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A new way of forming a calcium phosphate cement using bioactive glasses as a reactive precursor

Niall W. Kent [1], Robert G. Hill, Natalia Karpukhina *

Dental Physical Sciences, Institute of Dentistry, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, Mile End Road, London E1 4NS, UK

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ABSTRACT

Calcium phosphate cements (CPC)s are conventionally made by mixing crystalline calcium phosphates with aqueous solutions. In this study new CPCs are made by reacting bioactive glasses (BG)s with $Ca(H_2PO_4)_2$ to form cement. It is found that high P_2O_5 content of 4 mol% or greater is required in BG to produce a cement. The phases formed are dependent on glass composition; brushite and octacalcium phosphate (OCP) form first with 6 mol% P_2O_5 BG. Brushite dissolves, reforms as OCP, then transforms to apatite. These new cements offer new route to forming CPC that combine in-situ setting and injectability of CPCs with resorbability and bioactivity of BGs.

1. Introduction

CPCs were proposed by Le Geros [1] and successfully developed by Brown and Chow [2], though the first cement formation in calcium phosphates system was demonstrated in 1950 by Kingery [3]. These cements are conventionally made by mixing crystalline calcium phosphate salts with an aqueous solution. The salts dissolve and re-precipitate as calcium deficient apatite ($Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x} \cdot nH_2O$) or Brushite also named as dicalcium phosphate dihydrate (DCPD, $CaHPO_4 \cdot 2H_2O$) [4]. Recently monetite cements have been also considered as an alternative to the brushite cements [5].

CPCs have found widespread use in orthopaedic surgery and cranial facial surgery as a bone substitute. Existing CPCs suffer from a number of disadvantages [6]; they are:

i) not very resorbable *in-vivo*, this is specifically related to the existing apatite cements,
ii) prone to wash out on exposure to water before being completely set,
iii) relatively brittle and have low compressive strengths.

In contrast bioactive glasses [7] have osteo-stimulatory properties and are more resorbable but generally cannot be injected through a narrow bore syringe and do not set hard *in-vivo*. Recently a number of studies have investigated the incorporation of BGs into existing CPC formulations [8–10], where the BG was of a large particle size and acted as a bioactive filler.

In this pilot study we investigated CPCs made from BGs where the glass was used as reactive precursor as opposed to filler and took part in the cement forming reaction.

This approach has three major advantages:

i) The glass compositions are not restricted by the stoichiometry of crystal.
ii) The dissolution rate/reactivity of BG can be controlled via glass composition/structure.
iii) It is possible to incorporate a wide variety of therapeutic ions into the glass composition including: strontium to stimulate bone formation and provide radio-opacity [11], zinc for its beneficial effects for wound healing [12], cobalt to promote angiogenesis [13–14] and fluoride to up regulate osteoblasts and form fluorapatite [15–16].

In order to form cement from a BG it is important that the glass both dissolves and forms apatite rapidly. Recently it has been shown [17–19] that the speed of apatite formation with BGs is related to the glass network connectivity and the phosphate content.

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* Corresponding author.
E-mail address: n.karpukhina@qmul.ac.uk (N. Karpukhina).

[1] Present address: Department of Chemical Engineering, University College London, Torrington Place, London, United Kingdom.

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2. Materials and methods

The glasses (Table 1) were melted at 1480 °C for one hour; with the synthesis details described earlier [11]. 100 g of each glass was ground using a vibratory mill (Gy-Ro mill, Glen Creston) for 2 × 7 min and sieved to a particle size below 38 µm. The $Ca(H_2PO_4)_2$ (Sigma-Aldrich) was prepared by grinding 27 g using the vibratory mill for 4 minutes.

The cement mixture was prepared by mixing the sieved glass powder with the milled $Ca(H_2PO_4)_2$ powder with overall calcium to phosphorus ratio of 1.33, the stoichiometry of octacalcium phosphate (OCP, $Ca_8(HPO_4)_2(PO_4)_4 \cdot 5H_2O$). The powders were hand mixed for 30 s on a glass slab, with 2.5% Na_2HPO_4 solution with a liquid to powder ratio of 0.70 ml/g.

The setting time of each cement was measured using the Gilmore needle test according to the ISO 9917-1:2007(E). The compressive strength specimens were prepared according to the ISO 9917-1:2007(E) using split cylindrical moulds. The cements were stored at 37 °C for two hours. Each cylinder was immersed in 10 ml of Tris buffer solution at 37 °C for either of 1, 24, 168 and 672 h prior to testing.

Powder XRD was carried out for the cements after immersion using Bruker D8-A25-Advance diffractometer with the CuKα radiation at 40 kV and 40 mA.

The ^{31}P MAS-NMR experiments were run on Bruker NMR spectrometer at the 242.9 MHz frequency. The powder samples were packed into 4 mm rotor and spun at 11–12 kHz. The measurements were done using 60s recycle delay and 85% H_3PO_4 was used to reference the chemical shift scale.

The fracture surface of the cement cylinders were gold coated and examined using Hitachi S-3400 machine with the accelerating voltage at 20 kV and an emission current of 54 mA.

3. Results

Table 1 gives the initial and final setting times. The two cements P4 and P6 were produced as cylinders for the compressive strength experiments. However, the P4 cements disintegrated upon immersion and only the compressive strength of P6 cements was determined as shown in Table 2.

The XRD for the P4 and P6 cements are shown in Fig. 1. At 1 h Brushite is present for P4 (Fig. 1a). On increasing the immersion time the Brushite dissolves and is progressively replaced by OCP. OCP has an almost identical diffraction pattern to hydroxyapatite but also exhibits a diffraction line at 4.68° 2θ corresponding to the 100 water inter layer spacing (18.6 Å) [20–21]. The P6 cement also shows the presence of Brushite at 1 h and 24 h whilst the 4.68° 2θ line of OCP is present at 1, 24 and 168 h but is absent at 672 h (Fig. 1b).

Fig. 2 shows the ^{31}P MAS-NMR results for P4 and P6 glasses and cements. The assignment of the spectra was done based on the previous studies [22–25]. The glasses exhibit a broad peak at ≈ 5 ppm corresponding to mixed sodium calcium amorphous orthophosphate. In Fig. 2a the dominant peak at 1.4 ppm for the P4 at 1 h and 24 h indicates that Brushite is the primary phase. A small fraction of the signal at −1.4 and −0.2 ppm indicates that Monetite ($CaHPO_4$) is also present. At 168 h chemical shifts at −0.2,2.0,3.2 and 3.6 ppm are present indicative of the formation of OCP, in addition to Brushite and Monetite. At 672 h peaks at −0.2,2.0,3.2 and 3.6 ppm are found showing the presence of OCP.

Table 1

Glass compositions in mol% and initial and final setting time in minutes for the experimental cements. Theoretical network connectivity of all glass compositions is equal to 2.00.

Glass	SiO_2	P_2O_5	CaO	Na_2O	Initial Set	Final set
P0	50.0	0.0	45.0	5.0	> 90	> 90
P2	46.0	2.0	46.8	5.2	> 90	> 90
P4	42.0	4.0	48.6	5.4	35.5	89.0
P6	38.0	6.0	50.4	5.6	10.5	15.0

Table 2

Compressive strength in MPa of the P6 cements after each of the immersion period in Tris buffer (Figures in brackets give the SD for $n=8$).

Glass/Time	1 h	24 h	168 h	672 h
P6	9.65 (0.56)	12.90 (1.60)	10.30 (1.33)	5.67(0.94)

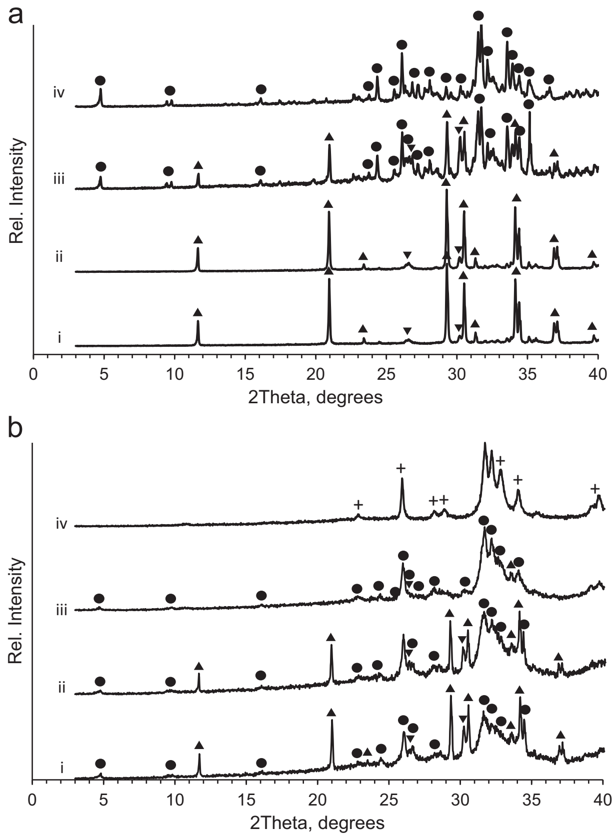

Fig. 1. X-ray diffraction patterns of cement formulation produced from glass P4 (a) and P6 (b) after immersion in Tris buffer solution for (i) 1 h, (ii) 24 h, (iii) 168 h and (iv) 672 h. ▲ – Brushite or DCPD; ▼ – Monetite or DCPA; ● – OCP; +Apatite.

Fig. 2b shows chemical shifts at −1.4,−0.2,1.4 and 3.3/3.1 ppm in 1 h and 24 h samples, these are Monetite (−1.4,−0.2 ppm), Brushite (1.4 ppm) and Apatite (3.1 ppm). The 672 h sample has a chemical shift at 2.9 ppm assigned as apatite.

The scanning electron micrographs (SEM) of the P4 and P6 cements are shown in Figs. 3 and 4. SEM showed porous structure with the crystals morphology and size changing over time. Small thin plate crystals were seen at 1 h in P4 cements. This changes to extremely elongated ribbon- or blade-like crystals at 168 h with the structure becomes much more open and remains the same by 672 h. In P6 cements with less open structure, a mixture of the crystals morphologies has been seen. The smaller whiskers-like crystals that were initially present disappeared with time and only plate-like crystals were remained.

4. Discussion

The two glasses with the lowest phosphate contents were not capable of forming cements that set within 90 minutes. The two