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Antibacterial Properties of a Tri-Sodium Citrate Modified Glass Polyalkenoate Cement

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Abstract: Primary deep infection following joint replacement surgery accounts for 7% of all revisions. Glass polyalkenoate cements (GPCs) have previously been shown to exhibit antibacterial properties. The present study had two objectives. The first was to determine if addition of tri-sodium citrate (TSC) to the powder phase of an Al-free GPC (0.04 SrO–0.12 CaO–0.36 ZnO–0.48 SiO2, by mole fraction) enhanced the resultant cement's antibacterial properties against three strains of bacteria that are commonly found in periprosthetic sites following total joint replacements (TJRs); namely, *E. coli*, *B. fragilis*, and *S. epidermidis*. Four cement sets were prepared, which contained 0 wt% TSC (control), 5 wt% TSC, 10 wt% TSC, and 15 wt% TSC. All the TSC-modified cements were found to exhibit large inhibition zones against all the bacterial strains, especially the cement containing 15 wt% TSC against *E. coli*. The antibacterial properties of the TSC containing GPCs are attributed to the release of Zn and Na ions from the cements and the presence of the TSC. The second objective was to investigate if, when a modified GPC is embedded in a bovine bone model, ionic transfer occurs. It was found that Zn ions migrated from the cement to the surrounding bone, particularly at the cement–bone interface. This is a desirable outcome as Zn ions are known to play a vital role in both bone metabolism and the regeneration of healthy bone. The present results point to the potential clinical benefits of using TSC-modified GPCs in TJRs. © 2009 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 90B: 700–709, 2009

Keywords: glass polyalkenoate; antibacterial; bone cement; tri-sodium citrate

INTRODUCTION

Polymethylmethacrylate (PMMA) bone cement is considered the gold standard in orthopedics, being widely used in anchoring total joint replacements (TJRs) as well as in the stand-alone augmentation of fractured osteoporotic vertebral bodies (vertebropasty and balloon kyphoplasty).[1,2] However, PMMA is known to have a number of drawbacks, such as a favorable environment for the proliferation of bacteria.[3]

Glass polyalkenoate cements (GPCs) were developed in the 1970s[4] and are predominantly used in luting and restorative dental applications.[5] However, due to their excellent biocompatibility,[6] their ability to adhere to surgical metals and bone, and their lack of volumetric shrinkage and negligible setting exotherm,[3] they have been considered for skeletal applications. GPCs set via an acid base reaction involving an ion leachable glass (base), and a polyalkenoic acid, usually polyacrylic acid (PAA). The metal cations in the glass serve to crosslink the polyacrylate chains resulting in a hard material comprising a polysalt matrix embedded with glass particles.[7,8] GPCs can be formulated to release clinically beneficial ions over time, such as fluoride[9]; responsible for the antibacterial nature of the dental cements.[10] The antibacterial nature of zinc (Zn) and strontium (Sr) ions in GPCs has also been previously established by the authors.[11,12]

One of the primary concerns associated with the use of conventional (Al-containing) GPCs in orthopedics is the release of Al^{3+} from the set cement *in vivo* because these ions have been implicated in the pathogenesis of degenerative neurological disorders such as Alzheimer's disease,[13] and have been identified as the primary cause of a patient's death in a case of otoneurosurgery performed using conventional Al-containing GPCs.[14] Al-free GPCs have been formulated using glasses from the $ZnO–CaO–SiO_2$ and

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$ZnO–CaO–SrO–SiO_2$ systems. These cements have many advantages; for example, Zn has a stimulatory effect on osteoblast DNA synthesis in newborn rats and on bone protein synthesis[15] and imparts an antibacterial nature to the cement,[16] while Sr increases osteoblastic activity and inhibits osteoclastic activity.[17,18]

Bacterial infection following TJRs is a common problem that is usually treated using antibiotic loaded PMMA cement. However, an increase in the use of these cements has led to an increase in the incidence of bacterial resistance to the antibiotics. Thus, in evaluating the suitability of a new fixation material for use in TJRs, particular attention should be paid to its antibacterial activity. The antibacterial properties of Al-free GPCs appear to be limited. Thus, there is a clinical need for methods enhancing this behavior. Addition of tri-sodium citrate (TSC) to the cement powder may be one such method. TSC is widely used in medical devices such as in catheters used in hemodialysis.[19–21] Furthermore, fibroblast and osteoblast interaction around an implanted TSC-modified hydroxyapatite (HA)/collagen composite material has been reported.[22]

The present study had two objectives. The first was to determine the influence of TSC on the antibacterial activity of an Al-free GPC against three bacterial strains that are commonly found in periprosthetic sites; namely, *E. coli*, *B. fragilis*, and *S. epidermidis*. The second objective was to investigate if, in case when a TSC-containing GPC is embedded in a bovine bone void model, ionic transfer from the cement into the bone occurred at the cement–bone interface.

MATERIALS AND METHODS

Glass Synthesis

A strontium–calcium–zinc–silicate glass formulation, designated BT101 (0.04 SrO/0.12 CaO/0.36 ZnO/0.48 SiO_2, by mole fraction) was synthesized. The glass was prepared by weighing out appropriate amounts of analytical grade reagents (Sigma-Aldrich, Dublin, Ireland) and ball milling for 1 h. The mixes were then dried in an oven (100°C, 1 h), then fired (1480°C, 1 h) in a mullite crucible and shock quenched in water. The resulting frit was dried, ground and sieved to retrieve a glass powder with a maximum particle size of 45 μm.

PAA

PAA used in this study (M_w, 80,800) was supplied by Advanced Healthcare Limited (Kent, UK). The PAA was ground and sieved to retrieve <90 μm particles.

TSC

The TSC used in this study was obtained from Reagacon (Shannon, Ireland) and was added to the cement at 5 wt%, 10 wt% and 15 wt% additions.

Cement Preparation

Cement formulations (Table I) were prepared by thoroughly mixing the glass powder with the PAA and distilled water on a clean glass plate using a dental spatula at a powder to liquid ratio of 2:1.5. Additions of 5 wt%, 10 wt%, and 15 wt% TSC were also added to cement formulation as outlined in Table I.

Complete mixing was undertaken within 30 s. Split ring molds were used to produce cement discs (1.5 mm $\times$ 15 mm Ø, $n = 3$). After mixing, the moulds were filled to excess with cement, covered with acetate and clamped between perspex plates. The clamped specimens were allowed to set in an oven (air atmosphere) at 37°C for 1 h after mixing. Baseline specimens (t-0) were identified as those which were removed from the mold and tested after 1 h dry setting at 37°C in an oven (air atmosphere). The t-0 specimens were not immersed in distilled water. Additional cements were stored in distilled water at 37°C and removed for testing after t-1, 7 and 14 days. The quantity of distilled water for incubation of t-1, 7 and 14 day specimens was calculated by using the equation;

$$V_s = \frac{S_a}{10} \quad (1)$$

where S_a represents the exposed surface area of the cement disc.[23]

Agar Disc-Diffusion Test

The antibacterial activity of the cements was evaluated against *E. coli* strain DH5α, *B. fragilis* strain 638, and *S. epidermidis* strain NCIMB 12721 using the agar disc-diffusion method. Luria agar and broth were used for the culture of *E. coli*, BHI agar supplemented with 5 vol% sheep blood was used for plate culture of *B. fragilis,* and BHI broth supplemented with hemin (5 mg/L), and cysteine (50 mg/L) used for liquid culture, BHI agar and Todd–Hewitt broth were used for culturing *S. epidermidis*. All organisms were grown at 37°C. *E. coli* and *S. epidermidis* were grown aerobically with agitation when appropriate. *B. fragilis* was grown in a gas pack jar with an anaerobic atmosphere provided by an AnaeroGen pack (Oxoid).

Preparation of the agar disc-diffusion plates involved seeding BHI agar plates with a sterile swab dipped in a 1/50 dilution of the appropriate 16 h culture of bacteria. Three discs of each material were placed on the inoculated plates

TABLE I. Cement Formulations

Cement Composition	Control	5 wt./wt%	10 wt./wt%	15 wt./wt%
BT 101[a] (g)	1.00	1.00	1.00	1.00
PAA (g)	0.37	0.37	0.37	0.37
TSC (g)	0	0.0375	0.075	0.11
H_2O (mL)	0.37	0.37	0.37	0.37

[a] Silicate glass component

and the plates were cultured for 36 h at 37°C either aerobically or anaerobically depending on the nature of the seeded organism. The agar diffusion test was performed under standard laboratory sterile conditions where bacteria were handled under a fumigation hood using sterile cotton swabs.

Calipers were used to measure zones of inhibition at three different diameters for each disc and zone sizes were calculated as follows:

$$\text{Inhibition zone (mm)} = \frac{\text{Halo}\varnothing - \text{Disc}\varnothing}{2} \qquad (2)$$

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

Statistical Analysis

One-way analysis of variance (ANOVA) was employed to compare the antibacterial efficacy of GPCs relative to: (1) the TSC content of cements (2) the effect of time on the size of inhibition zone. Comparison of relevant means was performed using the *post hoc* Bonferroni test. Differences between groups was deemed significant when $p \leq 0.005$. Statistical analysis was performed using SPSS software for windows version 15 (SPSS, Chicago, IL).

Preparation of Agar Specimens

Agar strips (3 × 14 × 5 mm) were prepared for investigation by EDX from the agar diffusion test. The specimens were cut from the assay, extending from the cement disc, through any inhibition zone, through to the bacterial colony. The agar specimens were then placed on a glass slide and incubated at 37°C in an oven (air atmosphere) for 24 h until dry.

Ion Release Profiles

The Zn, Sr, sodium (Na) and calcium (Ca) concentration of the distilled water extracts that contained the GPCs for t-1, 7 and 14 days, were measured using an Atomic Absorption Spectrometer (AAS) (Varian SpectrAA-44-400) and the conditions are listed in Table II.

Standard solutions were used for calibration of the system. NaCl was added to Sr and Na while LaCl was added to Ca to inhibit ionization of these elements. Three measurements were taken from each aliquot in order to determine the mean concentration of each element for each incubation period.

Preparation of Cement Implanted Bone Specimen

Two pieces (15 cm × 15 cm × 5 cm) of bovine femur were cleaned and a hole (5 mm Ø) was drilled through the center of one of the bone specimens. The bone specimens were then thoroughly washed with distilled water. The control cement (no TSC) was prepared as described in section Cement Preparation and used to fill the void in one specimen of bone, while the second specimen of bone was used as a control. Both ends of the cement implanted bone specimen was covered with sheets of acetate paper, then clamped between two steel plates and left to set in an oven (air atmosphere) at 37°C for 1 h. The construct was then removed from the clamp and each surface polished using 1200 grit silicon carbide paper. Both constructs were stored in distilled water for 7 days, following which they were removed and dried in an oven (air atmosphere) at 37°C.

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 of the bone-cement interface. EDX measurements were performed on the control specimens of bone and across the cement–bone interface of the other two specimens, extending towards the edge of the bone. 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.

RESULTS

Agar Diffusion

The results (Figures 1–3, Table III) indicate that the addition of TSC to the cements significantly increases their antibacterial efficacy, as determined by One-Way Anova and *post hoc* Bonferroni tests. The cements caused clear zones of inhibition from 2 to 13 mm and 0 to 11 mm when in contact with *E. coli* and *B. fragilis*, respectively at t-0 [Figures 1(A) and 2(A)], where t-0 is the time after which the cement specimen was tested after 1 h dry setting in an oven (air atmosphere) at 37°C. Inhibition zones were only present at time t-0 for *B. fragilis* however statistical analysis determined that increasing the TSC content of cements

TABLE II. AAS Operating Conditions

	Zn	Sr	Na	Ca
Lamp current (mA)	5	10	5	10
Fuel	Acetylene	Acetylene	Acetylene	Acetylene
Support	Nitrous oxide	Nitrous oxide	Air	Nitrous oxide
Wavelength (nm)	213.9	460.7	330.2	239.9

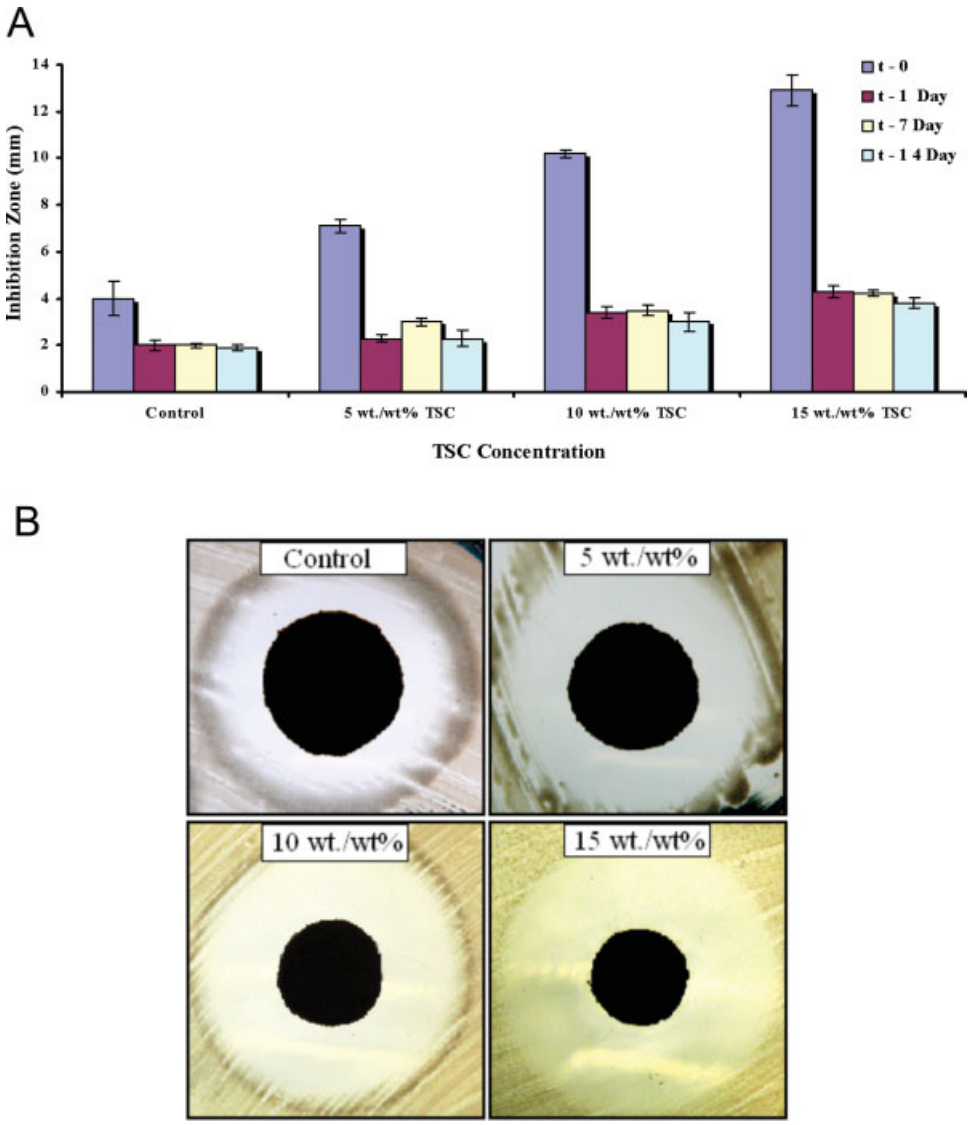

Figure 1. A: Inhibition zones of *E. coli* at t-0, 1, 7 and 14 days at 37°C. B: Photographic images of inhibition zones of *E. coli* at t-0. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

significantly ($p = 0.000$) increased their antibacterial efficacy at this time point. For cements in contact with *S. epidermidis,* inhibition zones existed, but were less clear than those observed for *E. coli* and *B. fragilis* as some bacterial growth was present within the inhibition zone. Therefore, the antibacterial effect of *S. epidermidis* was not tabulated and compared to *E. coli* and *B. fragilis*. It was also found that the cements exhibited significantly (Table IV) greater antibacterial properties when tested at t-0 as compared to 1, 7 and 14 days where the antibacterial properties proved to be predominantly insignificant.

The EDX analysis of agar taken from bacterial plates (Figure 4) indicates that there was a higher concentration of Zn at the cement–agar interface than at the edge of the inhibition zone, while the concentration of Sr showed no spatial variation (Figure 5).

Ion Release

Addition of TSC to the cements (Figure 6) increased the concentration of Sr, Zn and Na released from the cements. Comparable concentrations of Zn and Na were released and both of these levels were much higher than those for Sr. The levels of Sr release are very low for the control, 5 wt% and 10 wt% TSC in comparison to Zn and Na which makes illustration of these results difficult. Also no Na was detected in the control cement as Na is not part of the glass composition.

Ion Incorporation into Bovine Bone Void Model

EDX from the bovine bone implanted with the GPC (Figures 7 and 8) illustrate that Zn was released and taken up

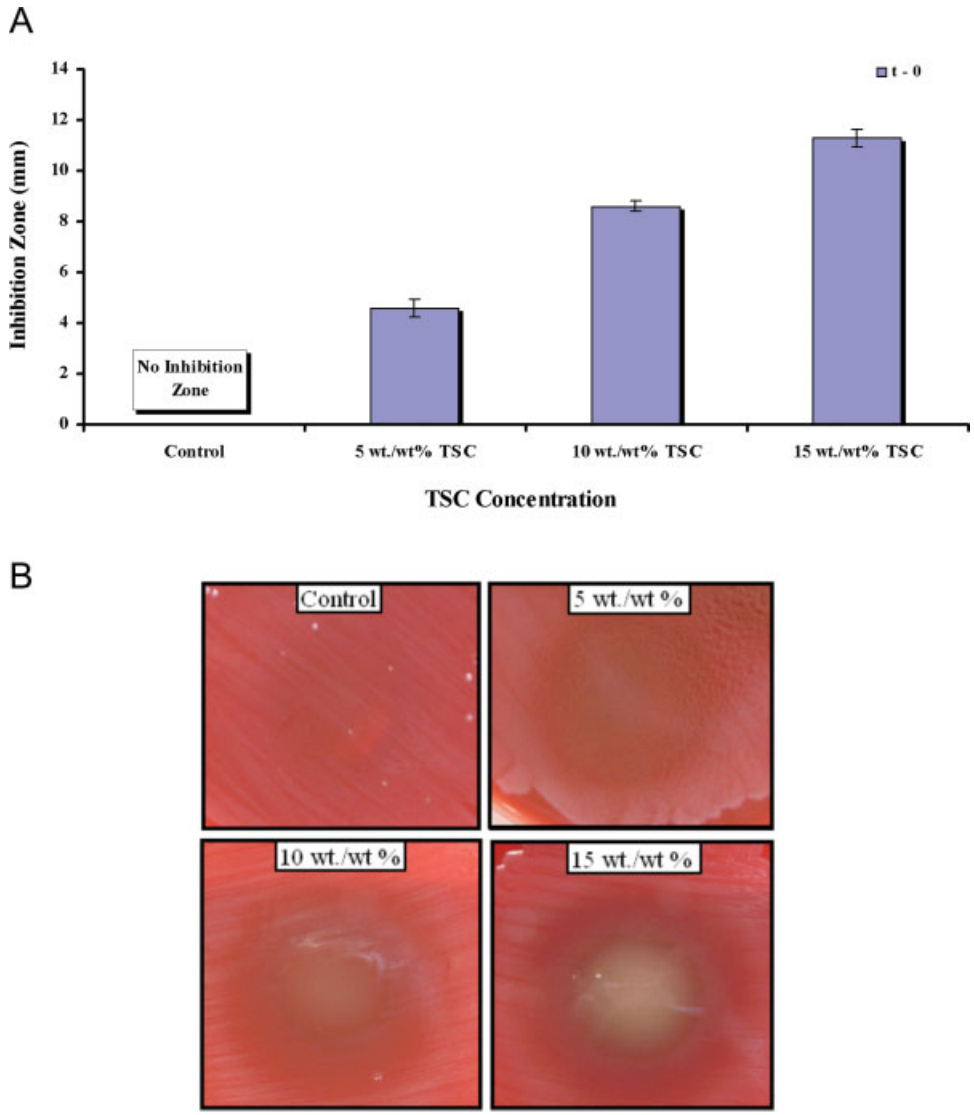

Figure 2. A: Inhibition zones of *B. fragilis* at t-0 at 37°C. B: Photographic images of inhibition zones of *B. fragilis* at t-0. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

by the bone tissue at distances up to 8 mm (point 3 in Figure 7) from the cement–bone interface. Zn was not detected by EDX in the bovine bone control specimen. The highest concentration of Zn (10 wt/wt%) was recorded near the cement–bone interface, with lowest concentrations (<3 wt/wt%) recorded about 8 mm into the specimen. Sr was undetectable at any point on the specimen.

DISCUSSION

The present results indicate that the antibacterial properties of the GPC were significantly improved with the addition of TSC to the cement powder. This finding is attributable to the TSC retarding the setting reaction of the cement allowing for high elution of Zn from the cement. It is known that Zn inhibits many activities in the bacterial cell, such as glycolysis, transmembrane proton translocation and acid tolerance.[11] Research has shown that Zn^{2+} inhibits the action of sphingomyelinase from *Bacillus cereus*. Zn^{2+} has also been found to inhibit the hemolytic activity of phospholipase C from *Legionella pneumophilia* and *Pseudomonas aeruginosa*.[24] It has also been reported that ZnO inhibits the formation of fibrin by the action of the coagulase enzyme of *Staphylococcus aureus*.[25] In addition, it is believed that zinc binds to the membranes of micro-organisms prolonging the lag phase of the growth cycle and increasing the generation time of the organism so it takes longer for each organism to complete cell division[26] resulting in decreased proliferation rates.

The results demonstrate that ions released from the cements prevent bacterial proliferation and that incorporating TSC into the cements significantly increases their antibacterial efficacy prior to immersion in water (Table III).

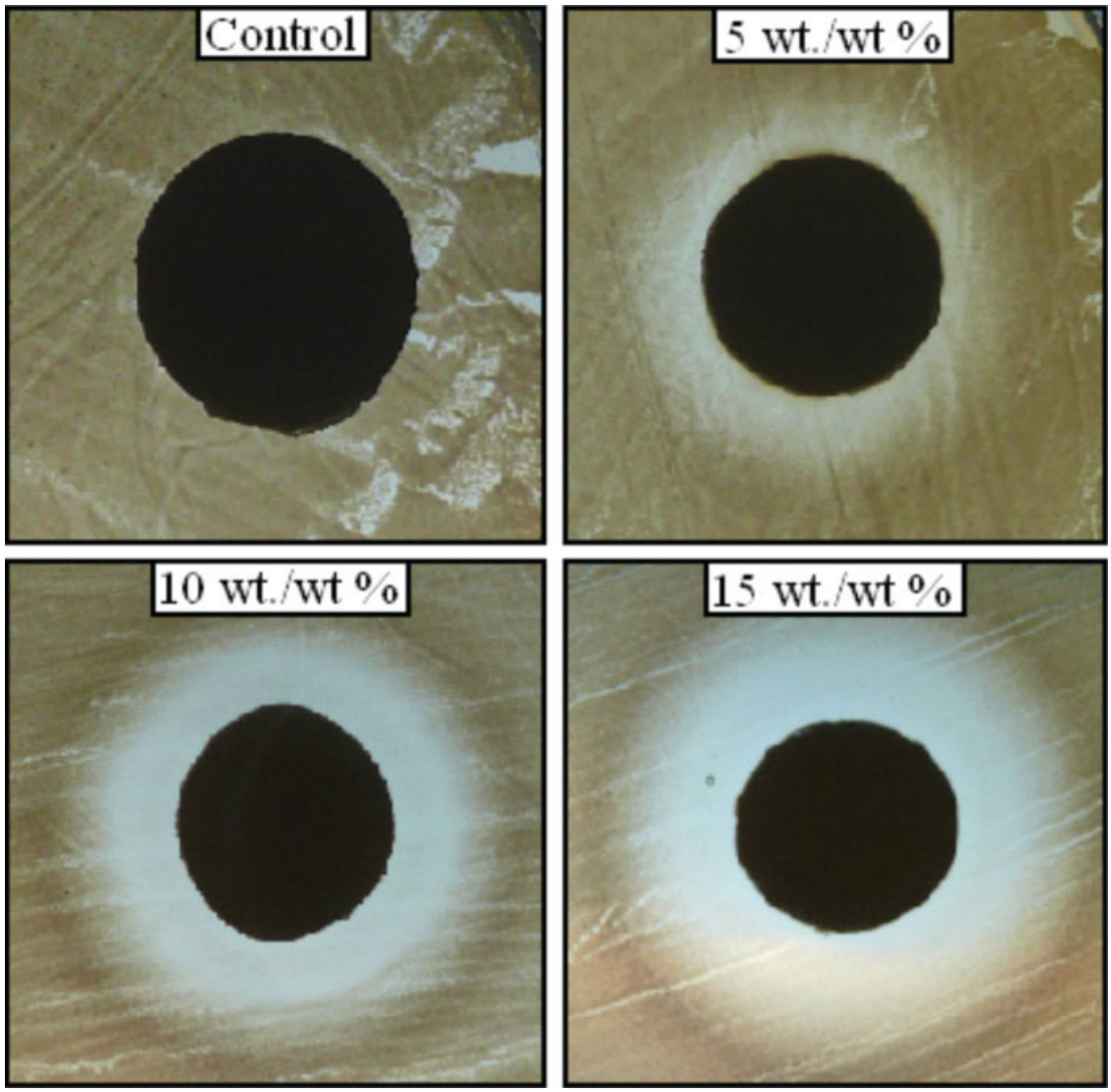

Figure 3. Photographic images of inhibition zones of *S. epidermidis* at t-0. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

In general for *E. coli*, the size of the zones of inhibition increased significantly (Table III) with increasing TSC content. However, some non-significant differences were noted between 0 wt/wt% *vs.* 5 wt/wt% ($p = 0.155$) after 1 day; 5 wt/wt% *vs.* 10 wt/wt% ($p = 0.470$) after 7 days; and both 0 wt/wt% *vs.* 5 wt/wt% ($p = 0.050$) and 5 wt/wt% *vs.* 10 wt/wt% ($p = 0.006$) after 14 days. The control cements (no TSC added) also exhibited antibacterial properties, but were significantly less potent than their TSC containing counterparts (Table III). It is evident from Figures 1(A) and 2(A) that those cements tested at t-0 resulted in the greatest inhibition zones (up to 13 mm in *E. coli* for the

TABLE III. Multiple Comparison (Bonferroni) of Mean Inhibition Zone Size with Respect to Increasing TCS Content for the Modified GPCs

		p-Values 0–15 wt./wt% TSC		
Means Compared	0 wt./wt%	5 wt./wt%	10 wt./wt%	15 wt./wt%
0 day *vs.* 1 day	0.0	0.0	0.0	0.0
1 day *vs.* 7 days	1.0	0.0	1.0	1.0
7 days *vs.* 14 days	1.0	0.001	0.053	0.095
0 days *vs.* 7 days	0.0	0.0	0.0	0.0
0 days *vs.* 14 days	0.0	0.0	0.0	0.0
1 day *vs.* 14 days	1.0	1.0	0.045	0.073

The mean difference is significant at the $p < 0.005$ level.

TABLE IV. Multiple Comparison (Bonferroni) of Mean Inhibition Zone Size with Respect to Time for the Modified GPCs

	p-Values at Time Intervals 0–14 days			
Means Compared	0 day	1 day	7 days	14 days
0 wt./wt% *vs.* 5 wt/wt%	0.0	0.155	0.0	0.050
5 wt./wt% *vs.* 10 wt/wt%	0.0	0.0	0.470	0.006
10 wt./wt% *vs.* 15 wt/wt%	0.0	0.0	0.0	0.0
0 wt./wt% *vs.* 10 wt/wt%	0.0	0.0	0.0	0.0
0 wt./wt% *vs.* 15 wt/wt%	0.0	0.0	0.0	0.0
5 wt./wt% *vs.* 15 wt/wt%	0.0	0.0	0.0	0.0

The mean difference is significant at the $p < 0.005$ level.

cement containing 15 wt/wt% TSC). The cements exhibited the greatest antibacterial activity with respect to time when tested in *E. coli* where the cements exhibited antibacterial properties up to 14 days. The greatest inhibition zone was determined at t-0 in each case and the overall greatest antibacterial effect was found with 15 wt/wt% TSC addition.

The mean inhibition zone size was also compared (Bonferroni) with respect to time. It was found that each cement tested at t-0 was significantly more antibacterial than those immersed in water (Table IV). In general, comparison of the mean size of inhibition zone for cements immersed in water after 1 day did not significantly decrease when compared with those immersed in water for up to 14 days (Table IV). This is of importance from a clinical perspective as it indicates the prolonged antibacterial nature of these cements in an aqueous environment.

The cements were originally intended to be tested up to 14 days in each bacterium; however, no inhibition zones existed with *B. fragilis* and *S. epidermidis* after t-0. It is also evident with *B. fragilis* and *S. epidermidis* that the control cement exhibited no antibacterial activity. This may be due to these bacteria being tolerant to low levels of Zn and Sr. However, subsequent TSC additions at t-0 increased the antibacterial efficacy of the cement. The antibacterial activity observed when testing against *B. fragilis* and *S. epidermidis* is due solely to TSC addition where the antibacterial response increases with increasing TSC content. The zones of inhibition for *S. epidermidis* were not completely clear of bacterial growth, as seen for *E. coli* and *B. fragilis*. This may be explained in part by previous calculations of kill time for TSC on *S. epidermidis*.[19] These authors showed that at 7.5 wt/wt% TSC the viability of *E. coli* was reduced by 2 log in 1 h and reduced to 0 in 4 h, whereas, for *S. epidermidis*, little effect on viability was seen after 2 h and was only reduced by 2 log after 4 h. Thus the effect of TSC on *S. epidermidis* is slower and may give rise to some low level growth in the inhibition zone.

The largest inhibition zones were obtained using cement specimens that were immediately immersed in agar following preparation, at t-0 [Figures 1(B) and 2(B)]. This situation more accurately resembles the clinical situation where cement is implanted immediately after mixing. Storage of the cement discs in water was found to reduce the antibacterial activity of the cements. This decrease is likely due to the leaching of both antibacterial ions and TSC from the cements into the aqueous environment from 1 to 14 days. This reduction however, is only significant between t-0 and 1 day (Table IV). After the initial decrease (i.e. from 1 day storage in water), the potency of the cement discs was generally not significantly reduced (Table IV) over a period from 1 to 14 days. The non-significant change in antibacterial properties from 1–14 days may be due to the fully set cement restricting ion release to the surrounding aqueous solution. TSC has been reported by the authors to extend the working time of this series of GPCs, from 70s to 300s (Ref. 27). However, the extended time is still less than 1 h; equating to a shorter duration than t-0. However, it is likely that the cement at t-0 has a high concentration of antibacterial ions that have diffused to its surface whereas the sample is clamped after mixing (1 h). These ions will be directly released into the agar causing immediate formation

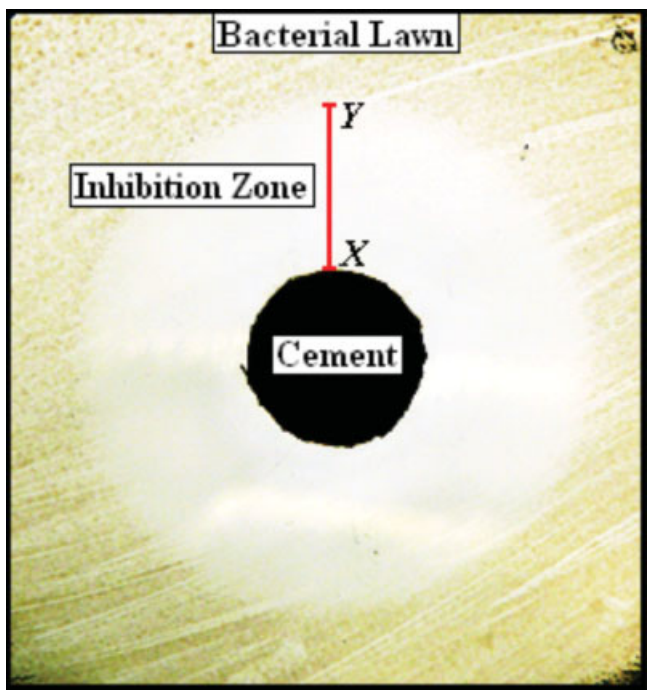

Figure 4. Photographic image of *E. coli* 15 wt/wt% TSC at t-0 illustrating points on agar for EDX testing. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

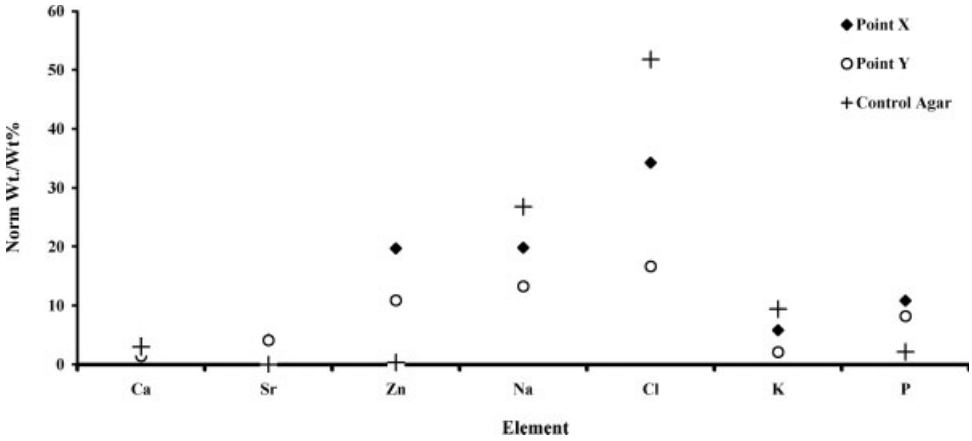

Figure 5. Quantitative EDX trace with control agar and agar from inhibition zone of *B. fragilis* at t-0.

of inhibition zones around the cement. This will be more evident in the samples at t-0 than those at longer storage times as, in these samples, these ions that have migrated to the surface will be released into the water during the extended storage. It is also possible that the release of these ions may cause a short term imbalance to pH of the agar, assisting in the inhibition zone formation. However, no pH measurement was undertaken so it is not possible to know if this was a contributory factor.

To relate the antibacterial effect to the release of ions from the cements, EDX was performed on the inhibition zone of the 15 wt/wt% TSC specimen of *E. coli* and *B. fragilis*. Figure 4 illustrates the inhibition zone around in the agar assay containing *E. coli*. Point *X* on Figure 4 illustrates an area of the inhibition zone on the agar assay closest to the cement; point *Y* illustrates an area of the inhibition zone furthest from the cement. EDX was performed at points *X* and *Y* on a sample of dehydrated agar from the *E. coli* and *B. fragilis* assays. A detection level of 0.2 wt/wt% is required for accurate EDX measurements. EDX was also performed on a sterile section of agar from a plate containing no cement disc and was used as a control. The results of the EDX from both organisms yielded similar traces. EDX on the control specimen of dehydrated agar showed that neither Zn nor Sr was present (Figure 5). The Ca content is constant in all three agar specimens, suggesting that little or no Ca ion release is occurring from the cements. Sr and Zn were not detected in the control specimen; however, for the test specimens the Sr content remains constant whereas the Zn concentration decreases with distance from the cement disc. As a result of the control agar specimen containing Na, EDX was not used to evaluate its elution from the cement into the gel layer, rather, AAS was performed on cement storage distilled water extracts to determine Na release profiles. From the EDX analysis it can be determined that there is Zn and Sr release from the cement in the inhibition zone, with a decreasing concentration of Zn when approaching the outer inhibition zone. However, the extent of Na and Ca ion release from the cements cannot be determined due to the concentrations of these ions already present in the agar.

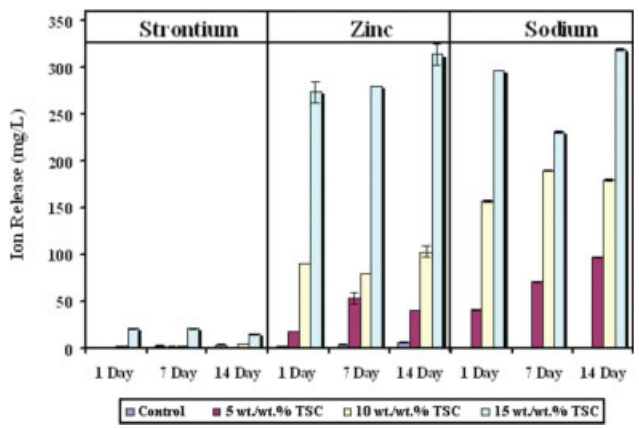

Figure 6. AAS profiles for strontium, zinc, and sodium. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

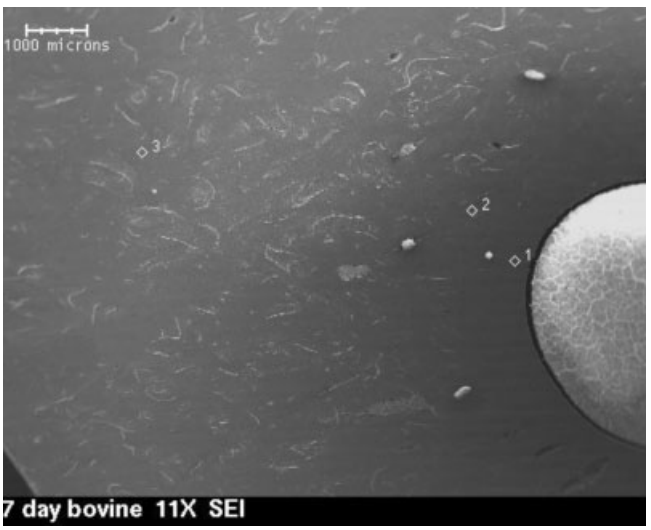

Figure 7. SEM image of bovine bone implanted with control cement specimen.

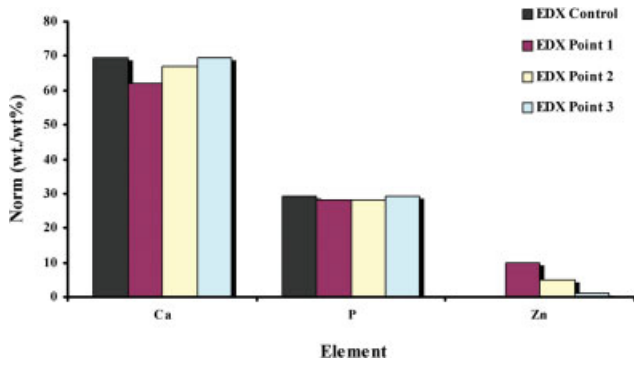

Figure 8. EDX results of Zn, Ca, and P ion transfer across the bone–cement interface for the control cement in a bovine bone void model. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

One possible explanation for the constant Sr content in the agar at either side of the inhibition zone is experimental error. There is some Sr release from the cement (as shown in Figure 6) into the agar, but the release is low, due to the low Sr content of the cement itself, as compared to the Zn concentration. Also the EDX cannot determine accurately between very low counts (0.2 wt/wt%) as it effectively acts as a qualitative measure at these low contents. Sr content would be expected to be lower at point *Y* compared with point *X*, and this maybe the case, but it may not be possible to resolve these two low counts using the EDX equipment.

To quantify ion release from the cement discs, AAS was performed on the t-1, 7, and 14 day distilled water extracts for Ca, Sr, Zn and Na ions. The resulting AAS profiles are shown in Figure 6. Ca^{2+} was not released from the cement after the material had set. This may be due to the low levels of Ca^{2+} in the cement being quickly incorporated into the glass network during the setting reaction thus facilitating its structural role. Figure 6 shows release profiles for Sr^{2+}, Zn^{2+} and Na^{+}. It is evident that there is little change in levels of ion release from 1 to 14 days and that the levels of Zn^{2+} and Na^{+} release are much higher than for Sr^{2+}. This is due to the low concentration of Sr present in the glass. It is also evident that increased TSC content results in increased levels of ion release. The reason for this effect is likely due to two synergistic mechanisms; firstly, TSC prolongs the setting reaction of these cements[27] leading to an increase in antibacterial ion (Zn, Sr) release (Figure 6). Secondly, TSC is itself an effective antibacterial agent[19] and will contribute to the overall antibacterial effects as a result of its leaching from the cements in an aqueous environment.

A bovine bone void model was used to determine if the ions released from the cements can be incorporated into the matrix of bone. It has been suggested that osteogenic cells are recruited by ions exchanged between the implant and the surrounding tissue.[28] Cortical bone is highly compact and consists principally of HA; predominantly calcium phosphate. The control cement (with no TSC) was used for this section of work; the rationale being that Zn and Sr are well known to have a beneficial effect on bone metabolism.[15,17] It is known that, half of the Na content in the body is present in extra-cellular fluids balanced by chlorides, while the other half is locked in the skeleton. Some authors suggest that Na is adsorbed onto HA crystals from contact with blood and is a reservoir which can be drawn upon when Na in the bloodstream is lost.[29]

The significantly higher contents of Ca and P compared with Zn at all points in the bovine bone void model (Figures 7 and 8) are attributable to Ca and P being the predominant ions present in bone mineral. EDX revealed that there was no Zn in the control specimen of bone whereas, after 7 days, three arbitrary points on the bovine bone specimen (1, 2 and 3 in Figure 7) were examined by EDX. The first was at the bone–bone cement interface (200 μm, point 1), while the second (1500 μm, point 2) and third (8200 μm, point 3) points were taken extending away from the implanted cement specimen and revealed that Zn was incorporated into the bovine bone void model. From Figure 8 it can be seen that the P levels in the cement remain relatively constant; however, there appears to be a reduction in the Ca content near the bone-cement interface as Zn is incorporated into the bone; though it remains unclear if the reduction in the Ca content is significant. What can also be observed is that there is a decrease in the Zn concentration as the EDX shifts toward the edge of the bovine bone specimen. No Sr was detected by EDX, which may be attributable to the relatively low concentrations being released for this cement (0.2 wt/wt% detection level). This work can conclude that Zn can be released from these GPCs and incorporated into bone matrix.

This agrees with the previous work by the authors where the biological properties of the cement were tested in simulated body fluid (SBF). The results of the SBF trial showed the precipitation of a surface CaP layer, which, as described by Kokubo et al.,[23] is regarded as a nucleation point for direct bonding to bone mineral. Studies have confirmed that the formation of a CaP layer on the surfaces of Bioglass 45S5, Sintered HA and glass ceramic A-W consequently bonded to living bone.[23] The authors also conducted transmission electron microscopy (TEM) on the CaP layer and determined that Zn–GPC can produce an amorphous CaP (ACP) surface layer.[30] In the natural mineralization process ACP is seen as the precursor to crystalline HA, which suggests that these cements may bond to bone tissue.[31]

CONCLUSION

An Al-free GPC to which TSC was added exhibited antibacterial activity, which is attributable to the dual action of TSC and Zn^{2+} ions as TSC retards the setting reaction of the GPC. The increasing levels of Zn^{2+} ions released into the environment are due to an increase in the TSC content of the cement. In a bovine bone void model, it was shown

that ion transfer (especially Zn) occurred between a GPC and the surrounding bone. Thus, it is an important finding given the important roles that Zn is known to play in bone metabolism.

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