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Cement from nanocrystalline hydroxyapatite: Effect of calcium phosphate ratio

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Nanocrystalline hydroxyapatite (nHA) can be mixed with phosphoric acid to form a brushite cement; a degradable inorganic bone filling material. nHA was precipitated from reactants of calcium to phosphate (Ca/P) ratio 0.8 to 2.0 and mixed with phosphoric acid, which resulted in the formation of a brushite cement. Cement was also formed by mixing microcrystalline calcium phosphates, β-tricalcium phosphate, hydroxyapatite and tetracalcium phosphate with phosphoric acid solution. Cement produced with nHA was stronger in compression than that formed with crystalline calcium phosphate phases. Setting time, strength and composition of cement produced with nHA was dependant on both the Ca/P ratio of nHA and the concentration of phosphoric acid in cement slurry. Increasing phosphoric acid concentration increased compressive strength whilst reducing the initial setting time of cement. Reducing the Ca/P ratio of nHA precipitation reactants retarded the setting and increased the extent of reaction of cements. This finding was unexpected and suggests that Ca/P ratio may strongly affect dissolution behaviour and this parameter is more important than stoichiometry in determining extent of reaction in this system. This study demonstrated that the wide variation in stoichiometry that may be attained in nanocrystalline apatite may be utilised to change cement performance and setting behaviour.

1. Introduction

Calcium phosphate cement (CPC) may be used to fill defects in bone that have occurred as a consequence of disease or trauma. CPCs are used clinically to fill non-load bearing defects in the cranio and maxillofacial region [1–3]. CPCs are mouldable and injectable and therefore may be more versatile in handling characteristics than prefabricated calcium phosphate granules or blocks [4–6]. Furthermore they are osteoconductive, allowing for ingrowth of new bone and integration of cement with surrounding tissues [7]. CPCs are produced from the setting of cement slurry comprised of calcium phosphate and an aqueous liquid [8, 9].

The formation of apatitic cement by the mixture of tetracalcium phosphate ($Ca_4(PO_4)_2O$) and dicalcium phosphate dihydrate (DCPD, $CaHPO_4{\cdot}2H_2O$) was reported by Brown and Chow in the mid 1980's [8–10]. The disadvantage of apatitic cements is that they dissolve at a slow rate in physiological conditions [11, 12] and are therefore resorbed mainly due to osteoclastic activity *in vivo*. A rate of resorption that is lower than the rate at which new bone is deposited may hinder the biological performance of a graft. In 1987 Lemaître *et al.* [13] reported the formation of cement consisting of predominantly brushite (DCPD). Brushite cements are more soluble than apatite cements in physiological conditions [14, 15] and may be resorbed due to dissolution of cement; therefore brushite cement may be resorbed at a greater rate than apatitic cement *in vivo*.

Brushite cement has been produced by the mixture of β-tricalcium phosphate (β-TCP), water and either monocalcium phosphate monohydrate or phosphoric acid [5, 11, 12]. However, more recently, we have reported the formation of brushite cement by the mixture

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of nanocrystalline hydroxyapatite (nHA) and phosphoric acid [16–18], Equation 1.

$$Ca_9(PO_4)_5(HPO_4)OH + 3H_3PO_4 + 17H_2O \rightarrow 9CaHPO_4 \cdot 2H_2O \quad (1)$$

Hydroxyapatite has only been investigated as a seed to accelerate cement setting previously [19–21]. However, nHA has a higher specific surface area than that of hydroxyapatite produced by thermal processes due to smaller crystal size and therefore has greater kinetic solubility. nHA dissolves in phosphoric acid to reprecipitated as brushite crystals that make up cement [16]. It has been reported that nHA may be precipitated at a calcium to phosphate (Ca/P) ratio as low as 1.3, which is closer to that of brushite (Ca/P ratio = 1) than β-TCP (Ca/P = 1.5) thus fewer phosphate ions are required to form brushite from nHA than β-TCP [9, 15]. If fewer phosphate ions are required in the setting reaction of cement then a more dilute phosphoric acid solution may be used as a cement reactant. In the current study cements were produced from nHA of various Ca/P ratios to determine the effect of stoichiometry on cement strength and setting time. Brushite forming cements were also made from microcrystalline calcium phosphates to further investigate stoichiometry and examine the effect of crystallinity of the reactants.

2. Methods and materials

Nanocrystalline hydroxyapatite (HA) was precipitated following the methods of Jarcho *et al.* [22]. Briefly, a 300 mM solution of diammonium phosphate ($(NH_4)_2HPO_4$) (Sigma, UK) was added, drop wise, to a calcium nitrate ($Ca(NO_3)_2{\cdot}4H_2O$) (Sigma, UK) solution at concentrations that delivered a Ca/P ratio of 0.8 to 2.0, under constant stirring at 25 °C [16, 17]. Both solutions were adjusted to a pH of 11 with concentrated aqueous ammonia (NH_4OH) (Fisher, UK) prior to mixing. The reaction mixtures were stirred overnight and then centrifuged (2000 rpm for 10 min), the supernatant decanted and the precipitate resuspended in double distilled water. This washing process was repeated twice more. Centrifuged precipitate was dried at 75 °C and ground with pestle and mortar and sieved to a size of $<300\,\mu$m. β-TCP (Plasma Biotal, UK) was used as a calcium phosphate of Ca/P ratio 1.50, hydroxyapatite (Plasma Biotal, UK) was used as Ca/P ratio 1.67 and tetracalcium phosphate (TTCP) used as Ca/P ratio 2. TTCP was prepared by sintering an equimolar mixture of DCPA (Baker) and calcium carbonate (Baker) at 1500 °C for 18 h followed by quenching in air. The sintered cake was crushed with pestle and mortar until it passed through a 355 μm sieve.

Cement was formed by mixing calcium phosphate powder with 2 or 3 M phosphoric acid solutions (Sigma, UK). Cements were mixed at a powder to liquid ratio of 1.5 g/ml. X-ray diffraction (XRD) patterns of HA precipitate and set cements were recorded from $2\theta = 10$–$100°$ with a step size of 0.02° and a count time of 12 s/step (Philips PW1070 diffractometer) with Ni filtered CuK_α radiation ($K_{\alpha 1} = 1.5406$ Å, $K_{\alpha 2} = 1.5444$ Å) at 40 kV and 30 mA. Rietveld refinement phase analyses of the precipitates and set cements were performed using TOPAS software. Setting time was determined using the Gillmore needle technique [23]. Phosphorus was determined by dissolving 100 mg of the powders in diluted HNO_3 (1 M) and measuring photometrically after complexation with ammonium molybdate by means of UV-Vis spectroscopy. Calcium content was determined with a calcium sensitive electrode against standard solutions of $CaCl_2$.

Cement cylinders of 6 mm diameter and 12 mm height were formed in split moulds. After 24 h storage in ambient conditions these cement cylinders were loaded in compression until failure (Instron 5544, UK) at a crosshead speed of 1 mm/min, with a 2 kN load cell, to determine compressive strength. Density of cement products was measured using helium pycnometry (Accupyc 1330, Micromeritics). Relative density was calculated by dividing the apparent density, as determined from the geometry and mass of cements, by measured density. In order to detect unreacted acid pH of 3 ml of double distilled water in which 1 g of cement, ground with a pestle and mortar, was stored for 12 h was measured for cements made from nHA of varying stoichiometry.

3. Results

All precipitated calcium phosphates were predominantly composed of nHA, as determined using Rietveld refinement phase analysis of XRD data, Table I, the remainder being brushite. Crystallite size determination showed that the apatite was nanocrystalline (10–15 nm). The weight percent nHA in precipitate increased as the Ca/P ratio of precipitation reactants was increased. Measured Ca/P ratio demonstrated that all nHA precipitates were calcium deficient, ranging from 1.27 to 1.60, Table I. XRD with Rietveld refinement phase analysis confirmed that cement was composed of brushite, increasing the concentration of acid in the cement slurry (from 2 to 3 M phosphoric acid) resulted in a higher extent of reaction at higher Ca/P ratios (>1.48), Table II. XRD was also used to determine that cements produced with microcrystalline components β-TCP and HA were composed of brushite product and unreacted component, however cement produced with TTCP consisted of unreacted TTCP and a small amount of monetite, Fig. 1. While unreacted HA could be detected in the sample made from crystalline HA,

TABLE I Composition of calcium phosphate precipitates from which cements were prepared

Ca/P ratio of precipitation reactants	Wt % Hydroxyapatite in crystalline phase	Hydroxyapatite crystal size (nm)	Measured Ca/P ratio
0.80	89.7	10.1 ± 0.2	1.27 ± 0.01
1.00	92.8	9.9 ± 0.2	1.42 ± 0.01
1.33	91.2	10.4 ± 0.2	1.48 ± 0.01
1.40	95.3	14.8 ± 0.3	1.55 ± 0.01
1.67	94.7	11.9 ± 0.2	1.48 ± 0.01
2.00	93.7	10.1 ± 0.2	1. 60 ± 0.01

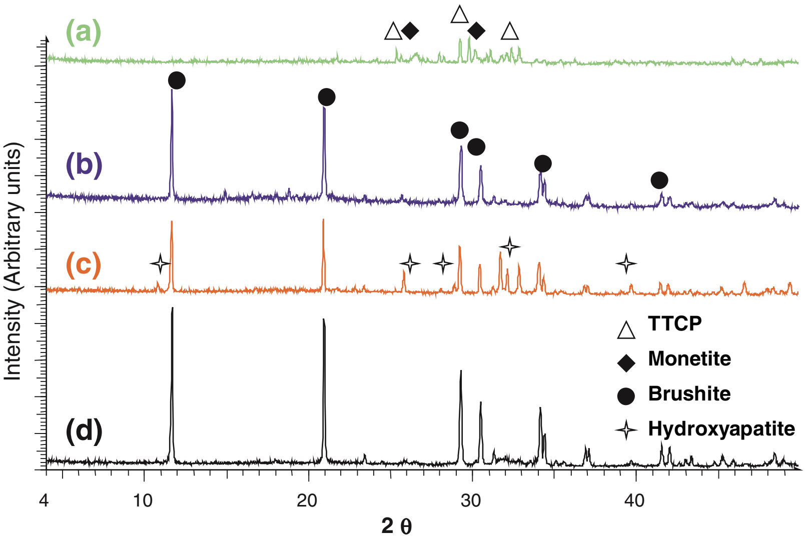

Figure 1 XRD spectra obtained when cement formed from 3 M phosphoric acid and (a) TTCP, (b) β-TCP, (c) crystalline HA and (d) nHA (Ca/P 1.55) were examined.

only minor traces could be observed in cement made from nHA, (Ca/P = 1.55).

Increasing both the Ca/P ratio of nHA precipitation reactants and the concentration of phosphoric acid in cement slurry resulted in a decrease in the final setting time of cement, Fig. 2(a). Increasing acid concentration also resulted in a decrease in setting time when microcrystalline calcium phosphates were investigated, Fig. 2(b). Cement formed with TTCP set within 0.5 mins, Fig. 2(b). Cement formed with nHA had similar setting times to that of cement formed with calcium phosphates except cement formed with HA that had a much longer final setting time than that of any other cement, Fig. 2(a) and (b), and TTCP reacted vigorously when cement components were mixed, producing noticeable heat and set within 0.5 mins.

Cement produced from nHA with a Ca/P ratio of 1.55 was the strongest in compression, Fig. 3. Increasing the concentration of acid in the liquid phase of cement resulted in increased strength of all cement produced from nHA, Fig. 3. Cement produced from nHA with a Ca/P ratio of 1.27 and 2 M phosphoric had a mean strength of less than 1 MPa, and with 3 M acid 5 MPa. Cement produced from nHA was equal or stronger in compression than cement formed with microcrystalline calcium phosphate phases, Fig. 3. The lowest strength cements were formed from microcrystalline HA.

TABLE II Composition of cement formed with nHA.

Ca/P ratio of precipitation reactants	$[H_3PO_4]$ (M)	Crystalline phase composition of cement (wt%) Brushite	Unreacted nHA	Brushite crystal size (nm)
0.80	2	84.2	15.8	185.5 ± 3.7
1.00	2	83.0	17.0	117.6 ± 3.0
1.33	2	83.5	16.5	201.9 ± 4.2
1.40	2	77.7	22.3	136.6 ± 2.6
1.66	2	77.4	22.6	197.4 ± 4.2
2.00	2	68 .1	31.9	159.3 ± 3.1
0.80	3	85.6	14.4	130.9 ± 2.3
1.00	3	83.8	16.2	123.5 ± 2.0
1.33	3	84.1	15.9	163.8 ± 2.9
1.40	3	85.9	14.1	134.1 ± 2.6
1.66	3	79.4	20.6	172.9 ± 4.0
2.00	3	77.8	22.2	161.7 ± 3.1

Cement formed from nHA with a Ca/P ratio of 1.55 had the lowest porosity. Increasing the concentration of acid in cement slurry resulted in a decrease in the porosity of cement formed, Table III, all cements were highly porous (typically 50–60% relative porosity). As Ca/P ratio of the nHA cement component increased the pH of the solution in which the set cement was stored decreased, indicating a higher amount of residual unreacted phosphoric acid in these cements, (Table IV).

4. Discussion

There have been many investigations into the preparation of nano and micro crystalline hydroxyapatite, [15, 22, 24] but the successful use of hydroxyapatite (nHA) as a reactant for the formation of brushite cement has only been reported recently [16, 17]. In previous studies, were nHA has been used as a cement reactant, nHA has only been precipitated from calcium phosphates of Ca/P ratio 1.4 [16–18]. In the current study nHA was precipitated from calcium phosphates of Ca/P ratio 0.8 to 2.0, Table I. All precipitates contained approximately 90 to 95 wt% nHA in the crystalline phase of the precipitate. These precipitates were found to be calcium deficient, e.g. nHA precipitated from solutions with a Ca/P ratio of 1.67 was determined to have an actual Ca/P ratio of 1.48. In the current study nHA was produced with a Ca/P ratio as low as 1.27.

XRD was used to confirm that cement produced from nHA in this study was composed of unreacted nHA and brushite. nHA may not have been fully converted to brushite as at a powder mass: liquid volume ratio of 1.5 g/ml; 2 and 3 M solutions of phosphoric acid contained insufficient phosphate ions and excess water for a stoichiometric reaction to proceed

TABLE III Relative porosity of cement tested in compression

Calcium phosphate	Ca/P of precipitation reactants	$[H_3PO_4]$ (M)	Mean density (g/cm^3) Apparent	Mean density (g/cm^3) Measured	Relative porosity (%)
nHA	1	2	1.07 ± 0.03	2.51 ± 0.02	57.4
nHA	1.4	2	1.43 ± 0.06	2.84 ± 0.03	49.6
nHA	1.67	2	1.03 ± 0.20	2.76 ± 0.01	60.2
nHA	2	2	1.47 ± 0.13	2.92 ± 0.03	52.4
β-TCP	1.5	2	1.33 ± 0.10	2.73 ± 0.04	51.1
Hydroxyapatite	1.67	2	1.19 ± 0.10	2.76 ± 0.05	57.0
TTCP	2	2	1.13 ± 0.03	2.79 ± 0.06	59.5
nHA	1	3	1.15 ± 0.07	2.62 ± 0.02	56.1
nHA	1.4	3	1.27 ± 0.04	2.41 ± 0.02	46.9
nHA	1.67	3	1.12 ± 0.11	2.44 ± 0.01	54.0
nHA	2	3	1.27 ± 0.13	2.48 ± 0.01	48.8
β-TCP	1.5	3	1.47 ± 0.08	2.59 ± 0.04	43.2
Hydroxyapatite	1.67	3	1.20 ± 0.04	2.59 ± 0.05	53.5
TTCP	2	3	1.15 ± 0.09	2.73 ± 0.04	57.8

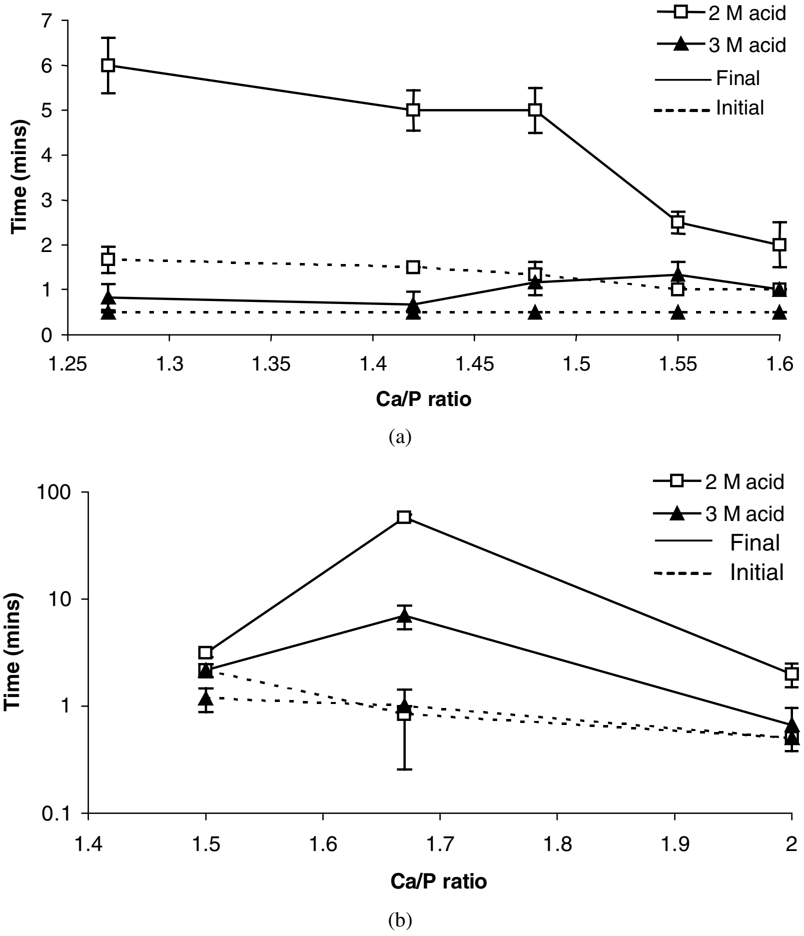

Figure 2 (a) Effect of Ca/P ratio on cement formed with nHA and 2 or 3 M phosphoric acid. Dashed line = initial setting time, Line = final setting time. (b) Initial (dashed line) and final (line) setting times of cement formed with calcium phosphate of known Ca/P ratio, Y-axis is logarithmic.

following Equation 1. 1.5 g of the nHA shown in Equation 1 is equivalent to 1.6 mmoles, which would require 4.8 mmoles of phosphoric acid and 27.2 mmoles of water (i.e. 490 μl of 9.8 M phosphoric acid solution) to react stoichiometrically. Thus it can be seen that only 41% (62%) of the required phosphoric acid and double the amount of water was present in our system for 2 M (3 M) phosphoric acid solutions. While excess water is required to form workable slurries of brushite cement systems, as reported previously [5, 11, 12], limiting this excess as far as possible should improve cement mechanical performance as unreacted water is a major source of porosity in CPC. Brushite has a Ca/P ratio of 1 and hydroxyapatite has a Ca/P ratio of 1.67, so clearly additional phosphate is required to be added to hydroxyapatite to produce an overall Ca/P ratio of 1 to form brushite. nHA made in this study with a Ca/P ratio of 1.27, (approximately 9/7), would, by analogy with Equation 1, have required a third less phosphoric acid (1.92 mmol H_3PO_4 for 1 mmol nHA) for complete reaction, yet still the cement mixture was phosphate deficient by 27% for 2 M acid but was stoichiometric with

TABLE IV Mean pH (±1 s.d.) of 100 ml of water after soaking 1 g of set cement, crushed in a pestle and mortar, for 12 h at room temperature ($n = 3$), control double distilled water alone

Calcium phosphate	Ca/P ratio	pH
–	–	6.57 ± 0.05
nHA	1.27	5.58 ± 0.05
nHA	1.55	5.18 ± 0.07
nHA	1.60	4.76 ± 0.02

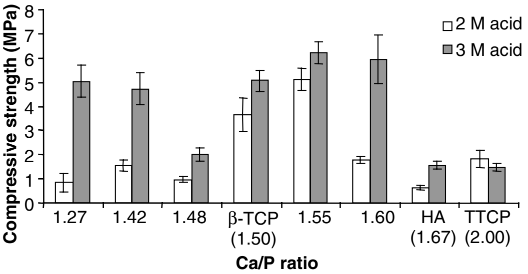

Figure 3 Compressive strength of cement formed with nHA or calcium phosphate of known Ca/P ratio and 2 or 3 M phosphoric acid.

respect to phosphate for 3 M acid. Table II shows this cement reactant resulted in a higher degree of conversion than nHA with higher Ca/P ratios. Table IV illustrates that practically this resulted in less unreacted acid in the cement after hardening which may reduce initial inflammation caused by phosphoric acid release after implantation. This finding is unexpected since it might be anticipated that an excess of calcium ions would minimise the potential for unreacted phosphoric acid to remain in the cement. This would suggest that Ca/P ratio may strongly affect dissolution behaviour of nHA and it was this parameter rather than stoichiometry that was the more important in determining extent of reaction in this system. Indeed, the kinetic solubility of HA is reported to be influenced by stoichiometry [15, 24], Mavropoulos *et. al.* [25] have reported that the dissolution of HA in aqueous solution is dependant on the Ca/P ratio of the HA and that the dissolution of HA of lower Ca/P ratio (as low as 1.49) was enhanced compared to that of stoichiometric HA (Ca/P = 1.67). The lowest Ca/P ratio reported for calcium deficient HA is 1.21 [24], which is similar to that reported here.

It is well established [5, 11, 13] that mixing microcrystalline β-TCP with orthophosphoric acid results in the formation of brushite cement, however here we also found that the substitution of β-TCP with HA resulted in the formation of brushite too, Fig. 1. However, when TTCP was mixed with orthophosphoric acid a vigorous acid base reaction occurred and no brushite was formed, instead, a mixture of TTCP and a small amount of monetite (a dehydrated form of brushite [15]) was present, Fig. 1. Brushite may have not been formed due to the strongly basic nature of TTCP. It is likely that this prevented a pH of the cement slurry below 4 at which brushite can be precipitated. Possibly a predominantly amorphous precipitate was formed during the vigorous reaction, which could not be detected using X-ray diffraction. A small amount of monetite may have formed on the surface of TTCP agglomerates due to crystallisation of this amorphous phase or small amounts of brushite may have been formed and water lost due to excessive heat produced as a consequence of the vigorous nature of the reaction observed [26].

Cement produced from 2 M phosphoric acid and nHA precipitated with a Ca/P ratio of 1.55 was the stronger in compression, Fig. 3. However, when 3 M phosphoric acid was used as a cement reactant there appeared to be little difference in strength between cement formed from nHA precipitated at a Ca/P ratio of 1.4 and 2.0. This may be due to the actual Ca/P ratio of nHA, as determined using wet chemical analysis. Both nHA precipitated at Ca/P ratio 1.4 and 2 had a measured Ca/P ratio in excess of 1.5, which is close to that of β-TCP (Ca/P = 1.5) used previously to form brushite cement [5]. Cements formed with nHA were stronger than cement formed with microcrystalline calcium phosphates at the same Ca/P ratio. This may have been due to variation in porosity, composition and microstructure as a consequence of differences in the kinetics of reactant dissolution. Cement produced from nHA of measured Ca/P ratio of 1.5 had relatively poor mechanical performance compared to cement formed with calcium phosphates of Ca/P ratio greater than 1.5, Table II and Fig. 3. Further study is required to determine whether differences in kinetic solubility were responsible for this effect.

5. Conclusions

In the current study nHA was precipitated with a Ca/P ratio as low as 1.27. Mixing nHA with phosphoric acid solution resulted in the formation of slurry that set to cement predominantly composed of brushite. Cement formed with nHA had a compressive strength greater than that of cement formed with microcrystalline calcium phosphates of the same Ca/P ratio. Cements made with nHA with the lowest Ca/P ratios had the highest degree of conversion and longest setting times, despite having a lesser deficiency of phosphate in the cement mixture less unreacted phosphoric acid was detected in these cements. This suggests that differences in solubility rather than cement stoichiometry may have been responsible for this effect.

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