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Cements from nanocrystalline hydroxyapatite

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Calcium phosphate cements are used as bone substitute materials because they may be moulded to fill a void or defect in bone and are osteoconductive. Although apatite cements are stronger than brushite cements, they are potentially less resorbable *in vivo*. Brushite cements are three-component systems whereby phosphate ions and water react with a soluble calcium phosphate to form brushite ($CaHPO_4 \cdot 2H_2O$). Previously reported brushite cement formulations set following the mixture of a calcium phosphate, such as β-tricalcium phosphate (β-TCP), with an acidic component such as H_3PO_4 or monocalcium phosphate monohydrate (MCPM). Due to its low solubility, hydroxyapatite (HA) is yet to be reported as a reactive component in calcium phosphate cement systems. Here we report a new cement system setting to form a matrix consisting predominantly of brushite following the mixture of phosphoric acid with nanocrystalline HA. As a result of the relative ease with which ionic substitutions may be made in apatite this route may offer a novel way to control cement composition or setting characteristics. Since kinetic solubility is dependent on particle size and precipitation temperature is known to affect precipitated HA crystal size, the phase composition and mechanical properties of cements made from HA precipitated at temperatures between 4 and 60 °C were investigated.

1. Introduction

There has been great interest in calcium phosphate cements as bone substitute materials [1–4] because they can be moulded to fill a void or defect and are osteoconductive [5–7]. In 1985, Brown and Chow reported [8–10] a calcium phosphate paste that set and hardened to form a cement at ambient temperature. This cement was composed of mostly apatite, which can have a low rate of resorption *in vivo* [4, 11, 12]. Shortly after 1987 Lemaître *et al.* [13] reported another calcium phosphate cement system whereby cement consisted of predominantly brushite. Brushite cements are thought to be more soluble than apatite cements in physiological conditions and hence resorbable *in vivo* [4, 11, 14].

Brushite cements are three-component systems whereby phosphate ions and water react with a calcium phosphate to form brushite ($CaHPO_4 \cdot 2H_2O$). In previous reports the calcium phosphate component has been β-tricalcium phosphate (β-TCP) reacted with monocalcium phosphate monohydrate (MCPM), Equation 1, or simply phosphoric acid solution, Equation 2 [15, 16].

$$\beta\text{-}\ Ca_3(PO_4)_2 + Ca(H_2PO_4)_2 \cdot H_2O + 7H_2O \rightarrow 4CaHPO_4 \cdot 2H_2O \qquad (1)$$

$$\beta\text{-}\ Ca_3(PO_4)_2 + H_3PO_4 + 6H_2O \rightarrow 3CaHPO_4 \cdot 2H_2O \qquad (2)$$

Hydroxyapatite (HA) has not previously been reported as a brushite cement reactant. HA is the least soluble calcium orthophosphate at physiological conditions. However, at pH 4.2 in the system $Ca(OH)_2$–H_3PO_4–H_2O at 25 °C it is as soluble as brushite [10, 14, 17]. Depending on reaction conditions, it might be expected that cements formed from HA could be biphasic; consisting of unreacted HA in a brushite matrix. Factors that would affect extent of reaction are relative concentration of reactants, temperature and the particle size of solid reactants [18]. Greater kinetic solubility may be achieved by increasing HA surface area. Surface area may be increased by reducing the particle size of solid reactants by milling/grinding or altering precipitation conditions. Precipitated HA has a

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greater specific surface area than HA produced by thermal treatment. Furthermore, precipitation temperature is well known to affect the degree of supersaturation and hence crystal size [19].

We report the effect of HA precipitation temperature on composition and mechanical properties of cements formed with 3 M phosphoric acid solution. Mixing precipitated HA and phosphoric acid produced cements predominantly composed of brushite.

2. Methods and materials

Nanocrystalline HA was precipitated following Jarcho *et al.* [20]. Briefly, 1.4 mol of calcium nitrate ($Ca(NO_3)_2$) (Sigma) were dissolved 900 ml of double distilled water (DDW). The pH of this solution was brought between 11 and 12 by adding concentrated aqueous ammonia (NH_4OH) (Fisher) and the solution diluted to 1.8 l. Diammonium phosphate solution was prepared by adding 1 mol (Sigma) to 1.5 l of DDW. Concentrated aqueous ammonia was added to this solution to bring pH between 11 and 12. The solution was diluted to 3.2 l. The calcium nitrate solution was vigorously stirred and temperature was maintained at 4, 20 or 60 °C and the ammonium phosphate solution added drop wise over 30–40 min. The reaction mixture was stirred overnight ($>$ 12 h) and then centrifuged (2000 rpm for 10 min) and the supernant decanted and the precipitate was re-suspended in DDW. This washing procedure was repeated twice more. Centrifuged precipitate was dried at 75 °C, ground with a pestle and mortar and sieved to a size of $<$ 300 μm in diameter. The calcium phosphate ratio (Ca/P) of precipitated HA was calculated using chemical titration and photometry. Dried HA precipitate (18 mg) was dissolved in 1 ml of 1 M hydrochloric acid solution (Sigma). The amount of calcium present was determined by titrating the dissolved HA solution against a 5 mM solution of ethylenediaminetetraacetic acid. The phosphate in dissolved HA solution was complexed with molybdate–vanadate reagent and its quantity calculated photometrically at 400 nm.

Cements were formed with precipitated HA and 3 M phosphoric acid (Sigma) containing 50 mM sodium citrate (Sigma) solution. Cements were mixed at a powder to liquid ratio of 1.5 g/ml. X-ray diffraction patterns of HA precipitates and set cements were recorded from $2\theta = 20$–$40°$ with a step size of 0.02° and a count time of 5–10 s/step (D5005 Siemens, Karlsruhe, Germany). The phase composition was determined by means of JCPDS reference patterns for β-TCP, HA and brushite. Crystal sizes and quantitative phase compositions of materials were calculated by means of total Rietveld refinement analyses with TOPAS software (Bruker AXS). As references, the system internal database structures of β-TCP, HA and brushite were used together with a Chebychev fourth order background model and a Cu Kα emission profile.

Scanning electron microscopy (SEM) was carried out with a JEOL JSM 6300 at an accelerating voltage of 20 kV. Transmission electron microscopy (TEM) was performed with a JEOL JEM 4000FX. Precipitates were suspended in 100% ethanol. Once precipitates had been suspended, 500 μl were transferred to a carbon-coated grid and suspended precipitates stored in ambient conditions to allow ethanol to evaporate leaving an even distribution of dried precipitate over the carbon-coated grid surface. TEM was performed at an accelerating voltage of 80 kV. Initial and final setting times were measured using the Gilmore needle technique [21] at ambient conditions. The time elapsing between cement mixing and no visible impression being made on the cement surface by the needles was recorded. Density of cement products was measured using helium pycnometry (Accupyc 1330, Micromeritics). Relative density was calculated by dividing the apparent density, as determined from geometry and mass of cements, by measured density.

Cylindrical cement specimens were formed with a diameter of 6 mm and a height of 12 mm. These cements were stored under ambient conditions for 24 h prior to testing. Cements were loaded under compression until failure (Instron 5544) at a crosshead speed of 1 mm/min, with a 2 kN load cell, to determine compressive strength.

3. Results

Total Rietveld refinement analysis of XRD data revealed that the precipitated calcium phosphate was low crystallinity phase pure HA with no other calcium phosphate phases detectable (Table I). The crystals of this HA varied in size as precipitation temperature was altered. HA precipitated at lower temperature had smaller crystals; HA precipitated at 4 °C had a mean crystal size 34.8 (± 6.1) nm whilst HA precipitated at 60 °C had a mean size around double the size, 60.8 (± 5.9) nm. As determined from XRD data cement formed with precipitated HA was made up of a combination of predominantly brushite and unreacted HA. HA remaining in the cement had crystal sizes of approximately half the size observed in the HA precipitate, for example, HA precipitated at 20 °C had crystals 44.2 (± 3.9) nm whilst unreacted HA in the cement was 16.6 (± 1.5) nm.

TABLE I Phase composition of precipitated HA and cements formed from precipitated HA and phosphoric acid solution

HA precipitation temperature (°C)	Calcium phosphate precipitate		Cement			
	wt % HA	HA crystal size (nm)	wt % HA	HA crystal size (nm)	wt % brushite	Brushite crystal size (nm)
4	100	34.8 (± 6.1)	31	14.1 (± 1.7)	69	195 (± 20)
20	100	44.2 (± 3.9)	33	16.6 (± 1.5)	67	121 (± 9)
60	100	60.8 (± 5.9)	30	27.3 (± 2.5)	70	170 (± 18)

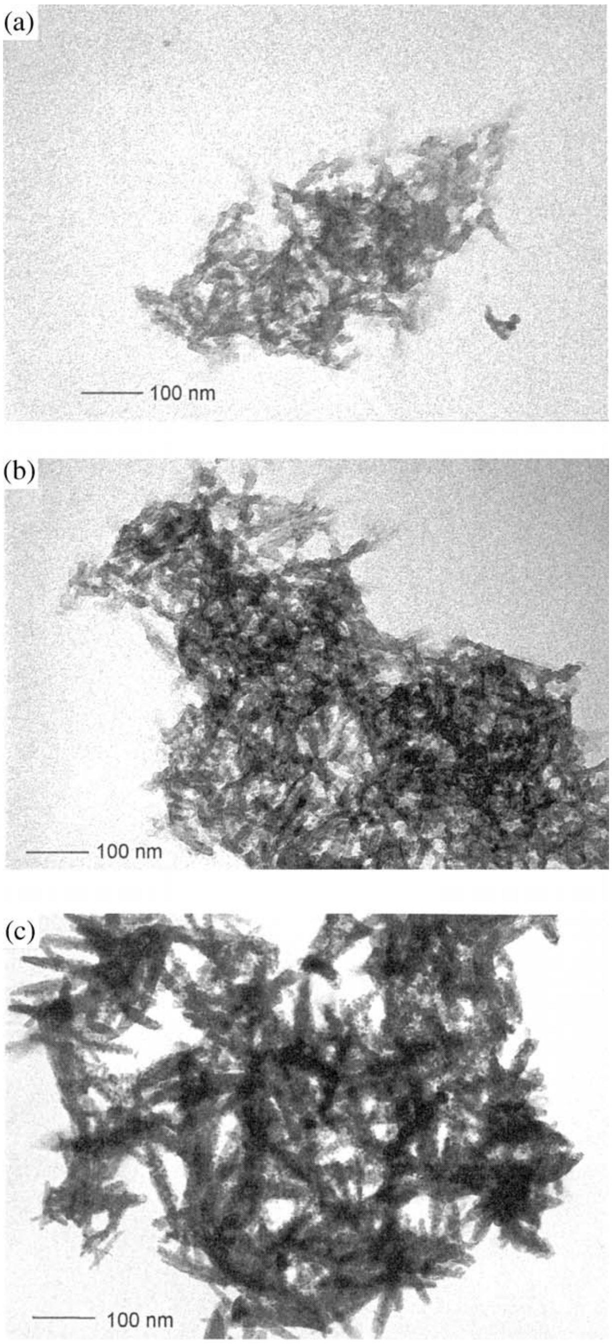

Figure 1 Transmission electron micrograph of HA precipitated at (a) 4 °C, (b) 20 °C and (c) 60 °C, at a magnification of 100 K.

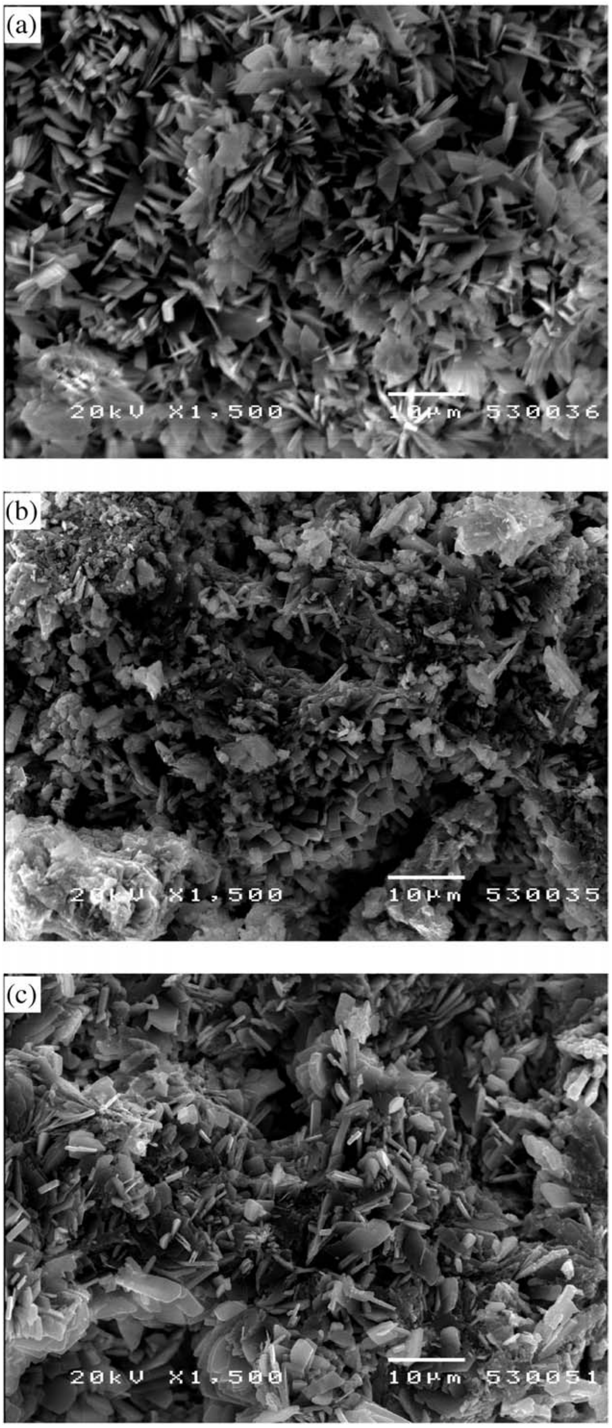

Figure 2 Scanning electron micrograph of the fracture surface of cement formed with 3 M phosphoric acid 50 mM sodium citrate and HA precipitated at (a) 4 °C, (b) 20 °C and (c) 60 °C.

Brushite crystals in cement ranged between 110 and 220 nm in size and varied with precipitation temperature of HA cement component. Cement formed from HA precipitated at 20 °C had the smallest crystals, 121.4 (± 8.9) nm. All precipitates were found to be calcium deficient, with Ca/P ratio of approximately 1.5, stoichiometric HA has a Ca/P ratio of 1.67 [18]. Calcium deficiency decreased as precipitation temperature increased such that when precipitated at 4, 20 and 60 °C, Ca/P ratio was 1.48, 1.53 and 1.55, respectively.

The TEM analysis confirmed crystal size determined from XRD data and revealed that nanocrystalline HA precipitated at 4 °C appeared to be comprised of small (20–30 nm, aspect ratio 1–1.5) spheroidal HA crystals whilst cements precipitated at higher temperatures were larger rod-like crystals (Fig. 1). The HA precipitated at 20 °C had crystals of approximately 45–70 nm in length (aspect ratio 2–2.5) whereas HA precipitated at 60 °C were less-rounded needles of higher aspect ratio, 4.5–5.5, approximately 100–150 nm in length. Blade-like crystals thought to be brushite could be observed using SEM on fracture surfaces of cement. There appeared to be little difference in the microstructure of cements formed from nanocrystalline HA precipitated at 4, 20 and 60 °C when observed using SEM (Fig. 2).

Varying HA precipitation temperature altered the setting time of cement. Cement produced by mixing 3 M phosphoric acid solution with HA precipitated at 4 °C had an initial setting time of 3.5 (± 0.3) min whilst cements formed with HA precipitated at 20 °C had initial setting times of 1.0 (± 0.0) min, cement formed from HA

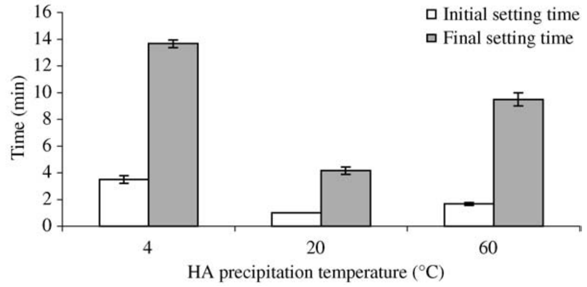

Figure 3 Effect of HA precipitation temperature on initial and final setting times of cement.

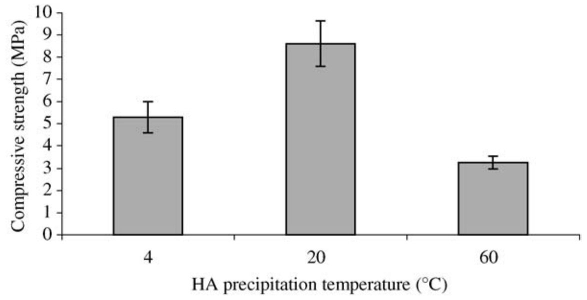

Figure 4 Effect of HA precipitation temperature on mean ultimate compressive strength.

precipitated at 60 °C had an initial setting time of 1.7 (± 0.1) min. Final setting times of cement produced with HA and 3 M phosphoric acid solution also varied with HA precipitation temperature; cement formed from HA precipitated at 20 °C setting the fastest and cement formed from HA precipitated at 4 °C setting the slowest (Fig. 3). Brushite crystal size and setting time appeared to be related, HA precipitated at 4 °C formed cement with the largest crystal size whilst cement formed from HA precipitated at 20 °C had the smallest crystal sizes (Table I, Fig. 3).

Cement formed with a 3 M solution of phosphoric acid and HA had relative densities between 50% and 55%. Cements formed with HA precipitated at 4 °C appeared to be less dense (50%) than cement formed with HA precipitated at 20 °C (54%) or 60 °C (55%).

Cement formed from HA precipitated at 20 °C had the greatest compressive strength of all cement tested. This cement had a mean compressive strength of 8.6 (± 1.0) MPa, whilst cement formed from HA precipitated at 4 °C had a compressive strength of 5.3 (± 0.7) MPa and cement formed from HA precipitated at 60 °C had a strength of 3.3 (± 0.3) MPa (Fig. 4).

4. Discussion

HA is the least soluble calcium phosphate at physiological pH [10, 14, 22] and it is only currently used as a setting accelerator in current cement systems [23, 24]. In this study the precipitates were nanocrystalline calcium-deficient HA (CDHA), as demonstrated using TEM, XRD and Ca/P ratio determinations. When precipitated CDHA was mixed with phosphoric acid it dissolved and reprecipitated as brushite (dicalcium phosphate dihydrate, DCPD) to form cement. The setting reaction may have proceeded as indicated in Equation 3. However, the reaction did not proceed to completion since unreacted partially dissolved CDHA (as evidenced by a decrease in crystal size) was found in the final structure of cement produced with precipitated CDHA.

$$\begin{aligned}&Ca_9(PO_4)_5(HPO_4)OH + 3H_3PO_4 + 17H_2O\\&\quad\rightarrow 9CaHPO_4\cdot 2H_2O\end{aligned}\tag{3}$$

Precipitated nanocrystalline HA is often one or more orders of magnitude smaller than HA obtained by thermal treatment [4, 11, 22], which is often microcrystalline. The smaller crystal size of nanocrystalline HA results in a large specific surface area which in turn increases kinetic solubility. An increase in precipitate crystal size and a more rod-like morphology were noted from TEM micrographs as precipitation temperature increased. It is, therefore, of interest that setting times were not related to crystal size, since the smallest and largest crystals took the longest to set (Fig. 3). Interestingly, cements formed with CDHA precipitated at 20 °C had a quicker setting time but higher strength than cements formed with material precipitated at either 4 °C or 60 °C. Nonstoichiometric HA contains vacancies on calcium and hydroxyl sites resulting in nonstoichiometric HA having different solubility products to stoichiometric HA [22]. However, the stoichiometry alone could explain neither the rapid setting time nor the greater strength. The cement formed from CDHA precipitated at 4 °C was more porous than other test cements and this may have been the cause of the low strength. Another possible explanation for the apparent lack of correlation between particle size and cement characteristics may have been that the 4 °C precipitate may have reacted too fast initially, such that the CDHA reactant became coated with an insoluble layer of cement matrix almost immediately after setting, thus setting time was increased. Similar deterioration in mechanical properties with increased reactivity has been reported in another system [27]. It is possible that optimisation of this system could be effected by appropriate secondary particle size control and or the use of retardants.

The ability to decrease the acidity of brushite cement mixes is considered desirable to reduce any local irritation during setting, however, the acidic phosphate solution is necessary to reduce the Ca/P ratio of the solid component (normally βTCP, Ca/P ratio 1.5) to 1 (the Ca/P ratio of brushite). Clearly nanocrystalline CDHAs or other nanoparticulate calcium phosphates may offer a route to achieving this since they can be prepared with Ca/P ratios as low as 1.3 [18].

5. Conclusion

The use of nanocrystalline HA as a reactant offers a novel route to the production of brushite cements. Mixing HA with phosphoric acid solution in this study resulted in the formation of cement composed of primarily brushite. Altering the temperature at which the HA component of cement is precipitated may act as a means to manipulate cement characteristics and properties, as shown in this study. Further investigation is needed to understand the difference in the behaviour of nanocrystalline HA and microcrystalline HA when used as cement reactants.

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