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# Comparison of antibacterial properties of commercial bone cements and fillers with a zinc-based glass polyalkenoate cement

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**Abstract** Postoperative infection following invasive surgical procedures is a significant cause for concern, particularly in spinal reconstructive surgery. The objective of this study is to compare the antibacterial efficacy of a novel zinc-based glass polyalkenoate cement (Zn-GPC) based on 0.04SrO–0.12CaO–0.36ZnO–0.48SiO<sub>2</sub> glass, to a number of commercially available bone cements and fillers including Simplex P + Tobramycin (S<sub>Tob</sub>), Spineplex (S<sub>pine</sub>) and Novabone Putty (N<sub>Put</sub>). The agar diffusion test was performed on each material against *Escherichia coli*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Staphylococcus Aureus*. S<sub>Tob</sub> was found to produce large inhibition zones in each of the bacteria tested and was statistically significantly higher than any other material. The experimental Zn-GPC (B<sub>TSC</sub>) was found to exhibit antibacterial properties in both *E. coli* and *S. epidermidis*. Neither S<sub>pine</sub> nor N<sub>Put</sub> showed any inhibitory effect in any of the bacteria tested. A study was also performed to determine the effect of antibiotic release from S<sub>Tob</sub> and Zn-GPC (B<sub>Tob</sub>) containing the antibiotic tobramycin (Tob). Antibacterial efficacy was found to increase with respect to maturation with B<sub>Tob</sub>, whereas S<sub>Tob</sub> was found to decrease significantly over the time period of 0–14 days. The final objective is to investigate any change in agar composition during the agar-diffusion test. Little change was observed for S<sub>Tob</sub> as antibiotic release cannot be determined using EDX. There was, however, an increase in Zn levels when

analysing B<sub>TSC</sub> which suggests that Zn is playing a role in the antimicrobial nature of the Zn-GPC. No significant changes were observed for S<sub>pine</sub> or N<sub>Put</sub>.

## Introduction

Biomaterial-associated infections are of major concern in surgery. Microorganisms are frequently introduced onto implant surfaces and subsequently into the body [1]. It has been reported that infection rates range from 0.8–1.2% in orthopaedic surgery (total hip arthroplasty) and 3.6–8.1% (closed fracture) to 17.5–21.2% (open fractures) in trauma surgery [2]. Sepsis can ultimately lead to failure of the arthroplasty, causing the need for revision surgery, prolonged hospitalization and even death [3]. The field of biomedical engineering relies heavily on the interaction between the biomaterials and the surrounding physiological environment [4] and recently additives have been included in biomaterial design to increase antibacterial properties in an effort to extend the longevity of the implant. Some examples include antibiotic coatings, antibiotic-loaded collagen sheets and cement spacers [5] and antibiotic-loaded bone cements (ALBC). Commercially, ALBCs consist of a PMMA-based cement with additions of antibiotics such as tobramycin, gentamicin [6, 7], vancomycin and erythromycin [7]. However, concerns related to the use of ALBCs include the long-term effectiveness of the released drug [6, 7] and promotion of bacterial resistance which is likely due to the long-term sub-therapeutic levels of antibiotic eluted from the cement [6]. Other additives such as chitosan and collagen have been added to materials to promote cell growth [7]. Chlorhexidine (CHX) has also been added to dental glass polyalkenoate cements (GPCs) to further improve antibacterial properties [8].

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However, CHX was found to interfere in the setting reaction of commercially tested GPCs resulting in reduced mechanical properties [8].

GPCs, traditionally used in dental applications, generally contain ions that impart an antibacterial nature when used in an oral aqueous environment. The antimicrobial nature of these cements is predominant due to the release of the fluoride ion ( $F^-$ ) [9–11] and also related to setting occurring at a low pH [12]. GPCs have the additional ability to uptake and re-release  $F^-$  ions in a  $F^-$ -rich aqueous environment, which can impart a prolonged antibacterial response [10]. However, translating GPCs from dental to orthopaedic applications highlights concerns relating to the composition of the material, in particular the presence of aluminium (Al) in the glass phase which can cause neurological [13, 14] and bone metabolic defects [15]. The GPCs produced in this work have zinc ions ( $Zn^{2+}$ ) substituting for Al ions ( $Al^{3+}$ ) as numerous studies report on the antibacterial efficiency [12, 16–18] and positive bone metabolic effects of Zn [19, 20].

For this study, a range of commercially available materials were selected for testing. Simplex + Tobramycin (Stryker Howmedica, Limerick, Ireland) and Spineplex (Stryker Howmedica, Limerick, Ireland) both of which are PMMA-based materials used in load-bearing applications in the skeleton. Novabone putty (Gian Medical, Birmingham, UK), a bioglass-based bone filler was also included alongside an experimental Zn-GPC formulation. Each material was tested against a number of known bacteria that cause infection in surgical procedures; *Escherichia coli*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Of these, *S. epidermidis* and *S. aureus* are responsible, in most instances, for biomaterial related infections [21], due to the presence of adherent, multilayered biofilms on the surfaces of implanted biomedical devices which are less susceptible to antibiotics when compared to their planktonic counterparts [22]. *P. aeruginosa* has been found to infect surgical procedures such as Total Knee Arthroplasty (TKA) [23], and is reported to be the cause of osteomyelitis in pelvic bones [24]. It has also, along with *E. coli*, been found to constitute 42% of polymicrobial infections in the spine [21]. This infection rate is preceded by *S. aureus* and *S. epidermidis* which are the primary cause of infection in the majority of postoperative spinal infections [21]. These species are prevalent in human epithelia residing on most skin and mucous membranes and are known to cause nosocomial infections in newborns, severely ill and immunocompromised patients [25]. Further studies on biopsies of the spine by Heyer et al. [26] reveal the presence of all these bacteria, highlighting the relevance of this study.

For this study, a novel GPC formulation alongside Simplex + Tobramycin, Spineplex and Novabone Putty,

were tested against *E. coli*, *S. epidermidis*, *P. aeruginosa* and *S. aureus*.

## Materials and methods

### Materials

- Simplex P + Tobramycin ( $S_{Tob}$ )—Stryker Howmedica, Limerick, Ireland (#0473S024).
- BT 101 + 10 wt% TSC ( $B_{TSC}$ )—experimental GPC.
- Spineplex ( $S_{pine}$ )—Stryker Howmedica, Limerick, Ireland (#V1104).
- Novabone Putty ( $N_{put}$ )—Gian Medical, Birmingham, UK (#0610D5).

### Glass synthesis

A strontium–calcium–zinc–silicate glass formulation (0.04SrO/0.12CaO/0.36 ZnO/0.48 SiO<sub>2</sub>, mol. 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 mixture was then dried in an oven (100 °C, 1 h), fired (1480 °C, 1 h) in a platinum 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.

### Polyacrylic acid (PAA)

The PAA used in this study ( $E9$ ,  $M_w$  80,800) was supplied by Advanced Healthcare Limited (Kent, UK). The polyacrylic acid was ground and sieved to retrieve <90-μm particles.

### Tri-sodium citrate (TSC)

The TSC used in this study was obtained from Reagacon (Shannon, Ireland) and was incorporated into the cement at 10 wt% addition.

### Tobramycin sulphate (Tob)

The Tob used in this study was obtained from Stryker Howmedica (Limerick, Ireland) and was incorporated into the cement at 2.5 wt% addition; a concentration similar to that contained in  $S_{Tob}$ .

### Cement preparation

Cements were prepared by thoroughly mixing the glass powder with the PAA and distilled water (BT 101) on a

**Table 1** Formulation of cements used in this study

	Cement formulations				
	Glass	PAA	H <sub>2</sub> O	TSC	Tob
BT 101	1	0.37	0.37	–	–
B <sub>TSC</sub>	1	0.37	0.37	0.075	–
B <sub>Tob</sub>	1	0.37	0.37	–	0.018

glass plate using a dental spatula at a powder to liquid ratio of 2:1.5 g/mL. Additions of 10-wt% TSC/2.5-wt% Tob were also added to the cement formulations as outlined in Table 1.

### Sample preparation

Three specimens of S<sub>Tob</sub>, B<sub>TSC</sub>, S<sub>pine</sub> and N<sub>Put</sub> were used for testing in each bacterium. Each sample was produced by filling moulds (2 mm × 8 mmØ) with the cement and leaving them to set for 1 h prior to testing. Samples were produced under standard sterile laboratory conditions. A short maturation study was undertaken to determine the antibacterial properties of the experimental GPC containing Tobramycin (B<sub>Tob</sub>) against S<sub>Tob</sub>. For this study, both the materials were immersed in similar concentrations of sterile water for 1, 7 and 14 days. The cement was then tested in *S. epidermidis* at 0, 1, 7 and 14 days. A similar quantity of antibiotic was added to the GPC formulation, 2.5 wt%, as is contained in S<sub>Tob</sub>.

### Agar disc-diffusion test

The antibacterial activity of the cements was evaluated against *E. coli* strain DH5 $\alpha$ , *B. S. epidermidis* strain NCIMB 12721, *P. aeruginosa* strain PA01 and *S. aureus* strain A35-4 using the agar disc-diffusion method. Luria agar and broth were used for the culture of *E. coli*, BHI agar and broth were used for culturing *S. epidermidis*, *P. aeruginosa* and *S. aureus*. All organisms were grown at 37 °C. Preparation of the agar disc-diffusion plates involved seeding the 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 and the plates were cultured for 24 h at 37 °C. The agar diffusion test was performed under standard laboratory sterile conditions using a fumigation hood with sterile swabs for inoculation of bacteria.

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

$$\text{Inhibition Zone (mm)} = \frac{\text{Halo } \phi - \text{Disc } \phi}{2} \quad (1)$$

All cements were analysed in triplicate and mean zone sizes  $\pm$  standard deviations were calculated.

### Preparation of agar specimens

Agar strips (3 × 10 × 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 air-assisted oven for 24 h until dry.

### Quantitative EDX

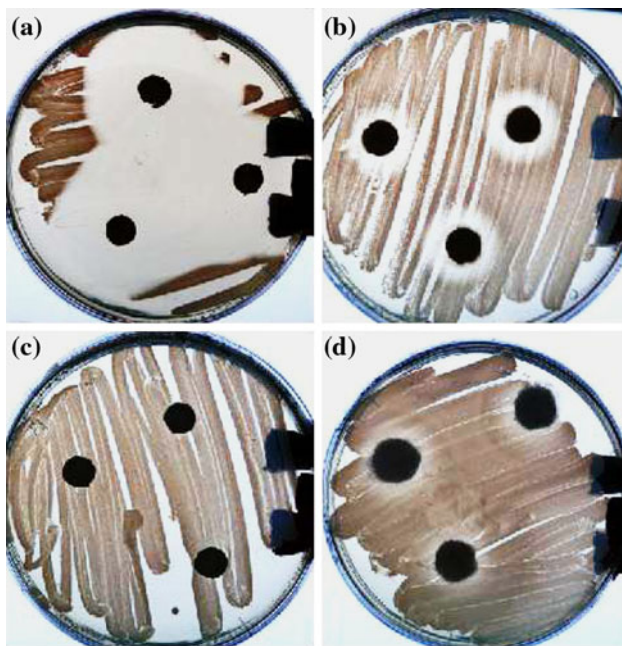
A Hitachi SU-70 field emission scanning electron microscope (FE-SEM) equipped with an Oxford Instruments Energy Dispersive X-ray spectroscopy (EDX) system was used to obtain secondary electron images and carry out chemical analysis of the agar samples. All EDX spectra were collected at 20 kV and quantitative EDX converted the collected spectra into concentration data using standard reference spectra obtained from pure elements under similar operating parameters.

### Statistical analysis

One-way analysis of variance (ANOVA) was employed to compare the antibacterial efficacy of the experimental materials in relation to (1) specific bacterial strains, (2) maturation when implanted with an antibiotic. Comparison of relevant means was performed using the post hoc Bonferroni test. Differences between groups was deemed significant when  $p \leq 0.05$ . Statistical analysis was performed using SPSS software for windows version 16 (SPSS Inc. Chicago, IL).

## Results and discussion

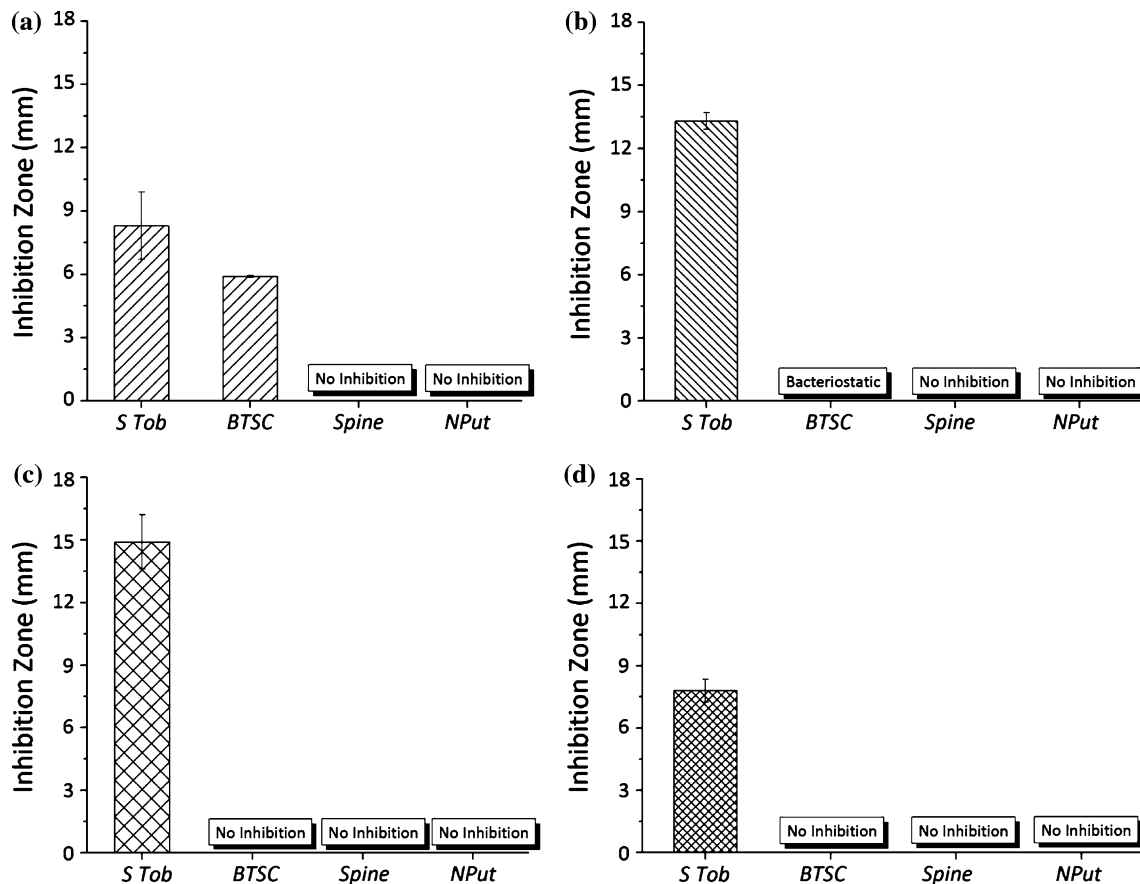
The primary objective of this study is to investigate the antibacterial response of a number of commercially available materials and one experimental material in an in vitro model. The first bacterium tested was a gram negative aerobic bacteria, *E. coli*, an opportunistic pathogen reported by Rafiq et al. [5] to account for 6% of all organisms infecting Total Hip Arthroplastys (THR) from the period 1974–2005. Research by van de Brand et al. [27] also found that *E. coli* was present in 60% of cultures where intermittent catheterization was required in joint



**Fig. 1** Cements tested in *S. epidermidis* where **a**  $S_{Tob}$ , **b**  $B_{TSC}$ , **c**  $S_{pine}$  and **d**  $N_{Put}$

arthroplasty. In relation to this study the biomaterials tested had more success in eliminating *E. coli* than any of the other bacteria examined. Figure 1 shows sample images of the agar diffusion test performed with *S. epidermidis*. Figure 2a shows the commercial materials and experimental GPC tested against *E. coli*.

From Fig. 2a, it can be seen that  $S_{Tob}$  proved to be the most effective in killing *E. coli*; achieving inhibition zones of  $8.3 \pm 1.69$  mm. However, the experimental GPC,  $B_{TSC}$  produced inhibition zones of  $5.9 \pm 0.05$  mm. Both  $S_{pine}$  and  $N_{Put}$  did not produce any zones of inhibition in *E. coli*. Statistical analysis was performed between each material for variance, and this was done for each bacterium. It was found that there are significant differences between the two cements that showed inhibition,  $S_{Tob}$  and  $B_{TSC}$  ( $p = 0.001$ ).  $S_{Tob}$  and  $B_{TSC}$  showed significant differences when compared with all other materials tested (Fig. 2a) also there was found to be no significant difference between  $S_{pine}$  and  $N_{Put}$  ( $p = 1.000$ ), as these materials showed no inhibition. This was similar for all bacterium tested where  $S_{pine}$  and  $N_{Put}$  showed no inhibition for *S. epidermidis*, *P. aeruginosa* and *S. aureus* ( $p = 1.000$ ) as shown in Fig. 2b–d.  $S_{Tob}$



**Fig. 2** Antibacterial properties of experimental materials in **a** *E. coli*, **b** *S. epidermidis*, **c** *P. aeruginosa* and **d** *S. aureus*



**Table 2** Analysis of variance (Bonferroni post hoc) between biomaterials and bacteria spp

	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. epidermidis</i>	<i>S. aureus</i>
S <sub>Tob</sub> vs. B <sub>TSC</sub>	0.001	0.000	0.000	0.000
S <sub>Tob</sub> vs. S <sub>pine</sub>	0.000	0.000	0.000	0.000
S <sub>Tob</sub> vs. N <sub>put</sub>	0.000	0.000	0.000	0.000
B <sub>TSC</sub> vs. S <sub>pine</sub>	0.000	1.000	1.000	1.000
B <sub>TSC</sub> vs. N <sub>put</sub>	0.000	1.000	1.000	1.000
S <sub>pine</sub> vs. N <sub>put</sub>	1.000	1.000	1.000	1.000

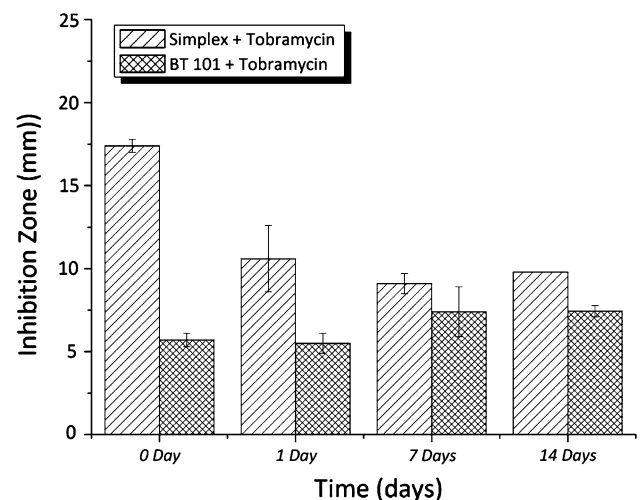
The mean difference is significant at the 0.05 level

showed significant differences in each bacterium where it produced inhibition zones of  $13.3 \pm 0.4$  mm for *S. epidermidis*,  $14.9 \pm 1.3$  mm for *P. aeruginosa* and  $7.8 \pm 0.5$  mm for *S. aureus*. These results were also significantly different to all other materials (Table 2) as no other material produced clear inhibition zones for *S. epidermidis*, *P. aeruginosa* and *S. aureus*. However, some antibacterial efficiency was observed with B<sub>TSC</sub> when tested in *S. epidermidis* (Fig. 1). Inhibition zones were not recorded as isolated colonies of bacteria were present within the inhibition zone. This indicates that B<sub>TSC</sub> is bacteriostatic in relation to *S. epidermidis* and not bacteriocidal. B<sub>TSC</sub> is likely to be inhibiting the growth and proliferation of these bacteria as opposed to killing the cells outright.

With respect to each bacterium, S<sub>Tob</sub> was found to be bacteriocidal and outperformed each of the competing materials. This is due to the presence of the antibiotic Tobramycin (Tob). Tob is generally used to treat gram negative bacterial infections such as skin or bone, lower respiratory tract and central nervous system (CNS) infections [28]. Studies by Le Goffic et al. describe Tob mechanism of action in relation to *E. coli*. Tob was found to occupy two binding sites on the ribosomes of the bacterial cell. The primary one is likely responsible for the inhibition of protein synthesis, whereas the secondary one is related to the misreading of the messenger RNA [29]. Tob has also previously been used to treat *P. aeruginosa* infections in patients with cystic fibrosis [30, 31], and has been described by D'Arrigo et al. to show excellent in vitro and clinical activities against susceptible strains of *E. coli* and *S. aureus* by inhibiting plasma membrane functions and by targeting ribosomal sites [32]. However, overuse of antibiotics in medicine promoted the evolution of some species of bacteria to infer resistance. These include the Methicillin-resistant *S. aureus* (MRSA) and *S. epidermidis* (MRSE) strains which are responsible for a considerable amount of hospital acquired infections [33–35]. The primary reason for increased usage of antibiotics in implant-related infections is that bacteria change their biological behaviour when adhering to implant surfaces [2]. They

produce a biofilm which forms a protective barrier and they can also reduce their metabolic activity and increase their generation time. As antibiotics act on growing bacteria, an increased minimum inhibitory concentration (MIC) of antibiotic is required to affect bacteria with reduced metabolic activity. Studies have found that 800-fold higher MIC for Tob in adhesive *P. aeruginosa* than for non-adhesive [2, 7]. Concerns also arise regarding the quantity of antibiotic released and the longevity around the implant site. After the high initial release of antibiotics, the long-term low concentration around implants may also contribute to antibiotic-resistant strains [2, 7]. The effectiveness of such materials is dependent on the rate and manner of which the drug is released, and this is directly related to the host material [7]. Drug release from materials is achieved through mechanisms of water pore penetration, soluble matrix dissolution and diffusion of the solubilized drug via matrix imperfections (pores/cracks). However, PMMA-based materials display a biphasic release pattern characterized by a high initial release followed by a long tail of low, ineffective and largely incomplete release that continues for days or months. Studies reveal that less than 10% of the trapped drug is eventually released from the cement [7]. Another concern is that antibiotics such as Tob can have harmful side effects in the body. Tob belongs to a group called aminoglycosides which are known to be nephrotoxic and ototoxic, depending on the choice of aminoglycoside and duration of therapy [36]. Increasing the dosage of antibiotic to reach the MIC when combating adherent biofilms may subsequently result in more traumas to local tissues.

To determine the efficacy of antibiotic release from S<sub>Tob</sub> and a GPC (B<sub>Tob</sub>), testing was performed on *S. epidermidis* over a period of 0–14 days. Figure 3 shows the comparison

**Fig. 3** Comparison of S<sub>Tob</sub> and B<sub>Tob</sub> in *S. epidermidis* with respect to maturation

**Table 3** Analysis of variance (Bonferroni post-hoc) between  $S_{Tob}$  and  $B_{Tob}$  in *S. epidermidis* with respect to maturation

Time (days)	$S_{Tob}$	$B_{Tob}$
a		
0 vs. 1	0.000	1.000
0 vs. 7	0.000	0.043
0 vs. 14	0.000	0.041
1 vs. 7	0.589	0.021
1 vs. 14	1.000	0.020
7 vs. 14	1.000	1.000
b		
$S_{Tob}$ vs. $B_{Tob}$	0 day	0.000
	14 day	0.001

The mean difference is significant at the 0.05 level

of  $S_{Tob}$  and  $B_{Tob}$  in *S. epidermidis* with respect to maturation.

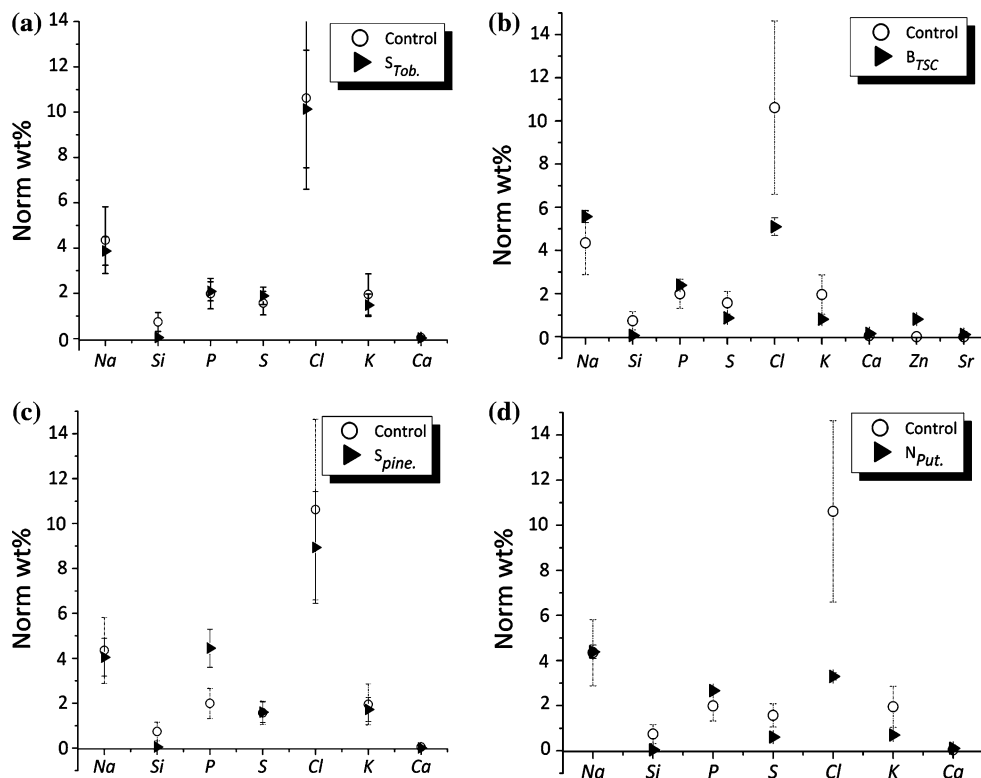
From Fig. 3 it can be seen that there is a significant decrease in antibacterial efficacy from 0 ( $17.4 \pm 0.4$  mm) to 14 days ( $9.8 \pm 0.01$  mm) regarding  $S_{Tob}$ . However, there is no significant change when comparing 1, 7 and 14 days ( $p = 0.589, 1.000, 1.000$ ; Table 3a). This suggests that the set material is retaining some of the antibiotic in the bulk and that the surface antibiotic has solubilized and was expended after initial testing at 0 days.  $B_{Tob}$ , on the other hand, was found to show a significant ( $p = 0.041$ ) increase in inhibition over the period of 0 ( $5.7 \pm 0.4$  mm)

to 14 days ( $7.4 \pm 0.3$  mm). It was expected that  $B_{Tob}$  would perform better than the results obtained here as GPCs are ion leachable materials. However, this may be the reason for the lower results found as compared to  $S_{Tob}$ . It may be possible that ion processes within the cement are reducing the efficacy of the antibiotic. Considering this, however, antibacterial efficacy was found to increase with respect to maturation. Table 3b confirms that there remains a significant change in inhibition between  $S_{Tob}$  and  $B_{Tob}$  at initial testing, 0 days and after 14 days. Further testing to 30 or 90 days may provide more conclusive results regarding the longevity and efficacy of antibiotic implanted materials.

Further testing was performed to determine what was causing the antibacterial efficacy of the materials. This was achieved by performing EDX on agar specimens from the agar extracted from the area immediately surrounding the samples, whether inhibition was present or not. Figure 4 shows the EDX results obtained from (a)  $S_{Tob}$ , (b)  $B_{TSC}$ , (c)  $S_{pine}$  and (d)  $N_{Put}$ .

It is evident from Fig. 4a that there is little change in the composition of the agar regarding  $S_{Tob}$ . In relation to  $S_{Tob}$ , the antibacterial properties are as a result of antibiotic release from the cement. Antibiotics are complex organic structures and techniques such as EDX are ineffective as only compositional elemental analysis is possible. Alternative methods would have to be employed to quantitatively determine antibiotic content. Figure 4b reveals the

**Fig. 4** EDX of agar samples comparing control agar with agar from plates containing materials. **a** Simplex + Tobramycin, **b** BT101 + 10 wt% TSC, **c** Spineplex, **d** Novabone Putty



presence of approximately 1-wt% Zn, whereas no Zn was found in the control agar. There was also a small quantity of Sr (0.1 wt%) detected, where again, no Sr was present in the control agar. It is difficult to determine the precise mechanism by which  $B_{TSC}$  is exhibiting antimicrobial properties as TSC present in the cement is known to be an antibacterial agent itself [37, 38]. Previous study by the authors determined that TSC prolongs the setting [39] of this cement facilitating greater ion release [40]. It has also been previously been reported that by increasing the concentration of TSC and Zn ion release, the antibacterial efficacy of the material increases [40]. Zn is an antibacterial ion which acts as an inhibitor of multiple activities in the bacterial cell, such as glycolysis, transmembrane proton translocation and acid tolerance [41]. It has also been cited by Shashibhushan et al. [18] that Zn can interfere with substrate transport and oxidation and can react with thiol groups on the bacterial cell wall. Zn can also change protein structure leading to inhibition of specific metabolic enzymes, thereby causing growth inhibition [18]. Another possible reason for inhibition by  $B_{TSC}$  could be explained by the mechanism of action of TSC, which is known to chelate Ca [42], which in this case slows the setting reaction in these cements [39]. However, regarding bacterial inhibition, TSC may be binding any Ca being used by the bacteria for metabolism. There was no antibacterial effect attributed to  $S_{pine}$  and  $N_{put}$ , however, there were slight changes in the EDX patterns. Figure 4c shows that there was an increase in the phosphorus (P) concentrations as compared to the control agar. This may be due to bacteria metabolising as they proliferate on the surface of the agar. There was also a very slight increase in P content in the agar surrounding  $N_{put}$  (Fig. 4d). These materials exhibited no antibacterial properties when tested against these bacteria as  $S_{pine}$ , in particular, contains no antimicrobial compounds or ions. This cement is implanted and remains as an inert material exhibiting no bioactivity.  $N_{put}$  is based on a Bioglass formulation suspended in a gelatin binder, however, the composition failed to inhibit the proliferation of the bacteria tested.

To conclude,  $S_{Tob}$  was the only material to exhibit antibacterial properties when tested in each bacterium. However, the ongoing discussion as regards the overuse of antibiotics suggests that additional ways of combating infection would be welcome.  $B_{TSC}$  was the only other material to exhibit antibacterial properties and this was only evident against *E. coli* and *S. epidermidis*. The advantage of using a GPC over an acrylic cement is that beneficial ions can be absorbed and re-released when in an ion-rich environment. However, further research is required to evaluate at what extent this occurs.  $S_{pine}$  and  $N_{put}$  are two commercially available materials that were found to exhibit no antibacterial properties, and as such this

leaves room for development of more bioactive materials with properties suitable for use in orthopaedics.

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