Cement from magnesium substituted hydroxyapatite

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Brushite cement may be used as a bone graft material and is more soluble than apatite in physiological conditions. Consequently it is considerably more resorbable *in vivo* than apatite forming cements. Brushite cement formation has previously been reported by our group following the mixture of nanocrystalline hydroxyapatite and phosphoric acid. In this study, brushite cement was formed from the reaction of nanocrystalline magnesium-substituted hydroxyapatite with phosphoric acid in an attempt to produce a magnesium substituted brushite cement. The presence of magnesium was shown to have a strong effect on cement composition and strength. Additionally the presence of magnesium in brushite cement was found to reduce the extent of brushite hydrolysis resulting in the formation of HA. By incorporating magnesium ions in the apatite reactant structure the concentration of magnesium ions in the liquid phase of the cement was controlled by the dissolution rate of the apatite. This approach may be used to supply other ions to cement systems during setting as a means to manipulate the clinical performance and characteristics of brushite cements.

1. Introduction

Calcium phosphate cements (CPCs) have been investigated as a means of meeting the need for synthetic bone 'grafts' and are used clinically to fill non-load bearing defects in the cranio and maxillofacial region [1–3]. CPCs are produced from the setting of a slurry comprised of solid calcium phosphate reactants and an aqueous liquid component [4]. An advantage of CPCs is that, unlike prefabricated calcium phosphate granules or blocks, used as bone substitutes, CPCs may be moulded to fill a void or defect in bone and are injectable [5, 6]. A further advantage is that they are osteoconductive, allowing for in growth of new bone and integration of cement with surrounding tissues [7].

Brown and Chow reported the formation of CPC in 1985 [3, 8]. These apatitic cements were formed by mixing tetracalcium phosphate ($Ca_4(PO_4)_2O$) and dicalcium phosphate dihydrate (DCPD, $CaHPO_4{\cdot}2H_2O$). The disadvantage of apatite cements is that they may be resorbed at a slow rate *in vivo* [9]. In 1987 Lemaître *et al.* [10] reported the formation of cement consisting of predominantly brushite (DCPD). Brushite cements are more soluble than apatite cements in physiological conditions and hence are resorbable *in vivo* [11].

Previously, brushite cements have been formed by the mixture of monocalcium phosphate monohydrate (MCPM), Equation (1), or phosphoric acid, Equation (2), with β-tricalcium phosphate (β-TCP) and water [12, 13]. We have

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

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

previously reported the production of brushite cement from the mixture of nanocrystalline HA and phosphoric

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acid, Equation (3) [14].

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

Brushite has a calcium to phosphate (Ca/P) ratio of 1 and therefore in previous cement systems the addition of phosphate ions was necessary in order to produce stoichiometric brushite because β-TCP has a Ca/P ratio of 1.5. However, MCPM or phosphoric acid, which lower the pH of the cement reactant slurry have been used as sources of phosphate ions. The advantage of using HA as a cement reactant is thought to be that fewer phosphate ions were required to form brushite as HA may be formed at Ca/P ratio as low as 1.3. Furthermore, ionic substitutions may be made in the HA crystal lattice during preparation [15], thereby creating a potential means by which ions may be introduced in a controlled manner and at a low concentration in the liquid phase of cement during setting.

The substitution of ions into HA has been extensively researched; apatitic components of bone and other calcified tissues contain ionic substitutions [15–17]. The substitution of magnesium into HA and its role in the precipitation of many calcium phosphates has been investigated [18, 19]. Magnesium is believed to play a role in the formation of dental caries, urinary calculi and bone deposition and mineralisation [16, 20, 22]. Decreased crystallinity and increased solubility have been observed after magnesium substitution into many calcium phosphates [23, 24]. Brushite cements that incorporate magnesium may offer a means of controlling microstructure, strength, composition and resorbability of the cement and its interaction with host tissues *in vivo*. Although magnesium substitution into HA has been reported previously, magnesium substituted HA has not been investigated as a brushite cement component. However, magnesium has been included in brushite cement formulations as a separate phase ($MgHPO_4\cdot 3H_2O$) in order to alter brushite hydrolysis and increase degradation [25]. The purpose of this study was to form a brushite cement from magnesium substituted HA and to characterise the cement properties and composition.

2. Methods and materials

Nanocrystalline hydroxyapatite (HA) was precipitated following the methods of Jarcho *et al.* [26]. Briefly, a 300 mM solution of diammonium phosphate ($(NH_4)_2HPO_4$) (Sigma, UK) was added, drop wise, to an 800 mM calcium nitrate ($Ca(NO_3)_2\cdot 4H_2O$) (Sigma, UK) solution under constant stirring at 25 °C. Both solutions were adjusted to a pH of 11 with concentrated aqueous ammonia (NH_4OH) (Fisher, UK) prior to mixing. Magnesium substituted HA was also produced following this method except magnesium nitrate ($Mg(NO_3)_2\cdot 6H_2O$) (Sigma, UK) was added to the calcium nitrate solution to give 1–8% magnesium concentration as a proportion of the total magnesium and calcium concentration. 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.

Calcium, magnesium and phosphate ion concentrations in HA precipitates were derived from ion chromatography data. Samples were dissolved in hydrochloric acid (Sigma, UK) and the resultant solution diluted. An ICS-1000 ion chromatography system (Dionex, UK) was used for cation determination. A 20 mM methanesulfonic acid (BDH, UK) solution was used as the eluent. Cations were eluted using a 4 × 250 mm IonPac® CS12A separator column. All results were calculated against a 4-point calibration curve. Chromeleon® software package was used for data analysis. Sodium chloride (Sigma, UK) and calcium chloride ($CaCl_2\cdot 2H_2O$) (BDH, UK) were used as cation reagents. A 100 ppm. mixed (sodium and calcium) stock solution was prepared; from which serially diluted 50, 20, 10 and 1 ppm standard solutions were prepared. Phosphate anion measurements were carried out using an ICS-2500 ion chromatography system (Dionex, UK), consisting of a gradient pump with a 25 μl sample loop. In this method, phosphates were eluted using a 4 × 250 mm IonPac® AS16 anion-exchange column packed with anion exchange resin. Anion Self-Regenerating Suppressor (Dionex ASRS®) was used at 242 mA. The Dionex EG40 eluent generator equipped with a potassium hydroxide (KOH) cartridge was used in conjunction with the ASRS®. The sample run time was set for 30 min, KOH concentration maintained at 60 mM. Chromeleon® software was used for data analysis.

Cement was formed by mixing precipitated HA or magnesium substituted HA with 3 M phosphoric acid solution (Sigma, UK). Cements were mixed at a powder to liquid ratio of 1.5 g/ml. 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. Setting time was determined using the Gillmore needle technique [27]. X-ray diffraction (XRD) patterns of HA precipitate and set cements were recorded from $2\theta = 10\text{–}100^\circ$ with a step size of 0.02° and a count time of 12 s/step (Philips PW1070 diffractometer) with Ni filtered Cu K_α radiation ($K_{\alpha 1} = 1.5406$ Å, $K_{\alpha 2} = 1.5444$ Å) at 40 kV and 30 mA). Rietveld refinement phase analysis was performed using TOPAS software.

To determine the effect that magnesium content had on brushite hydrolysis, ground cement samples were placed in 1 M Na_2HPO_4 (BDH, UK) solution at a liquid to solid mass ratio of 5 and stored at 37 °C for 16 h [28]. These samples were filtered, washed and then dried before XRD was performed to determine the extent of hydrolysis. Cement cylinders of 6 mm diameter and 12 mm height were formed. 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.

TABLE I Composition of calcium phosphate precipitate and set cement

Concentration of ions in precipitation solutions (%) $\frac{\text{Mg}}{(\text{Mg+Ca})}$	Measured composition of apatite (wt%)				Theoretical composition of cement mix (wt%)				Calculated composition of crystalline phases in set cement (wt%)		
	Ca	Mg	PO_4	$\frac{(\text{Mg+Ca})}{PO_4}$	Ca	Mg	PO_4	$\frac{(\text{Mg+Ca})}{PO_4}$	Ca	PO_4	$\frac{\text{Ca}}{PO_4}$
0	41.5	0.0	55.1	0.753	27.8	0	49.5	0.561	27.5	56.0	0.490
1	42.5	0.28	57.9	0.738	28.5	0.19	51.4	0.557	23.7	52.7	0.451
2	43.4	0.54	60.0	0.733	29.1	0.36	52.8	0.557	23.4	50.7	0.462
4	39.7	1.0	52.7	0.773	26.6	0.69	47.9	0.569	19.9	44.6	0.447
8	34.8	1.8	51.5	0.709	23.3	1.20	47.1	0.519	18.6	41.9	0.444

3. Results

Chromatography data were used to determine that precipitates were composed of 35–45 weight percent calcium and 50–60 weight percent phosphate, Table I. The amount of magnesium in the precipitate increased as the amount introduced as precipitation reactant increased. The amount of magnesium in precipitate as a percentage of the total number of moles of magnesium plus calcium was close to that in precipitation solution, Fig. 1. The XRD spectra obtained from calcium phosphate precipitates had broad peaks at $2\theta = 26$ and 32, characteristic of poorly crystalline hydroxyapatite. Analysis of XRD data revealed that cement produced from HA without magnesium was composed of HA in a predominantly brushite matrix. However, cements produced from HA containing magnesium consisted of monetite (dicalcium phosphate anhydrous, DCPA) as well as HA and brushite, Fig. 2. The proportion of brushite appeared to decrease as the amount of magnesium in the cement mix was increased, Fig. 2.

The theoretical composition of the cement mix was determined from knowledge of the proportions of reactants used to make cements, Table I. The calculated composition of crystalline phases in set cement was calculated from Rietveld refinement phase analysis data. Measured $[\text{Ca+Mg}]/[PO_4]$ in the HA was of course higher than the ratio within the cement mix since the phosphate ions in the added liquid component reduced it. However, the Ca/PO_4 ratio of the crystalline components of the set cement could not account for all of the calcium ions since it was much lower than the ratio used to make the cement formulation. While magnesium substitution could account for some of the expected calcium, it was insufficient to account for all of it. This suggests that any amorphous phases formed had a Ca/P ratio >1 or that the crystalline phases present deviated from expected stoichiometry. All brushite lattice parameters cement increased as magnesium content increased, Table II, which may also indicate substitution of magnesium into brushite crystals.

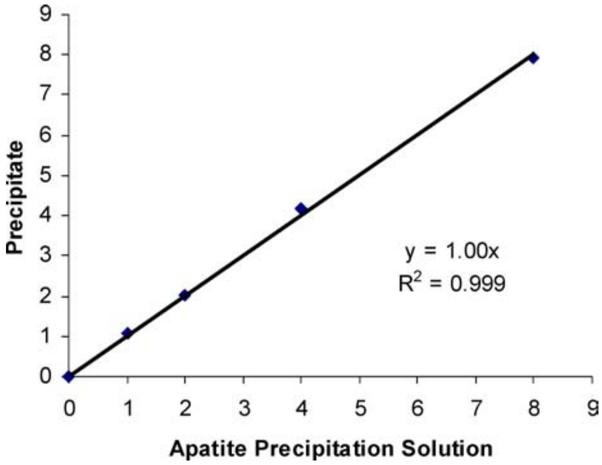

Figure 1 Relationship between magnesium content in precipitated HA relative to the amount of magnesium in the precipitation solution. Magnesium content calculated as the concentration of magnesium as a percentage of total magnesium plus calcium concentration.

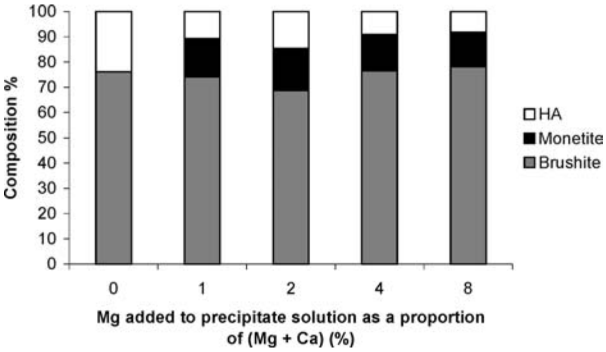

Figure 2 Composition of cement produced from HA containing varying amounts of magnesium ion.

The relative density of cement decreased as the amount of magnesium in the cement reactants was increased. Cement formed from HA containing no magnesium had a relative density of 53% whilst cement produced from HA containing the maximum amount of magnesium had a relative density of only 34%, Table III. The setting time was measured and all cements set within 5 min. The initial setting time was reduced from 1 min to 0.5 min when the largest amount of magnesium was added. The final setting time was extended from 2.7 ± 0.3 min to 3.7 ± 0.6 min.

Following accelerated *in vitro* aging in sodium phosphate solution cements produced from HA containing

TABLE II Lattice parameters of brushite crystals in set cements

concentration of ions in precipitation solutions $\frac{\text{Mg}}{(\text{Mg+Ca})}$	Lattice parameters (Å)		
	a	*b*	*c*
0	5.80732	15.16863	6.23661
1	5.80345	15.15580	6.23332
2	5.81538	15.19475	6.24552
4	5.81721	15.20645	6.24915

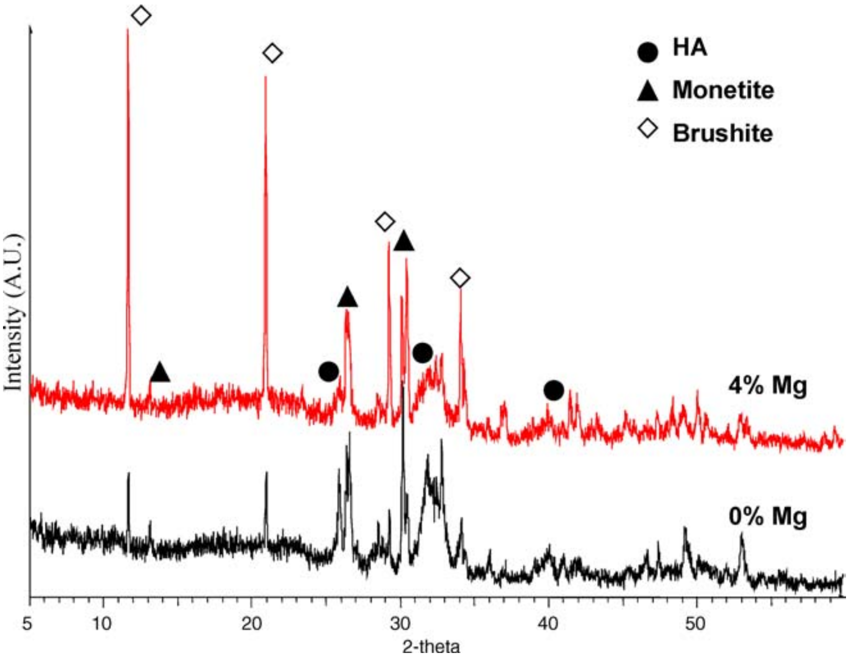

Figure 3 X-ray diffraction pattern obtained when cements formed from HA and HA containing 4% magnesium as a percentage of the total magnesium and calcium concentration of precipitate reactants were stored in Na_2HPO_4 solution for 16 h.

4% magnesium consisted of more brushite than those produced with unsubstituted HA, as could be determined by the relative intensities of the brushite and apatite peaks in XRD patterns (Fig. 3).

The addition of magnesium to the cement system resulted in a reduction in compressive strength of cement, Fig. 4. As the proportion of magnesium in the initial HA precipitate was increased the compressive strength of cement produced fell by approximately 95%. Cement formed with HA containing no magnesium had a mean compressive strength of 7.4 ± 0.7 MPa whilst cement formed from HA containing the maximum amount of magnesium had a compressive strength of 0.3 ± 0.0 MPa.

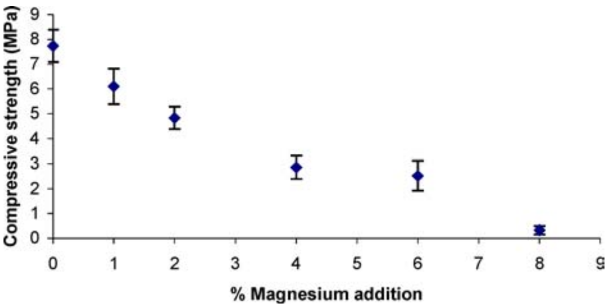

Figure 4 Compressive strength of cement produced from HA containing varying amounts of magnesium.

4. Discussion

Previously nanocrystalline hydroxyapatite (HA) has been precipitated and used as a component of brushite cement [14]. In the current study, HA was precipitated with the addition of magnesium for use as a component of brushite cement. It has been reported in the literature that the maximum amount of magnesium that may replace calcium in HA prepared by precipitation is 0.3 wt% [15]. However, analysis of chromatography data suggested that HA precipitated in this study contained up to 1.8 wt% magnesium, Table I. However, the presence of magnesium alone could not distinguish whether all of the magnesium was substituted into the HA lattice or if some magnesium was within a surface layer surrounding HA or within an amorphous phase. The ratio of calcium and magnesium to phosphate [Mg + Ca]/[PO_4] in HA precipitate was approximately constant (~0.75) regardless of the quantity of magnesium added to reactant solutions, Table I. The molar ratio of magnesium to total magnesium plus calcium [Mg]/[Mg + Ca] in precipitate was close to that expected from the amounts of magnesium and calcium added to the precipitation solution (Fig. 1); indicating that very little magnesium was lost during precipitation and thus magnesium was associated with precipitate, even after washing.

TABLE III Effect of magnesium concentration in HA reactants on the relative density of cement

Concentration of ions in precipitation solutions (%) $\frac{Mg}{(Mg+Ca)}$	Relative density of cement produced (%)
0	53.0 ± 0.8
1	46.9 ± 0.6
2	43.9 ± 0.4
4	40.0 ± 0.3
6	43.4 ± 0.4
8	34.3 ± 0.7

When dried, crushed and sieved HA precipitated with or without magnesium was mixed with phosphoric acid solution, a slurry was formed that set to form cement. In the absence of magnesium, cement was composed of predominantly brushite with some unreacted HA (Fig. 2). Yet in the presence of magnesium, cement was composed of brushite, HA and monetite, Fig. 2.

The formation of monetite has been reported where brushite has been precipitated in the presence of magnesium [22]. This different behaviour may be explained by the higher thermodynamic stability of monetite and the lower solubility of DCPA (ks = 1.83×10^{-7} mol L^{-1}) rather than DCPD (ks = 2.59×10^{-7} mol L^{-1}). Monetite is slightly more stable than brushite in the pH range between 2–7, however the rate of crystal growth of brushite is several orders of magnitude higher than that of monetite such that in normal cements only brushite is formed. However, in the presence of Mg ions the rate of crystal growth of brushite is probably lowered to a point similar to monetite such that both compounds are formed as setting products.

Notably in this study the proportion of unreacted HA in cement decreased as the amount of magnesium in the cement system was increased. Thus, magnesium could be used to adjust the composition and degree of reaction of the cement.

When the theoretical Ca/PO_4 ratio of cement was compared with that determined from Rietveld refinement phase analysis it was apparent that not all calcium ions were located in crystalline phases, (cf. theoretical and calculated compositions Table I). The calcium deficiency of the crystalline phases in the cement compared to the known theoretical composition was an indication that an amorphous phase was present in the cement, undetected by XRD. The formation of amorphous calcium phosphate phases have been reported previously when HA has been produced in the presence of magnesium [24, 29]. Magnesium has been reported as stabilising non-crystalline calcium phosphates and preventing crystallisation into other more stable calcium phosphate phases [30], in other studies the amount of brushite precipitated in solution has been reduced by the addition of magnesium [22].

Magnesium has been used previously as a separate component of brushite cement to reduce hydrolysis [25]. The hydrolysis of brushite to HA is reported to be dependant on the rate of brushite dissolution and precipitation of HA from the resultant solution [31].

The presence of magnesium has been shown to retard the formation of HA from brushite [30, 32], and was believed to result from adsorption of magnesium onto HA nuclei and brushite and the stabilisation of any amorphous calcium phosphate present in the reaction solution. The results obtained in this study when cements aged in sodium phosphate solution were examined using XRD indicated that the presence of magnesium in the cement structure retarded brushite hydrolysis to HA, Fig. 3. Brushite cement in which magnesium is a constituent of the brushite crystal, as in this study, could be more resistant to hydrolysis than cement produced without magnesium or cements produced with magnesium as a separate phase. This may be an advantage because the incorporation of magnesium into brushite crystals could be used as a means to controlling brushite hydrolysis *in vivo*. The incorporation of magnesium into brushite crystals was suggested by an increase in lattice parameters. Previously it has been suggested that magnesium ions may become trapped in interstitial sites in brushite, but in this case the a axis was unaffected by the presence of magnesium because magnesium ions have a smaller ionic radii compared with calcium ions [22].

The mechanical properties of cement decreased significantly as the amount of magnesium introduced into the cement system was increased, Fig. 4. The compressive strength was only reduced by a small proportion at low magnesium concentrations compared to the use of higher magnesium concentrations in the cement mix. The microhardness of brushite crystals has been reported to decrease after magnesium substitution but not at low concentrations [22]. Reduced hardness of brushite crystals could have contributed to the reduction of compressive strength observed after the introduction of magnesium into the cement system. However, a reduction in strength was noted at low magnesium levels and therefore reduced strength may be attributed to other factors, such as porosity, which has an inverse exponential relationship to compressive strength. Porosity was shown to increase as the amount of magnesium in the cement system was increased, Table III. Increased porosity may have been due to the formation of monetite, which consumes less water than brushite on formation. Changes in microstructure and the number of defects in the cement caused by the introduction of magnesium and its incorporation into brushite crystals may have also contributed to poor mechanical performance.

Further research is required to determine the effect that the incorporation of magnesium into the calcium phosphate component of brushite cement has on the *in vitro* hydrolysis of brushite and alteration of the mechanical performance of cement. Cement formulation may be altered to allow the incorporation of magnesium as an integral component of cement with optimised mechanical performance. Other ions may also be introduced into the cement systems following the methods outlined in this study. The introduction of other ions may be used as a means to further manipulate the structure, composition and performance of brushite cement.

5. Conclusion

A novel means by which magnesium may be incorporated into brushite cement to modify the hydrolysis of brushite has been reported. Nanocrystalline hydroxyapatite containing magnesium was precipitated and mixed with phosphoric acid solution to form brushite cement. When the cement was aged in sodium phosphate solution the hydrolysis of this novel cement appeared to be retarded. Cement containing magnesium was composed of a greater proportion of brushite than cement containing no magnesium after storage in sodium phosphate solution. Further investigation needs to be performed on the hydrolysis of brushite containing magnesium to conclusively determine the effect magnesium has on hydrolysis of magnesium containing brushite cement. Research is also required to determine the cause of reduced mechanical properties of magnesium containing cement and to find a way of optimising mechanical performance.

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