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The antibacterial effects of zinc ion migration from zinc-based glass polyalkenoate cements

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Abstract Zinc-based glass polyalkenoate cements have been synthesised and their potential use in orthopaedic applications investigated. Zinc ions were released from the materials in a rapid burst over the first 24 h after synthesis, with the release rate falling below detectable levels after 7 days. Cement-implanted bone samples were prepared and the released zinc was shown, using energy dispersive X-ray analysis, to penetrate from the cement into the adjacent bone by up to 40 μm. Finally, the cements exhibited antibacterial activity against *Streptococcus mutans* and *Actinomyces viscosus* that reflected the pattern of zinc release, with the inhibition of growth greatest shortly after cement synthesis and little or no inhibition measureable after 30 days.

1. Introduction

Poly (methyl methacrylate) (PMMA) bone cement is currently the only material used for anchoring total joint replacements to the contiguous bone in cemented arthroplasties. However, PMMA can impair the correct functioning of the immune system, leading to a safe haven for the proliferation of contaminating bacteria around cemented prostheses [1] and thereby contributing to the 7% of hip revision surgeries caused by primary deep infection [2]. Glass polyalkenoate cements (GPCs), commonly used in dentistry, are recognised for their ability to prevent secondary caries through the release of antibacterial agents such as fluoride and strontium [3]. However, the presence of aluminium in the glass phase of commercially available GPCs has restricted their use in orthopaedics as aluminium is believed to cause defective bone mineralisation [4, 5] and has been implicated in the pathogenesis of degenerative brain diseases such as Parkinson's and Alzheimer's disease [6–8].

Zinc oxide (ZnO) has the ability to act as both a network modifier and intermediate oxide in a similar method to alumina, and the authors have previously shown that aluminium-free GPCs can be produced based on calcium-zinc silicate glasses [9]. The properties and characteristics of the resultant cements are similar to their aluminium-based counterparts and the replacement of aluminium with zinc makes these materials suitable for widespread orthopaedic applications. The novel zinc releasing bone cements examined in this study offer advantages over conventional acrylic bone cements. They are not based on a toxic monomer, they exhibit no significant exotherm [10] and *in vitro* work has indicated that they may bond directly to bone [11].

Zinc-containing materials such as zinc phosphate and zinc polycarboxylate cements have been utilised for a number of years in clinical dentistry, due to their ability to release ions that inhibit the growth of caries-related bacteria such as *Streptococcus mutans* and *Actinomyces viscosus* [12, 13]. In addition to its antibacterial activities, zinc is important in healthy bone growth and development [14] and is required for effective functioning of the body's immune system [15, 16]. Furthermore, low bioavailability of zinc has been found to reduce resistance to infection in aging members of the population [17].

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Table 1 Glass formulations expressed as mole fractions

Glass	CaO	SrO	SiO_2	ZnO
A	0.05	0	0.42	0.53
B	0	0.05	0.42	0.53

The aim of this study, therefore, was to highlight the potential of these zinc-based GPCs as alternative bone cements to PMMA, by establishing the extent of zinc release from them, evaluating the depth of penetration of these ions into surrounding bone *in vitro* and measuring the antibacterial properties of the released zinc.

2. Materials and methods

2.1. Glass compositions

Two glass compositions were produced, as described in Table 1. Glass A contains calcium, zinc and silica, while in glass B the calcium was replaced by strontium. Appropriate amounts of analytical grade silica, zinc oxide and either calcium carbonate or strontium carbonate were weighed out in a plastic tub and mixed in a ball mill (1 h), followed by drying in a vacuum oven (100°C, 1 h). The pre-fired glass batch was transferred to a mullite crucible for firing (1580°C, 1 h). The glass melts were then shock quenched into demineralised water and the resulting frit was dried, ground and sieved. The glass that passed through a 45 μm sieve was used to produce the cements.

2.2. Polyacrylic acid (PAA)

PAA was supplied in aqueous solution (25% w/v) by Ciba speciality polymers (Bradford, UK). The acid was coded E7 and had an average molar mass (M_n) of 8,140 [18]. The acid was freeze-dried, ground and sieved through a <90 μm filter.

2.3. Preparation of cement discs and extracts

Two cement formulations were prepared, A and B, based on glasses A and B, respectively. Each formulation used 1 g of the corresponding glass, 0.36 g of PAA and 0.55 ml of water, resulting in a P:L ratio of 1:0.91 for both cements. Split ring moulds were used to produce cement discs ($n = 3$) with a thickness of 1 mm and an internal diameter of 5 mm. The cements were mixed on a clean glass slab with a stainless steel dental spatula. Thirty seconds after mixing the moulds were filled to excess with cement, covered with acetate and clamped between Perspex plates. The clamped samples were maintained at 37°C for 1 h after the start of mixing. Flash was subsequently removed by grinding samples in their moulds with 1200 grit silicon carbide paper. Samples were de-moulded, washed with ethanol and stored in 10 ml aliquots of distilled water. Cement discs were removed from the water after 30 min, 1 h, 2 h, 4 h, 24 h, 7 days and 30 days. The zinc concentration of the extracts was measured using a Varian SpectrAA-400 Atomic Absorption Spectrometer, with a lamp-current of 5 mA in an air/acetylene flame with wavelength 213.9 nm. Standard solutions of 0.5 ppm, 1.0 ppm, 1.5 ppm and 2.0 ppm were used to calibrate the system prior to use and three measurements were taken from each aliquot in order to determine the mean concentration of zinc at each time interval.

2.4. Preparation of cement implanted bone sample

A piece of bovine femur (5 cm $\times$ 5 cm $\times$ 2.5 cm) was cleaned and a hole (5 mm $\emptyset$) was drilled through the center of the bone, followed by thorough washing with distilled water. Cement A was mixed as described previously and used to fill the hole in the bone to excess. This was covered at either end with acetate sheets, clamped between two steel plates and allowed to set for 1 h at 37°C. The construct was then removed from the clamp and each surface polished gently using 1200 grit silicon carbide paper. The construct was stored in distilled water for 30 days, following which it was removed, dried and lightly polished.

2.5. Scanning electron microscopy and energy dispersive X-ray analysis

A JOEL JSM-840 scanning electron microscope (SEM) equipped with a Princeton Gamma Tech (PGT) energy dispersive X-ray (EDX) system was used to obtain secondary electron images and to carry out chemical analysis of the bone-cement interface. EDX measurements were performed across the bone-cement interface in order to examine the extent of ion migration into the bone. Sites at which zinc and calcium measurements were taken are illustrated in Fig. 1 and ranged from 60 μm on the bone side of the bone-cement inteface (Figure, point 1), through the interface, to 60 μm into the cement (Fig. 1, point 7), with samples taken 20 μm distances apart. All EDX spectra were collected at 20 kV, using a beam current of 0.26 nA. Quantitative EDX converted the collected spectra into concentration data by using standard reference spectra obtained from pure elements under similar operating parameters, according to standard procedures.

2.6. Agar disc-diffusion test

The antibacterial activity of the cements was evaluated against the oral bacterial species *S. mutans* (American Type Culture Collection, ATCC, 25175) and *A. viscosus* (ATCC

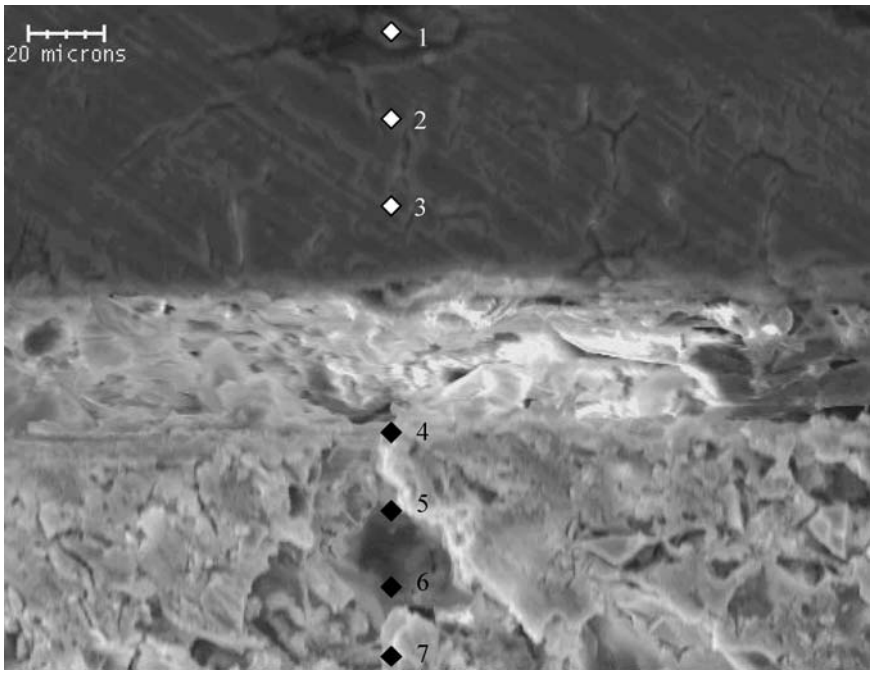

Fig. 1 Secondary electron microscope image of the bone-cement interface. Points for quantitative EDX are labelled 1–7 (spaced 20 μm apart). Control EDX data for bone was measured 2 cm from interface and at the centre of the cement implant for cement.

19246), using the agar disc-diffusion method [3]. The bacteria were grown from stock cultures on brain heart infusion (BHI) agar at 37°C for 16 h and isolated colonies were used to seed fresh cultures in 10 ml Luria Broth (LB). After incubation at 37°C for 12–16 h with shaking (200 rpm), the cultures were diluted in Mueller Hinton (MH) broth to give an OD_{600} of 0.05 for *S. mutans* and 0.1 for *A. viscosus*. A 350-μl volume of each bacterial suspension was streaked using clinical swabs on MH agar plates containing agar of 4 mm height, following which 2–3 discs of each material were placed on the agar. The plates were inverted and incubated under aerobic conditions (36 h, 37°C). Callipers were used to measure zones of inhibition at three different diameters for each disc and zone sizes were calculated as follows:

$$\text{Size of inhibition zone (mm)} = (\text{halo } \emptyset - \text{disc } \emptyset)/2.$$

All cements were analysed in triplicate and mean zone sizes ± standard deviations were calculated.

3. Results and discussion

3.1. Zinc release measurements

Cumulative zinc release from each cement was measured at intervals up to 30 days as outlined in section 2.3. In both cements the rate of zinc release was seen to decrease with respect to time, with the highest release rates measured immediately after production and no detectable zinc being released after 7 days (Figs 2 and 3). The absolute levels of zinc

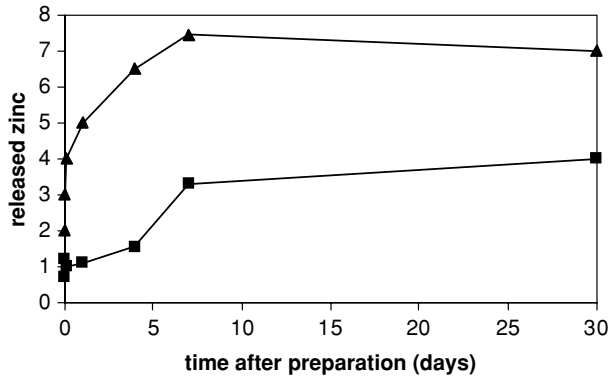

Fig. 2 Cumulative zinc release from cements A and B up to 30 days after synthesis.

released were lower in the case of cement B than cement A, even though the glasses used as the base for the two cements contain identical amounts of zinc (Table 1). There is no obvious reason why this should be the case but it is possible that the replacement of calcium with strontium in glass B results in a more integral network, thereby inhibiting the release of zinc. Further investigation of the glass structure and the mechanical properties of the cements would be necessary to confirm this.

The observed pattern of a sudden zinc release concurs with other published studies: Osinaga and co-workers [13] reported that the highest zinc release was detected in their zinc-added glass ionomer-based cements within 24 h of preparation and that zinc release was completed within the 15 day period of the study. Meanwhile, Foley and Blackwell [12] have reported the highest rate of zinc release from zinc-containing

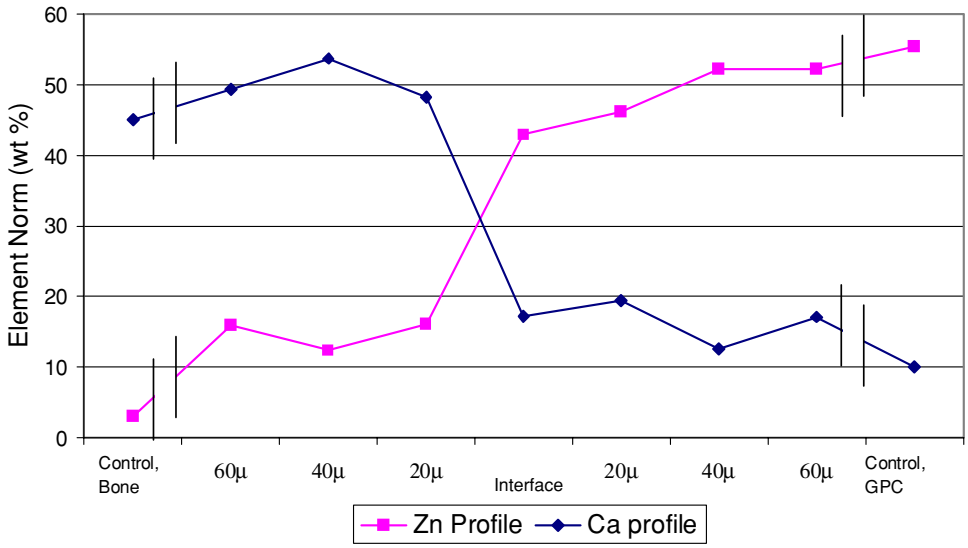

Fig. 3 Normal weight % EDX data for calcium and zinc content across the interface of GPC implanted into bovine bone. EDX points are illustrated in Fig. 1. Control values are included.

cements to occur after 2 days – the shortest incubation period investigated in their analysis – and a zinc release rate of "effectively zero" after 28 days.

3.2. Investigation of bone-cement interface

SEM and EDX were used to study the interface of the GPC implanted into bone and to generate calcium and zinc profiles across the interface. Control EDX data were measured for bone 2 cm from the bone-cement interface and at the centre of the implant for the cement. The EDX data indicated significant migration of zinc into the bone from a depth of 20–40 μm in the cement. This migration increased the normal zinc content in the bone from 3.1 wt%, as determined in the bone control sample, to 14.8 wt% 60 μm away from the cement interface in the bone. Given that zinc ions have been shown to have significant antibacterial effects *in vivo* and *in vitro*, this increased concentration of zinc ions in the cement-contiguous bone, and at the bone-cement interface in particular, may have important implications for the inhibition of growth of contaminating bacteria in prosthetic joints.

3.3. Antibacterial activity of cements

Inhibition of bacterial growth showed a clear correlation with released zinc, as zones of growth inhibition were largest with freshly prepared cements and became smaller as incubation times increased (Figs 4 and 5). This trend was observed with both cements and with both bacterial species tested. It correlates well with the atomic absorption results which indicate that the leachable zinc was lost rapidly to the storage solution (Fig. 2), so that by 7 days most of the leachable zinc had been lost from the cement sample and was unavailable for release into the agar plate. Accordingly, after 30 days' incubation in distilled water, zones of inhibition were not significantly above zero in 3 of the 4 cement-bacterial combinations tested (Figs 4 and 5), indicating that the antibacterial effects of the samples had all but disappeared. Other researchers have also reported the greatest inhibition of *S. mutans* growth to be associated with an initial high level of ion release from zinc-containing cements, followed by reduced antibacterial activity over time due to significantly lower ion release levels [14]. In one such study, inhibition of *S. mutans* growth was detectable only with 1 h aged cements and no inhibition was detectable in materials that had been aged for 15 days, though no measurements were taken in the intervening time as in this work [15].

The antibacterial activity measured with cement A was greater than that of cement B, which correlated with the higher zinc release rates observed with the former (Fig. 2). The strontium included in cement B was not found to increase the antibacterial effects of the cements, even though it has previously been shown to be inhibitory to bacterial growth when incorporated into similar materials [3]. In the previous published analysis, however, strontium concentrations were considerably higher than those investigated in this work, which may account for the discrepancy.

There was no significant difference between the inhibitory effects of the cements on *S. mutans* and *A. viscosus*. Such comparisons between different cultures are difficult to make, however, as agar diffusion assays are extremely sensitive to the density and growth phase of the individual cultures at the time of harvesting. Nevertheless, the two cements exhibited significant inhibition of both bacterial species.

Numerous studies have outlined the complications associated with bacterial infection after total hip arthroplasty (THA) [19, 20]. The bacteria most commonly found in

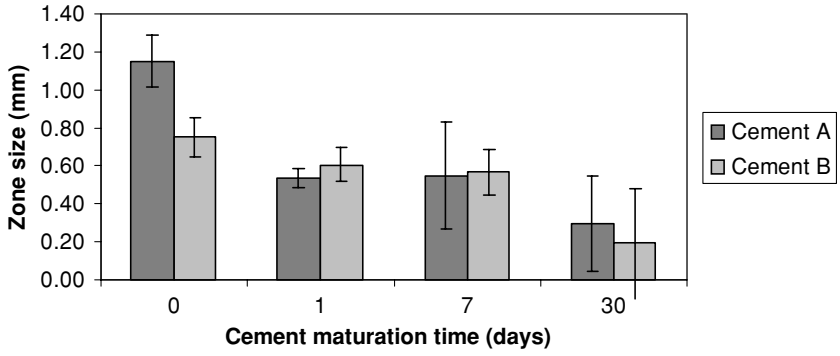

Fig. 4 Inhibition of growth of *A. viscosus*.

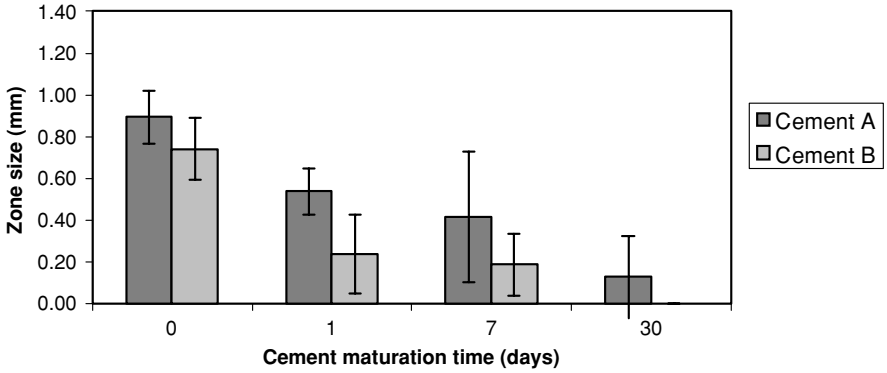

Fig. 5 Inhibition of growth of *S. mutans*.

infected hip joints are *Streptococci* and *Staphylococci* [21, 22], though members of numerous other genera such as *Actinomyces* [23, 24], including *Actinomyces viscosus* [25], *Prevotella* [26] and *Pseudomonas* [27] have also been reported. Thus, the two bacteria analysed in this study, as well as being common caries-associated species, were selected as representative of the many bacterial species isolated from infected hip joints. In addition, there is a recognised link between caries-causing microorganisms and bacterial infection of prosthetic hip joints, with a number of studies reporting THA infections that were preceded by dental work and associated with bacteria that are normally found exclusively in the mouth [28–31]. The growth inhibitory effects observed with *S. mutans* and *A. viscosus* in this work are therefore clearly of relevance to prevention of THA infections in a clinical setting.

Bacterial infections of prosthetic hip joints have a considerable financial consequence, both for the patient and the healthcare provider, and can necessitate extended antibiotic treatment and numerous surgical interventions, as well as causing long-term physical and mental hardship for the patient. In an attempt to avoid such infections, antibiotic-impregnated cement spacers have been utilised in THA in order to achieve localised, extremely high concentrations of antibiotics that minimise bacterial growth [20, 31]. Problems associated with such antibiotic-impregnated cements, however, include weakening of the cement itself and the generation of antibiotic-resistant bacteria in infected implant sites [31].

As zinc inhibits multiple activities in the bacterial cell, such as glycolysis, transmembrane proton translocation and acid tolerance [14], it has been shown to exhibit an antibacterial effect at considerably lower concentrations than many antimicrobial agents [32]. Furthermore, though generally regarded as bacteriostatic, it can have bactericidal effects also, particularly when used in combination with other ions, such as fluoride [33], or antibacterial agents [34]. Therefore, the use of zinc-containing cements such as those investigated here might permit incorporation of antibiotics at considerably lower concentrations than are currently necessary to avoid bacterial infections in THA. This would not only reduce sample weakening but, due to the simultaneous presence of the two antibacterial agents, might be expected to reduce the rate at which antibiotic resistant bacteria arise.

4. Conclusions

Bacterial infection after THA is a serious complication of hip replacement surgery that frequently necessitates revision surgeries. In this work, we have synthesised and characterised zinc-containing glass polyalkenoate cements to investigate their potential use in THA. Zinc was released from the study cements in a rapid burst after synthesis and released zinc ions

could penetrate into contiguous bone by up to 40 μm. The growth of two characteristic bacterial species, *Streptococcus mutans* and *Actinomyces viscosus*, was significantly inhibited by the released zinc in *in vitro* studies, while maximal zinc release rates corresponded with the greatest inhibition of the two bacteria. We suggest that such zinc-containing materials may have potential in hip replacement surgery due to this inhibitory activity and may be particularly effective if used in conjunction with impregnated antibiotics. This dual selective pressure would allow the use of lower concentrations of antibiotics, thus reducing cement weakening associated with antibiotic impregnation, and would reduce the frequency with which resistant bacteria colonise such joints.

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