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Antibacterial coatings for medical devices based on glass polyalkenoate cement chemistry

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Abstract A biofilm is an accumulation of micro-organisms and their extracellular products forming a structured community on a surface. Biofilm formation on medical devices has severe health consequences as bacteria growing in this lifestyle are tolerant to both host defense mechanisms and antibiotic therapies. However, silver and zinc ions inhibit the attachment and proliferation of immature biofilms. The objective of this study is to evaluate whether it is possible to produce silver and zinc-containing glass polyalkenoate cement (GPC) coatings for medical devices that have antibacterial activity and which may therefore inhibit biofilm formation on a material surface. Two silver and zinc-containing GPC coatings (A and B) were synthesised and coated onto Ti6Al4V discs. Their handling properties were characterised and atomic absorption spectrometery was employed to determine zinc and silver ion release with coating maturation up to 30 days. The antibacterial properties of the coatings were also evaluated against *Staphylococcus aureus* and a clinical isolate of *Pseudomonas aeruginosa* using an agar diffusion assay method. The majority of the zinc and silver ions were released within the first 24 h; both coatings exhibited antibacterial effect against the two bacterial strains, but the effect was more intense for B which contained more silver and less zinc than A. Both coatings produced clear zones of inhibition with each of the two organisms tested. In this assay, *Ps. aeruginosa* was more sensitive than *S. aureus*. The diameters of these zones were reduced after the coating had been immersed in water for varying periods due to the resultant effect on ion release.

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1 Introduction

Biofilms are microbially derived sessile communities characterized by cells that are irreversibly attached to a substratum or to each other and embedded in a matrix of extracellular polymeric substances that they have produced. Such micro-organisms exhibit an altered phenotype with respect to growth rate and gene transcription [1]. Biofilms that form on implanted medical devices are particularly problematic since the extra cellular matrix exported by the micro-organisms along with the changes in their physiology results in the requirement to remove the device to effect a microbiological cure [2, 3]. However, the mechanisms of biofilm formation are poorly understood and effective prevention and therapeutic strategies still need to be developed for device-associated infections. Treatment with antibiotics can slow down biofilm progression by eliminating planktonic cells and interfering with biofilm metabolism [4], but cure is rare. Other eradication methods that have been employed include prevention of initial attachment of bacterial cells by constructing materials into which antimicrobial agents (e.g. silver ions) have been incorporated [4] and minimising biofilm formation by the disruption of quorum-signalling

molecules, which may, in turn, disrupt the biofilm structure allowing for improved inactivation and removal [5].

Glass polyalkenoate cements (GPCs), formed by the reaction between an ion-leachable glass and an aqueous solution of polyacrylic acid (PAA) [6], have proven to be both antibacterial and cariostatic [7]; properties related to their ability to release beneficial amounts of therapeutic ions [8, 9]. Commercially available GPCs are all based on aluminium glass chemistry [10]. The composition of the glass is critical to the setting of these cements and aluminium is present because it can isomorphically replace the SiO_4 tetrahedra within the glass structure. This causes a local charge imbalance within the structure, resulting in the acid degradability of the glass [11]. However, the presence of aluminium retards the medical and surgical applications of such cements as aluminium ion (Al^{3+}) release in vivo can cause demineralisation of the bone [12] and has been implicated in the pathogenesis of degenerative brain diseases including Parkinson's and Alzheimer's disease [13, 14].

The zinc ion (Zn^{2+}) performs a similar role in glass forming to the Al^{3+} ion, in that it has the ability to act as both a network modifier and an intermediate oxide, but it does not cause defective bone mineralisation [12, 15]. Studies have shown that inhibition of bacterial growth correlates with released zinc from zinc phosphate cements (ZPC) [16] and zinc sulphate incorporated GPCs [17, 18]. The minimum zinc concentration required for bacterial inhibition is $>6.53 \times 10^{-4}$ ppm [19]. Zinc inhibits multiple activities in the bacterial cell including glycolysis, transmembrane proton translocation and acid tolerance [20]. Silver is also a known antibacterial agent [21, 22] as the silver ion (Ag^{+}) binds to negatively charged components in proteins and nucleic acids, thereby causing structural changes in bacterial cell walls, membranes and nucleic acids that affect bacterial cell viability. To have antimicrobial efficacy, silver ions must be released in biocidal concentrations. The minimum silver required for inhibition of bacteria is 3.25 ppm [23].

The objective of this study is to evaluate the proof of concept that GPC coatings can be produced containing silver and zinc ions, and these coatings will adhere to metal surfaces and release concentrations of active ions which can be shown to be antibacterial.

2 Materials and methods

2.1 Glass compositions

Two glass formulations were synthesised (Table 1).

Appropriate amounts of analytical grade reagents were weighed out in a plastic tub and mixed in a ball mill (1 h), then dried in a vacuum oven (100°C, 1 h). The reagents were fired in mullite crucibles (1480°C, 1 h) and shock quenched into water. The resulting frit underwent grinding in a gyromill (15 min) and the glass powder was passed through a 25 μm sieve. All further work was undertaken on the sub 25 μm particles.

Table 1 Glass compositions (mol. fraction)

Glass	SiO_2	ZnO	Ag_2O	Na_2O
A	56.04	32.98	0.11	10.87
B	56.04	32.76	0.33	10.87

2.2 Polyacrylic acid (PAA)

Ciba specialty polymers (Bradford, UK) supplied the polyacrylic acid (PAA), which was coded E11 (Mw, 210,000) in aqueous solution (25 vol%). The PAA was subsequently freeze dried and ground (maximum particle size, 90 μm).

2.3 Glass characterisation

2.3.1 X-ray diffraction

Diffraction patterns for both glasses were collected using a Philips Xpert MPD Pro 3040/60 X-ray Diffraction Unit (Philips, Netherlands) using Cu Kα radiation. A generator voltage of 40 kV and a tube current of 35 mA were employed. Diffractograms were collected in the range $5° < 2\theta < 80°$, at a scan step size 0.0083° and a step time of 10 s.

2.3.2 Differential thermal analysis

A differential thermal analyser-thermal gravimetric analyser (DTA-TGA, Stanton Redcroft STA 1640, Rheometric Scientific, UK) was used to measure the glass transition temperature (T_g) for both glasses. A heating rate of 10°C/min was used in an air atmosphere up to a maximum temperature of 1000°C, using alumina as a reference in a matched platinum crucible.

2.3.3 Network connectivity

The network connectivity of both glass networks was determined from the molar composition using Eq. 1. Calculations were based on the assumption that silver and zinc were acting as network modifiers in the glass network.

$$\mathrm{NC} = \frac{\text{No. BOs} - \text{No. NBOs}}{\text{Total no. bridging species}} \quad (1)$$

where BO = bridging oxygens, NBO = non-bridging oxygens.

2.4 Cement preparation

Two GPCs, A and B, were prepared by mixing 0.5 g glass (A and B, respectively), with 0.2 g PAA and 0.2 ml distilled water. Mixing was undertaken on a clean glass plate with a dental spatula in ambient laboratory conditions.

2.5 Cement rheology

Working times (W_t) and setting times (S_t) of the GPCs were determined. W_t was considered to be the duration from commencement of mixing to the point when the cement is no longer pliable. S_t is defined in ISO9917 [24].

2.6 Coating preparation

Coatings were produced by spreading cements onto Ti6Al4V discs (Engineering Sheets Limited, Limerick, Ireland) of known size (25 mm Ø, 2 mm thick) and weight. Each cement/disc construct was weighed prior to being clamped to another Ti6Al4V disc, separated from the coating by an acetate sheet (Fig. 1). This sandwich structure was then stored in an oven (37°C, 24 h), prior to declamping, to ensure that coatings were as well bonded to the metal as possible.

The second disc and acetate were removed and the construct reweighed. The cement weight was calculated by Eq. 2.

$$\text{GPC weight} = \text{construct weight} - \text{disc weight} \quad (2)$$

2.7 Ion release evaluation

Evaluation of both zinc (Zn^{2+}) and silver (Ag^{+}) ion release was undertaken at 1, 7 and 30 days. GPC/disc constructs were matured in purified water (Reagecon Limited, Shannon, Ireland); with up to 65 ml of water immersing the samples. Three samples of both cements were produced.

Standard solutions of silver and zinc were formulated as per the literature [25], for zinc concentrations of 0.1, 0.5, 1.0 and 2.0 parts per million (ppm) and for silver concentrations of 0.5, 1.0, 5.0 and 10.0 ppm.

Ion release was evaluated using the Varian Spectra AA-220 FS atomic absorption spectrometer (AAS, Varian, Australia) from each cement construct immersed in water ($n = 3$). Five 1 ml extracts were taken from each sample. The zinc concentrations released from the coatings exceeded the detection limit of the apparatus and, for this reason, the solutions for evaluation of zinc release were diluted (1:10) with purified water. The pH of the solutions was also evaluated using an Accumet Portable pH/mV/°C Meter (Fisher Scientific, Ireland). Calibration was with pH4 and pH6 buffers.

2.8 Antimicrobial assay

Staphylococcus aureus strain Oxford and a clinical isolate of *Pseudomonas aeruginosa* were grown overnight at 37°C on Brain Heart Infusion agar (BHI; Oxoid Ltd, Poole, UK). Suspensions of each organism were prepared in sterile phosphate buffered saline (PBS), pH 7.2, to a density of approximately 10^7/ml and 200 µl of each were spread evenly on separate BHI agar plates. Titanium discs (25 mm Ø) coated with either of the GPCs were sterilised by exposure to UV light (350 nm) for 2 h at room temperature. One disc was then placed on each lawn of bacteria and incubated (16 h, 37°C); these samples were designated as control coatings. The diameters of any inhibitory zones produced were measured at 3 points with callipers and values were expressed as diameters of zones (mm) minus the diameter of the titanium discs, with each assay being performed in triplicate. Data presented are means of these triplicate zones with three measurements per zone ± standard deviation. The cement determined to be the most antibacterial from this study underwent further testing by being left to elute in distilled water for 1, 7 and 30 days before assaying for antimicrobial activity as above.

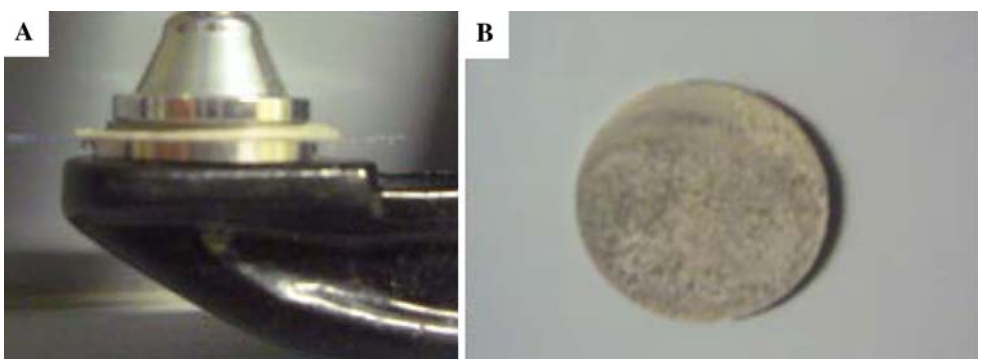

Fig. 1 (**a**) Sandwich structure clamped. (**b**) Thin GPC coating on Ti6Al4V disc

3 Results and discussion

3.1 Glass characterisation

Glasses were produced and characterised as outlined in the methods section. Both glasses were determined to be amorphous with identical T_gs (597°C) and network connectivity (2.43).

3.2 Cement characterisation

W_t and S_t were evaluated for both GPCs and are compiled in Table 2. The W_t of both cements ensured that the cement coatings were placed evenly on the disc. The W_t of the cements facilitated the deployment of a uniform coating on the metal discs. Regarding the long S_ts of the cements compared to previous work on GPCs [26, 27], these may be a result of the high molecular weight PAA, E11 (Mw, 210,000) employed for formulating the cements. The literature confirms that zinc based GPCs can form set cement bodies at the mixing ratio employed herein [28, 29], however all previous work used lower molecular weight PAA (Mw, 80,800) for this purpose. The extended chain lengths of E11 in the same volume of water used in the mixing regimes highlighted in the literature may result in compromised hydrolysis leading to incomplete uncoiling of the PAA chains retarding entanglement and formation of partially covalent bonds [30] between the zinc ion and the carboxylate groups.

3.3 Ion release evaluation

Given the extended S_t for the cements (Table 2), cement/metal constructs for ion release evaluation and antibacterial assays were prepared as outlined in Sect. 2.6 to ensure that the materials had fully set. In agreement with the literature [18], the majority of the Zn^{2+} ion release (1.5 ppm) occurred in the first 24 h (Fig. 2), with another 1 ppm being release after 7 days and the remaining 1 ppm being released over 30 days.

Only coating B showed appreciable Ag^{+} release; the majority of which (0.2 ppm) occurred within the first 24 h (Fig. 3). This was due to a greater mol % of silver in the construct. Over the next 29 days, only an additional 0.1 ppm Ag^{+} was released by the coatings. With reference to the literature, Zn^{2+} release was in biocidal concentrations (>6.53×10^{-4} ppm) [19], but below toxic levels for humans [31]. However, the concentration of silver ions released were both below biocidal concentrations (3.25 ppm) [23] and toxic levels for humans [32].

As illustrated in Fig. 3, coating B releases more Zn ions than coating A, despite the fact that coating A contains more Zn. This result is anomalous given that the only difference in each coating is the substitution of 0.22 mol. fraction Ag for Zn. Moreover, each coating is produced using identical powder: liquid (P:L) ratios with completely amorphous glasses having identical T_g values of 597°C. Nevertheless, the results of increased ion release of Ag and Zn in coating B versus coating A is uniform across all time frames examined. In order to identify the cause of this effect further work is necessary and shall comprise a detailed structural analysis of each glass using MAS-NMR, and cement structural characterization using FTIR.

Table 2 Working and setting times of the cement formulations

Cement	W_t	S_t
A	4m12s	16h07m41s
B	5m22s	16h15m23s

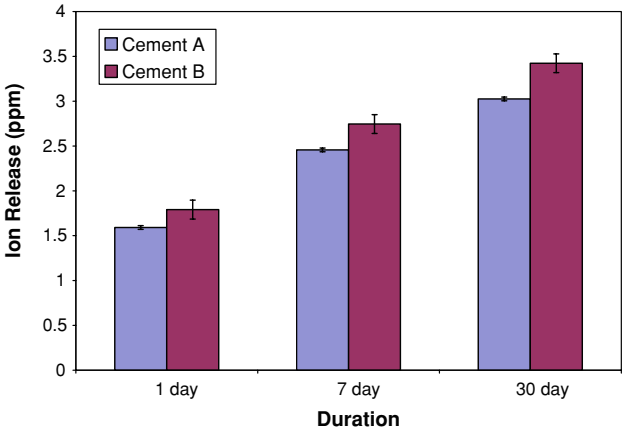

Fig. 2 Cumulative zinc ion release for cements A and B

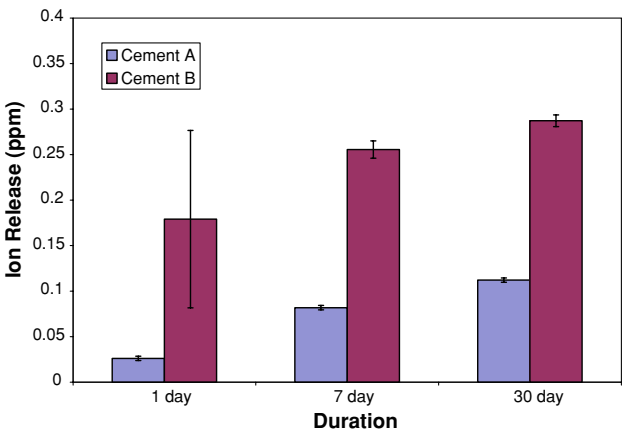

Fig. 3 Cumulative silver ion release for cements A and B

3.4 Antimicrobial assay

Two organisms were selected for this initial study, *S. aureus* and *Ps. aeruginosa.* These represent Gram-positive and Gram-negative bacteria respectively and both species are common aetiological agents of hospital-acquired infections. Contamination of surfaces by these organisms is a potential reservoir for spread of infection within health-care settings and they can readily form biofilms on medical devices.

Both coatings produced clear zones of inhibition with each of the two organisms tested and Table 3 shows the mean diameters of the zones of inhibition obtained. This antibacterial efficacy is independent of pH, given that a pH of 7 was recorded for all solutions at all time frames, inferring that the metal ion release, and not H^+ release from the polyacrylic acid, is responsible for the coatings' efficacy.

It is evident that cement B was more antibacterial to both strains and that *Ps. aeruginosa* was more sensitive than *S. aureus*. The diameters of these zones were reduced after the coating had been immersed in water for varying periods. There is no clear trend in inhibition zone size with respect to elution time for the two bacteria, probably due to the high standard deviations caused by loosening of the coatings in some instances, but results from all elution times were substantially lower than for the untreated coatings (student *t*-test *S. aureus* eluted v untreated $P < 0.005$; *Ps. aeruginosa* eluted vs. untreated $P < 0.0005$. Elution *S. aureus* 1 day vs. 7 day $P = 1$; 7 day vs. 30 day $P = 0.69$; 1 day vs. 30 day $P = 0.62$; *Ps. aeruginosa* 1 day vs. 7 day $P = 0.26$; 7 day vs. 30 day $P = 0.67$; 1 day vs. 30 day $P = 0.45$). This confirms that inhibitory ions were lost from the material by elution in water.

In the case of the coatings that had been immersed in water, some of the material on the discs became dislodged from the surface, resulting in a non uniform coating. Inhibition of growth was only observed where material was retained at the edge of the disc. This explains the large standard deviations in the measurements. However, where material was present at the edge of the disc, the width of the inhibitory zone was still reduced compared with untreated (not eluted) material. So, for example, before elution (30 days) the mean width of the inhibition zone from the edge of the titanium discs for *S. aureus* was 2.3 mm and after immersion the maximum width was 1.25 mm, while for *Ps. aeruginosa* before elution it was 5.15 mm and afterwards the maximum was 2.25 mm.

It can be seen from the above results that by increasing the silver and decreasing the zinc content between cements A and B in the constructs improves antibacterial capability. Although the silver being released is not completely biocidal on its own, it has a disruptive effect on the cement structure, causing the zinc to release more ions. It is this cumulative effect that imparts the antibacterial nature to the cements [33].

Table 3 Mean growth inhibitory zones (mm ± SD) produced by coatings

Construct	*S. aureus*	*Ps. aeruginosa*
Uncoated titanium	0 mm	0 mm
Control coating A	0.73 ± 0.3 mm	4.6 ± 1.1 mm
Control coating B	4.6 ± 1.3 mm	10.3 ± 0.6 mm
Coating B after elution in H_2O for		
1 day	1.0 ± 0.9 mm	2.8 ± 1.6 mm
7 days	1.2 ± 1.7 mm	4.1 ± 2.0 mm
30 days	1.42 ± 1.5 mm	3.6 ± 1.7 mm

4 Conclusions

Biofilm formation on medical devices has severe health consequences as it provides a sanctuary for bacteria which are tolerant to both host defense mechanisms and antibiotic therapies. However, silver and zinc ions are known to inhibit the attachment and proliferation of immature biofilms. The objective of this work was to determine the feasibility of formulating coatings from GPCs containing both zinc and silver in the glass phase that exhibit antibacterial activity. The work has shown that it is possible to form thin film GPC coatings which adhere to surgical metals and, in solution, can release zinc and silver ions which retard bacterial growth and thereby should inhibit biofilm formation.

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