 MPa (±4 MPa) after 5 days soaking in SBF at 37 °C.

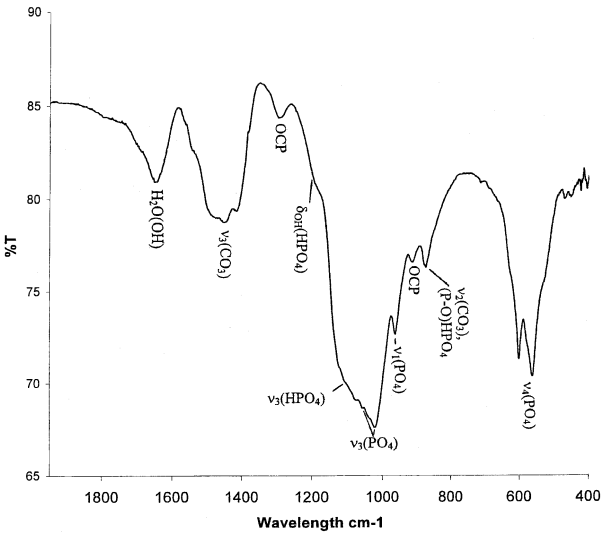
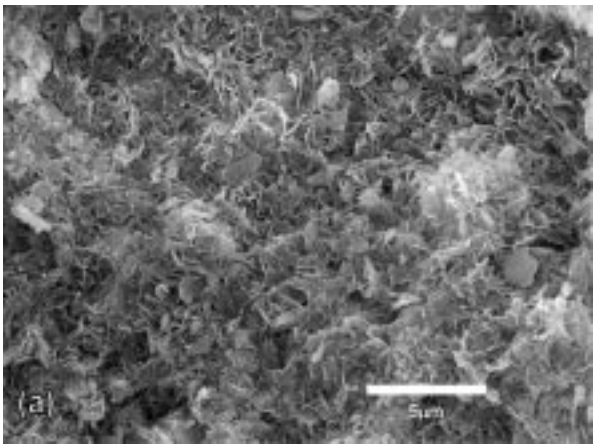


Figure 3 FTIR spectrum of foam cement after soaking in SBF for 5 days. The spectrum is consistent with that of calcium deficient apatite together with a small amount of OCP. Suggested band assignments are shown [8–10].



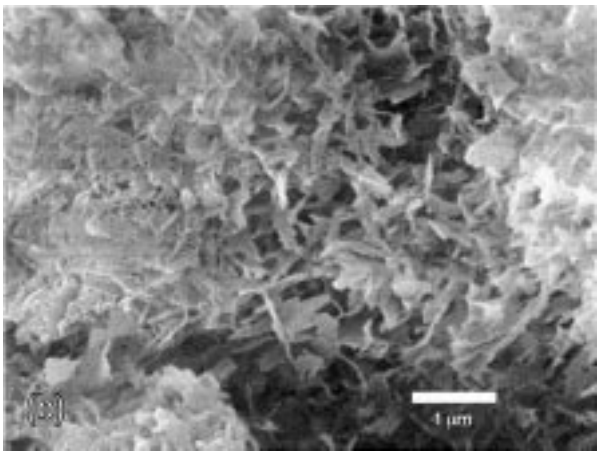


Figure 4 SEM micrographs of a foam cement (a) 24 h after mixing, (b) after 5 days soaking in SBF solution at 37 °C.

4. Discussion

The final compressive strength of the DCPD/TCPM system solid support cement of 27 MPa was within the 9 [12] to 34 MPa [1] range given in previous reports for this cement.

XRD measurements of the foam cement forming system indicate that the initial reaction was between the

Figure 5 Cross-sections of cement/foam cement composites of different sizes and foam cement cores only.

acidic MCPM and basic $CaCO_3$ components, resulting in the formation of DCPD:

$$CaCO_3 + Ca(H_2PO_4)_2 \cdot H_2O \rightarrow 2CaHPO_4 \cdot 2H_2O + CO_2 \quad (1)$$

The rapid evolution of CO_2 resulted in the open framework morphology of the foam preparation (Figs 4, 5). Changes in the pH of the cement mixture and fall off in the degree of effervescence suggest that this initial reaction occurred largely within the first 10 min of mixing. The particle sizes of the reagents may also be significant in the formation of a stable foam structure, since foams prepared with ball milled compounds were found to collapse before setting. Reaction between MCPM and TCPM may be minimal due to the relatively large size of the TCPM crystals ($\sim$ 13.9 μm), however this reaction also kinetically favors the formation of DCPD initially [13, 14]

$$Ca_4(PO_4)_2O + 2Ca(H_2PO_4)_2 \cdot H_2O + 9H_2O \rightarrow 6CaHPO_4 \cdot 2H_2O \quad (2)$$

pH measurements of a freshly prepared foam cement paste showed the mixture was initially at low pH due to the fast dissolving acidic MCPM component. The pH increased rapidly as the MCPM was consumed in its reaction with $CaCO_3$, and continued to rise to a maximum of 8.5 as the basic TCPM reached an equilibrium with the freshly precipitated DCPD. The pH then slowly fell to near neutral as the TCPM and DCPD reacted to form apatite.

The formation of HAp or calcium deficient apatite from the separate hydrolysis of DCPD or TCPM is kinetically very slow [15]. When DCPD and TCPM are both present in solution the rate limiting step for the reaction is thought to be associated with the dissolution rate of DCPD [16] since TCPM is more soluble than DCPD below pH 8.5 [15]. The foam cement had an open framework structure allowing extensive diffusion of solution throughout the cement, which allowed the components to react readily resulting in a calcium deficient apatite (Ca/P = 1.5), as shown in Equation 3.

$$3CaHPO_4 2H_2O + 1.5Ca_4(PO_4)_2O \rightarrow Ca_9(HPO_4)(PO_4)_5OH + 6.5H_2O \quad (3)$$

The overall approximate $CaHPO_4 : Ca_4(PO_4)_2O$ of 2 : 1 ratio allowed this reaction to be more rapid than if equimolar quantities were involved [17]. The presence of low levels of OCP at all stages was detected by XRD measurements, which may indicate that the DCPD to apatite reaction proceeded via an OCP phase.

Some degree of control over the pore size could be achieved by alteration of the amount of stirring prior to packing the foam into molds, a longer stirring producing a lower average bubble size.

Completely compacted cements using a lower solution/ powder ratio produced a solid cement with a final compressive strength of 17 MPa ($\pm$4MPa). This was in part due to the continued slow evolution of CO_2 during setting, resulting in small fissures that weakened the cement [18]. To allow a high stress application a known DCPD/TCPM cement formulation of higher strength was therefore employed for the solid support coating. In principle however, many existing cement formulations with various properties could be used, depending on the nature of the application.

Interestingly, the foam-like cement preparations stored at 37 °C exhibited the phenomenon of pseudomorphy in that plate-like apatite was formed of the same morphology of the DCPD precursor crystals. The results suggest that at this temperature the TCPM and DCPD components were consumed as apatite formed on the DCPD surface eventually largely replacing it, whilst retaining the original morphology of the plate [16]. Apatite nucleation is favored on the DCPD rather than TCPM crystals due to the higher local supersaturation around the plates [19, 20]. This epitaxial apatite growth process was completed within 2–3 days of SBF soaking at 37 °C (Fig. 2). There also may be some fusing of plates in contact as the compressive strength was found to rise on soaking.

Bone also consists of apatite plates, however, the overall dimensions of the cement plates produced here are considerablely larger than the $\sim$ 45 nm long plates present in bone [21]. The broad peaks obtained from the XRD measurements indicate that the foam cement plates are not single crystals but are composed of much smaller crystallites, formed during the gradual epitaxial growth process.

5. Conclusions

The inorganic component of bone is known to consist of a plate like calcium deficient carbonated HAp. Implant cements that mimic this composition should be advantageous, the degree of porosity is also an important factor in promoting bone ingrowth. The high porosity of the foam cement structure suggests the structure should be osteoconductive and the bone-like HAp composition, together with the presence of OCP, should allow the material to show good biological resorption properties. An outer coat of solid cement is used to reinforce the low-density core, to form a bone section implant material than can be shaped as required.

Acknowledgments

We thank the Japan International Science and Technology Exchange Center for financial support for this study and Mr Y. Suetsugu for assistance with carbonate determination.

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Received 13 April
and accepted 14 December 1999