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A preliminary investigation of the *in vitro* bioactivity of white Portland cement

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Abstract

Freshly-mixed and partially-cured ordinary Portland cement (OPC) pastes have been shown to exhibit good biological compatibility with a range of cells and tissue-types; particularly those associated with bone formation. Formulations based on OPC have been used as dental restoratives and are now being investigated for their potential use in orthopaedic repair. Despite the current clinical interest in OPCs, very little is known about their chemistry in the physiological environment. In this respect, research to investigate aspects of the interactions between a white Portland cement (WPC) paste and simulated body fluid (SBF) has been carried out *in vitro*. Exposure to SBF has been found to promote the precipitation of a layer of 'bone-like' hydroxyapatite on the surface of WPC paste which underpins its ability to integrate with living tissue. The dissolution of portlandite and formation of calcite were also observed on contact with SBF.

Keywords: Cement paste: Portland cement; Bioactivity

1. Introduction

In general, the body's defence mechanism will stimulate the formation of a fibrous capsule around an artificial implant material in an attempt to isolate it from the surrounding tissue; however, certain glass and ceramic formulations have been shown to form stable bonds with living bone tissue *in vivo* [1]. This ability to elicit a mechanically compliant interface with living tissue was first observed in 1969 and is referred to as 'bioactivity' [1]. Materials which exhibit this property can be used to repair diseased or damaged bone tissue and can be designed to remain *in situ* indefinitely or to degrade as the normal functions of the host tissue are restored. Moreover, bioactive substrates which release soluble silicate species have been shown to accelerate the formation of new bone tissue by promoting the genetic activity of bone-regulating cells [2,3].

With the exception of β-tricalcium phosphate and natural calcite, which bond directly to living bone, this material–cell interface develops *via* the deposition of a layer of substituted hydroxyapatite (HA), $(Ca,Na)_{10}(PO_4,CO_3)_6(OH)_2$, which forms by the precipitation of ions present in human plasma [4–6]. Human plasma is supersaturated with respect to HA, and the presence of certain functional groups, such as Si–OH, on the surface of a material induces the nucleation of apatite crystals which then continue to grow spontaneously. The resulting superficial HA layer is similar in structure to the mineral component of bone and provides a focus for the attachment and proliferation of new bone-forming cells.

Inorganic bioactive materials are used in a variety of forms, including: cements; coatings; monolithic articles; granules and particles. Materials in granular and particulate form can be packed directly into the bone defect site or applied as composite formulations in combination with organic gels, glues, putties and polymers [7].

A range of hydraulic calcium (alumino)silicate cements is currently being investigated with respect to their potential clinical use in dental and bone-contact applications [8–12]. For the past decade, Mineral trioxide aggregate (MTA), a proprietary formulation comprising 80 wt.% Portland cement and 20 wt.% bismuth oxide (to confer radiopacity), has been used as a root repair material in dentistry [13]. Its reported biocompatibility and clinical success in the sealing of communications between the root canal system and the surrounding tissues has prompted

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Table 1
Ionic compositions of simulated body fluid and human plasma

Species	Simulated body fluid (mM)	Human blood plasma (mM)
Na^+	142	142
K^+	5.0	5.0
Mg^{2+}	1.5	1.5
Ca^{2+}	2.5	2.5
Cl^-	147.8	103
HCO_3^-	4.2	27
HPO_4^{2-}	1.0	1.0
SO_4^{2-}	0.5	0.5
pH	7.40	7.2–7.4

speculation that hydraulic calcium (alumino)silicate cements may also be appropriate for use in orthopaedic repair [8]. In this respect, recent research has indicated that MTA and ordinary Portland cement pastes can support the production of new hard and soft tissues *in vivo* with minimal inflammatory response although the mechanism of this bioactivity has not yet been established [8–12].

In 1991 Kokubo devised an *in vitro* method for predicting *in vivo* bone-bonding capability in which a candidate material is exposed to an acellular 'simulated body fluid' (SBF) whose ionic composition approximates to that of human plasma (as shown in Table 1) [6]. The precipitation of HA on the surface of a material within 4 weeks of exposure to SBF *in vitro* gives an indication that the material is bioactive and will form a bond with living bone tissue.

In spite of the increasing speculation regarding the potential clinical applications of hydraulic calcium (alumino)silicate cements, their bioactivity and dissolution characteristics in SBF are not yet documented. Accordingly, a preliminary *in vitro* assessment of the bioactivity of a white Portland cement in particulate form has been carried out and the concomitant dissolution of aluminium, calcium and silicon from the cement matrix has been measured.

2. Experimental

2.1. Materials, preparation and characterisation

The WPC used in this investigation was supplied by Lafarge, and is commercially available as 'Snowcrete'. The chemical composition and principal phases of WPC are presented in Table 2. Cement paste samples were prepared in triplicate with distilled water at a water:solids ratio of 0.5 by mass. In each case 200 g of WPC were manually blended with 100 g of distilled water using a polypropylene spatula. The specimens were then cast into 52 mm × 35 mm plastic cylinders with hermetically sealing lids, vibrated to remove entrapped air, sealed and cured at 22 ± 2 °C for 28 days. After curing, the samples were removed from the mould and broken into pieces of approximately 5 mm diameter, a proportion of which were retained for characterisation and the remainder ground by mortar and pestle to pass 500 µm. The ground cement samples were immediately contacted with simulated body fluid (as outlined in Section 2.2). The 5 mm cement paste samples retained for characterisation were dehydrated by solvent exchange with propan-2-ol [14] to halt the hydration processes prior to analysis by powder X-ray diffraction (XRD) and energy dispersive X-ray analysis (EDX) as outlined elsewhere [14,15].

2.2. In vitro bioactivity and dissolution

Simulated body fluid was prepared in accordance with the method described in Ref. [6]. 0.15 g of ground WPC paste were contacted with 150 cm^3 of SBF in hermetically sealed polypropylene containers under sessile conditions at 37 °C for 7 days. Each analysis was carried out in triplicate. The pH values of the supernatant liquors were measured using a Corning 140 pH meter and solution concentrations of Si, Ca, Al and P species were monitored by inductively coupled plasma analysis (ICP) using a TJA Iris simultaneous ICP-OES. Multi-element standards for ICP were matrix-matched with sodium chloride at the appropriate dilutions and the instrument detection limits for the elements of interest were: Si, 0.002 mM; Ca, 0.003 mM; P, 0.006 mM; and Al, 0.004 mM. The solid specimens were recovered by filtration, washed once with distilled water and dried in air at 37 °C for 24 h prior to analysis by Fourier transform infrared spectroscopy (FTIR) using a Perkin Elmer Paragon 1000 FTIR spectrophotometer. Spectra were recorded between 4000 and 500 cm^{-1} using KBr discs. The recovered specimens were also analysed by powder XRD but were not reground prior to analysis as grinding can dilute the surface concentration of HA to below the detection limit.

3. Results

3.1. Characterisation of WPC

Powder XRD analysis indicated that the principal crystalline phases of hydrated WPC paste used in this investigation are portlandite, ettringite, unreacted β-dicalcium silicate and calcium carbonate (as calcite), the latter phase having arisen from atmospheric carbonation (Fig. 1(a)). An EDX spectrum of the carbon-coated fracture surface of WPC paste confirms the presence of the main elemental constituents; calcium, silicon, aluminium, oxygen, magnesium and sulphur (Fig. 2(a)).

3.2. In vitro bioactivity

An EDX spectrum of the surface of the WPC particles, following a residence time of 7 days in SBF, is shown in Fig. 2(b). These data demonstrate that phosphorus and chlorine species are

Table 2
Composition of WPC

Major oxide components		Minor oxide components		Major crystalline phases	
Formula	Mass (%)	Formula	Mass (%)	Formula	Mass (%)
CaO	69.2	MgO	0.49	Ca_3SiO_5	65
SiO_2	25.0	P_2O_5	0.43	Ca_2SiO_4	22
Al_2O_3	1.76	Fe_2O_3	0.33	$Ca_3Al_2O_6$	4.1
SO_3	2.00	SrO	0.14	$Ca_2(Al/Fe)O_5$	1.0

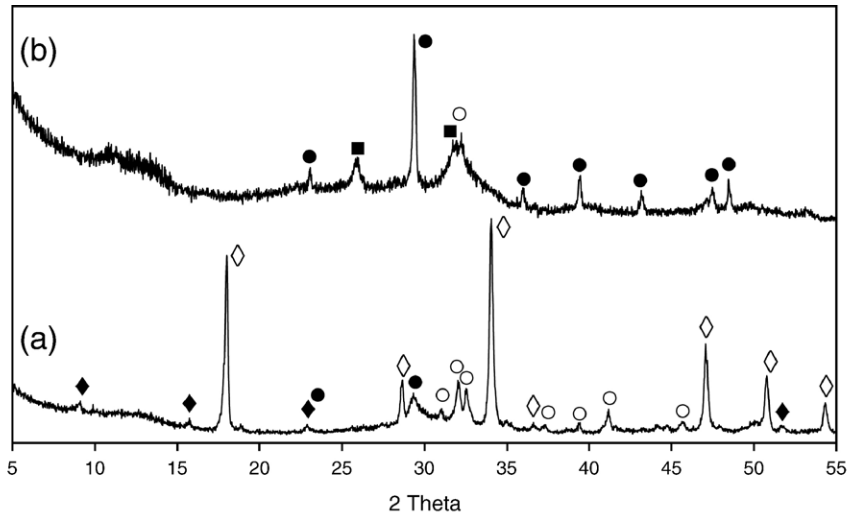

Fig. 1. Powder XRD data for (a) WPC paste (showing; ◆ ettringite, ◇ portlandite, ○ β-dicalcium silicate and ● calcite) and (b) WPC paste after immersion in SBF for 7 days (showing; ■ hydroxyapatite, ○ β-dicalcium silicate and ● calcite).

now present, along with increased proportions of sodium, magnesium and potassium. The signal-to-noise ratio of the corresponding powder XRD data of WPC paste after immersion in SBF is lower than that of the original WPC paste as the recovered solid matter was not re-ground prior to analysis (Fig. 1(b)). The characteristic (0 0 2) and (2 1 1) reflections of HA are observed at $2\theta \sim 25.8°$ and ~31.8°, respectively. Unreacted β-dicalcium silicate and increased proportions of calcite are also noted; whereas

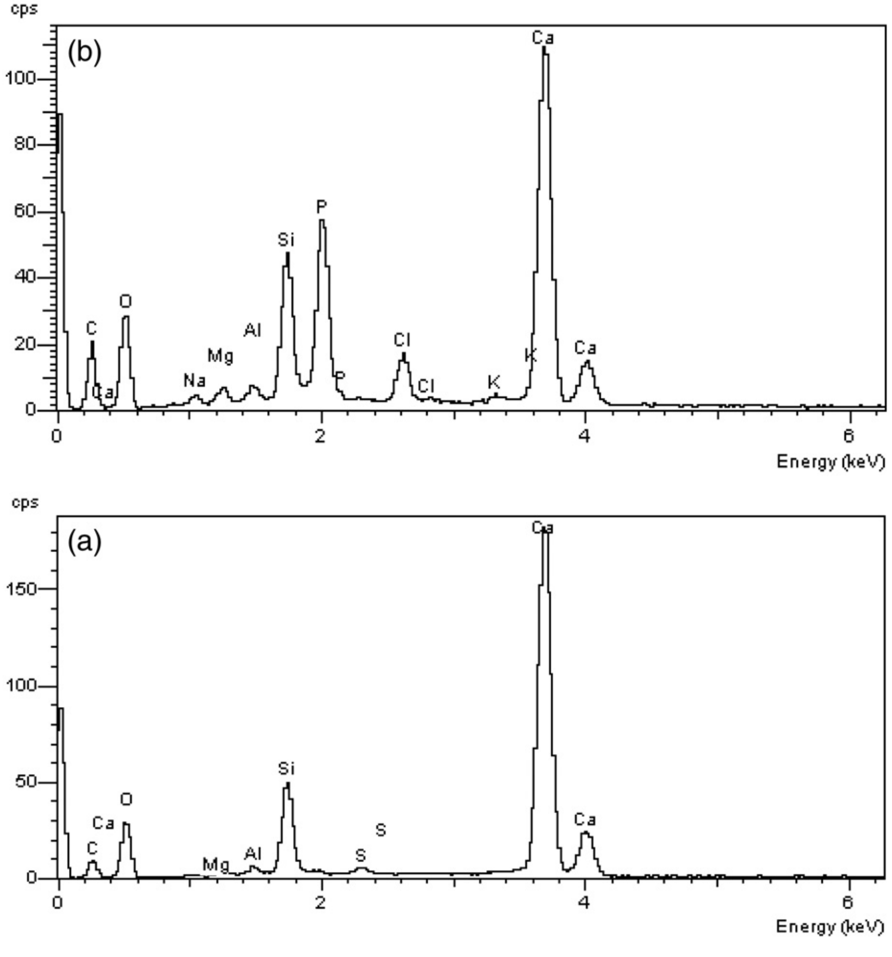

Fig. 2. EDX spectra of (a) WPC paste and (b) WPC paste after immersion in SBF for 7 days.

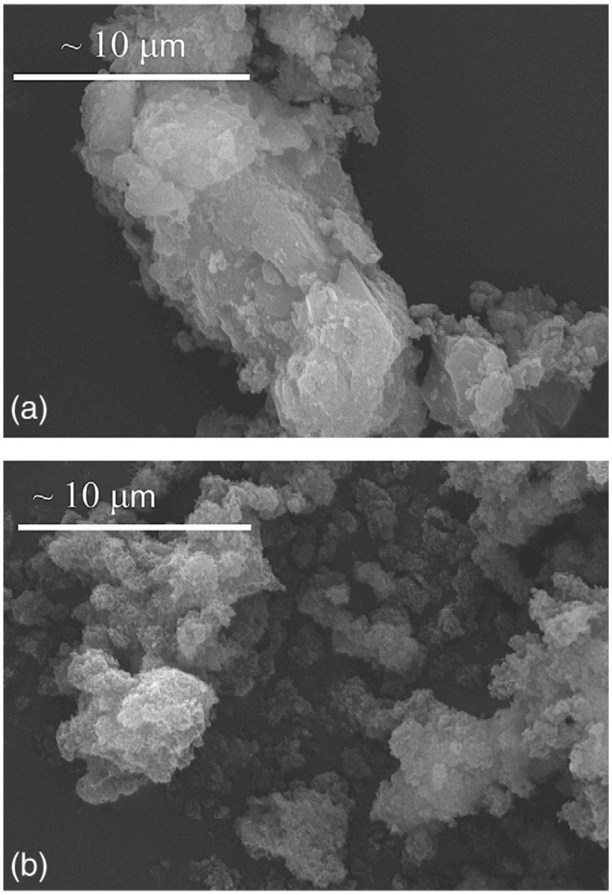

Fig. 3. Electron micrographs depicting the surfaces of (a) ground WPC paste after 28 days hydration, and (b) WPC paste following 7 days immersion in SBF.

reflections corresponding to ettringite and portlandite are no longer detected after exposure to SBF.

SEM analysis of WPC paste particles prior to and following immersion in SBF solution for 7 days (Figs. 3(a) and (b), respectively) confirms the formation of an HA layer. The surface of WPC after reaction with SBF (Fig. 3(b)) is seen to support a contiguous foliaceous deposit of similar morphology to the apatite layer reported to form on bioactive glass-ceramic [16].

FTIR spectroscopy was also used to detect the formation of HA on the surface of WPC and to confirm the dissolution of portlandite following immersion in SBF. For comparison, the FTIR spectra of hydroxyapatite and WPC are shown in Figs. 4(a) and (b), respectively. The wavebands at 570 and 605 cm^{-1} in the FTIR spectrum of HA are assigned to P–O bending modes; P–O stretching vibrations give rise to the bands at 960, 1060 and 1100 cm^{-1}, and signals at 620, 1610 and 3490 cm^{-1} are assigned to O–H vibrations (Fig. 4(a)) [17].

The band at ~970 cm^{-1} in the spectrum of WPC arises from Si–O stretching modes in C–S–H gel with contributions at 870 and 1120 cm^{-1} from carbonate and sulphate groups, respectively (Fig. 4(b)) [18]. The partially resolved doublet at 1420 and 1490 cm^{-1} is also attributed to carbonate species and the broad signals at 1660 and 3470 cm^{-1} arise from O–H vibrations of water and hydroxyl species. The stretching mode of O–H in crystalline calcium hydroxide gives rise to the sharp signal at 3670 cm^{-1} and the weak signal at ~550 cm^{-1} is assigned to octahedrally co-ordinated AlO_6 units in ettringite [19].

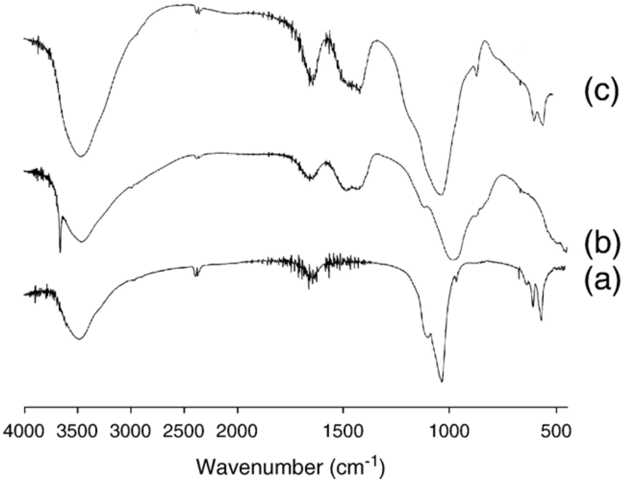

Fig. 4. FTIR spectra of (a) HA, (b) WPC paste and (c) WPC paste after exposure to SBF for 7 days.

The FTIR spectrum of WPC following immersion in SBF for 7 days is shown in Fig. 4(c). The doublet at 570 and 605 cm^{-1} is indicative of the deposition of crystalline HA on the surface of WPC, since these two wavebands are unresolved in amorphous HA [20]. And the dissolution of calcium hydroxide is also denoted by the disappearance of the sharp O–H signal at 3670 cm^{-1} in the FTIR spectrum of WPC after contact with SBF (Figs. 4(b) and (c), respectively).

3.3. Precipitation and dissolution characteristics

The concentrations of phosphorous, calcium, silicon and aluminium in human blood plasma, freshly prepared SBF and SBF following 7 days contact with WPC, are listed in Table 3 along with the corresponding pH values [6]. After 1 week of contact with WPC, the concentration of phosphorous species in SBF decreases from ~1.0 mM to below the limit of detection (0.006 mM); and the concentration of calcium ions increases from 2.4 to 8.5 mM. The depletion of phosphorus species from the SBF solution corresponds with the precipitation of HA and the concomitant release of calcium ions from WPC is principally attributed to the dissolution of portlandite. The associated increase in pH from 7.40 to 7.95 also arises from the dissolution of hydroxyl ions from portlandite, although this pH-excursion is

Table 3
Concentrations of P, Ca, Si and Al species in human plasma and SBF prior to and after 7 days of exposure to WPC

Solution	Concentration (mM)				pH
	P	Ca	Si	Al	
Human plasma	1.00	2.50	0.00	0.00	7.2–7.4
Initial SBF	1.01 (±0.01)	2.40 (±0.01)	b.d.l.	b.d.l.	7.40
Final SBF	b.d.l.	8.5 (±0.1)	1.32 (±0.05)	b.d.l.	7.95

b.d.l. indicates that the analyte is ‘below the detection limit’.

partially buffered by the components of SBF and is also mitigated by the incorporation of hydroxyl ions into the HA structure. It should be noted that, human blood plasma is also similarly buffered by a complex suite of organic and inorganic constituents. The dissolution of silicate species from the WPC matrix is denoted by a final silicon concentration of 1.32 mM and, conversely, the release of aluminium species, if any, was found to be below the limit of detection (0.004 mM).

4. Discussion

An indication of bioactivity (the ability to form a chemical bond with living bone tissue) can be obtained by the formation of an HA layer on the surface of a substrate in SBF *in vitro* [6]. Accordingly, this research has demonstrated that WPC, a material of increasing significance with respect to dental and hard tissue restoration, does indeed exhibit *in vitro* bioactivity.

When placed in contact with SBF, calcium hydroxide, in the form of portlandite, is leached from the WPC matrix. The released OH^- ions are partially buffered by the supernatant liquor and afford a moderate increase in pH from 7.40 to 7.95. These increases in both pH and concentration of Ca^{2+} ions (shown in Table 3) enhance the supersaturation of SBF with respect to hydroxyapatite which promotes the precipitation of the HA layer.

Functional groups, such as Si–OH, on the surface of calcium silicate glasses and ceramics have been shown to act as nucleation centres for HA precipitation [6,21]. Hence, it is thought that the deposition of HA onto WPC is attributed to both the dissolution of portlandite and to the high proportion of pre-existing Si–OH nucleation sites presented by the nanoporous calcium silicate hydrate (C–S–H) gel structure.

Recent research has shown that bioactive materials which release silicate species enhance the rate of new bone-growth [2,3]. This therapeutic effect arises from the influence of soluble silicate species on the genetic expression of bone-forming cells. In this respect, it is envisaged that the release of silicates, as indicated in Table 3, will confer additional *in vivo* bioactivity on WPC. It should also be noted that the solution concentrations of aluminate species leached from WPC, which are neurotoxic and can cause defects in adjacent bone-growth [22], were below the limits of detection (0.004 mM) after a 7-day immersion period.

Exposure to simulated body fluid, which contains 4.2 mM of HCO_3^- ions, is also seen to promote the formation of calcite on the surface of WPC. This phase arises from the direct carbonation of portlandite and other calcium-bearing cement matrix components and may also be formed by precipitation from the SBF solution constituents. In comparison, human blood plasma comprises 27.0 mM of HCO_3^-, and so, it is possible that the rate and extent of carbonation *in vivo* will exceed that observed *in vitro*.

Portland cement-based materials have been used successfully in dental applications for the past decade. Their ability to support the sustained attachment and proliferation of living tissues has been acknowledged and has prompted speculation that they may be appropriate for use in orthopaedic surgery [8]. This research has indicated that the likely mechanism of bonding between the Portland cement paste matrix and viable bone tissue is the spontaneous formation of an intermediate layer of HA on contact with human plasma.

Despite increasing clinical interest in Portland cements, very little is currently known about their chemistry in the physiological environment. This research has shown that, in addition to the precipitation of HA, rapid carbonation takes place when the Portland cement matrix is exposed to physiological liquors. This finding is fairly predictable and confirms the well-established reactivity of the cement phases to soluble carbonate species. Further research is now warranted to establish the setting characteristics, chemistry and durability of Portland cement articles *in vivo*; especially those intended for load-bearing applications. In a clinical environment, implant materials are likely to be exposed to blood, anaesthetics and antiseptics. If Portland cements are to be considered for use in orthopaedics the impact of these agents on their physicochemical and mechanical properties cannot be overlooked. Thus far, research in this field has been dominated by clinical and biological concerns, and accordingly, there are many outstanding research and development considerations which may be of interest to cement chemists and technologists.

5. Conclusions

This research has demonstrated that, on exposure to simulated body fluid *in vitro*, a layer of hydroxyapatite is precipitated on the surface of white Portland cement within 7 days of contact. It is speculated that this hydroxyapatite layer will provide a focus for the attachment and proliferation of living bone cells *in vivo* and that soluble silicate species released from the cement matrix are likely to enhance the rate at which new bone tissue is formed. Moreover, the observed retention of aluminate species by the cement matrix is also significant from a clinical perspective as soluble aluminium ions are neurotoxic and are reported to cause defects in the structure of bone.

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