01 Sep 2013

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A. W. Wren

A. Coughlan

M. M. Hall

M. J. German

et. al. For a complete list of authors, see https://scholarsmine.mst.edu/che_bioeng_facwork/1144

Recommended Citation

A. W. Wren et al., "Comparison of a SiO2-CaO-ZnO-SrO Glass Polyalkenoate Cement to Commercial Dental Materials: Ion Release, Biocompatibility and Antibacterial Properties," *Journal of Materials Science: Materials in Medicine*, vol. 24, no. 9, pp. 2255 - 2264, Springer, Sep 2013.
The definitive version is available at https://doi.org/10.1007/s10856-013-4974-6

J Mater Sci: Mater Med (2013) 24:2255–2264
DOI 10.1007/s10856-013-4974-6

Comparison of a SiO_2–CaO–ZnO–SrO glass polyalkenoate cement to commercial dental materials: ion release, biocompatibility and antibacterial properties

A. W. Wren · A. Coughlan · M. M. Hall · M. J. German · M. R. Towler

Received: 1 February 2013 / Accepted: 10 June 2013 / Published online: 21 June 2013

Abstract Ion Release and biocompatibility of a CaO–SrO–ZnO–SiO_2 (*BT 101*) based glass polyalkenoate cement (GPC) was compared against commercial GPCs, *Fuji IX* and *Ketac Molar*. The radiopacity (*R*) was similar for each material, 2.0–2.8. Ion release was evaluated on each material over 1, 7, 30 and 90 days. *BT 101* release included Ca (23 mg/L), Sr (23 mg/L) Zn (13 mg/L), Si (203 mg/L). *Fuji IX* release includes Ca (0.7 mg/L), Al (3 mg/L) Si (26 mg/L), Na (60 mg/L) and P (0.5 mg/L) while *Ketac Molar* release includes Ca (1 mg/L), Al (0.6 mg/L) Si (23 mg/L), Na (76 mg/L) and P (0.7 mg/L). Simulated body fluid trials revealed CaP surface precipitation on *BT 101*. No evidence of precipitation was found on *Fuji IX* or *Ketac Molar*. Cytotoxicity testing found similar cell viability values for each material ($\sim$60 %, $P = 1.000$). Antibacterial testing determined a reduced CFU count with *BT 101* (2.5×10^3) when compared to the control bacteria (2.4×10^4), *Fuji IX* (1.5×10^4) and *Ketac Molar* (1.2×10^4).

1 Introduction

Glass polyalkenoate cements (GPCs) are traditionally used in dental restorative and adhesive applications [1]. GPCs can adhere to tooth structures and base metals, they are biocompatible and exhibit low cytotoxicity [2, 3]. The commercially available GPCs typically consist of an alumino-silicate based glass and a polyalkenoic acid or acid copolymer. The majority of the glasses used to formulate GPCs also include CaO, CaF, Na_2O and PO_4^{3-}, however, glasses have also been formulated where the calcium concentration is replaced by strontium or lanthanum, which impart radiopacity to the cement [1]. The acid component is typically a homopolymer of polyacrylic acid (PAA) or a copolymer of itaconic-acrylic or maleic-acrylic acid. When the glass and acid components are mixed with water the materials undergo an acid base setting reaction where H^+ ions liberated from the carboxylate groups on the PAA chains promote partial dissolution of the glass surface. This results in ions from the glass leaching out into a polysalt matrix [1, 4, 5]. The ions liberated from the glass (Al^{3+}, Ca^{2+}) form crosslinks with the COO− groups from the PAA chains resulting in a set cement [5]. GPCs have been successfully used in dentistry due to a number of desirable properties such as the ability to aesthetically match the material to the surrounding tissue [6]. They also set with negligible exotherm [3, 7, 8] and shrinkage [9]. Previous studies have cited that the mechanical properties of GPCs are low and unsuitable for high stress sites in class I and II restorations in dentistry, and as such modifications have been made to improve the strength by including alumina fibers and carbon fibers [1], and by replacing the water component with a water-HEMA (hydroxymethyl methacrylate) mixture which can be used to influence both the setting and mechanical characteristics of this class of GPCs [2].

A significant clinical advantage to using GPCs over dental amalgams is the ability to leach antibacterial ions from the materials. Regarding dental applications, the

A. W. Wren (✉) · A. Coughlan · M. M. Hall
Inamori School of Engineering, Alfred University, Alfred, NY 14802, USA
e-mail: wren@alfred.edu

M. J. German
School of Dental Sciences, Newcastle University, Newcastle upon Tyne, UK

M. R. Towler
Department of Mechanical & Industrial Engineering, Ryerson University, Toronto, ON, Canada

leaching of fluoride (F^-) has been associated to impart an antibacterial nature, which results in the prevention of secondary caries formation [6, 10, 11]. Due to their therapeutic effect in dentistry, GPCs have been utilized in orthopedic applications such as bone cementation and maxillofacial and cranial surgery [8, 12], as they can form a strong chemical bond to hard tissues such as enamel, dentin and bone, which consists of tightly packed hydroxyapatite crystals forming a micro-porous structure [13, 14]. This chemical bond exists through ion exchange at the interface between the GPC and the hydroxyapatite. PAA chains can enter the hydroxyapatite surface, replacing a concentration of calcium and phosphate ions. Ion exchange between the GPC and the COO− groups from the PAA chains results in a strong interfacial bond [6, 10]. One potential drawback of using these materials in orthopedics is that aluminum (Al^{3+}) ion release from the cement can have negative neurological side effects if released into body tissue fluids over prolonged periods of time [8]. Cell culture studies report cell inhibition which has been attributed to both ion release (Al^{3+} and F^-) and pH effects [3]. There are also reports that Al^{3+} release can negatively affect mineralization of skeletal tissue adjacent to the GPCs [3, 15] as Al^{3+} may interfere with the initial stages of crystallization of calcium phosphate in vivo or reduce collagen synthesis [16].

Previous studies have looked at modifying the composition of the glass in order to tailor GPCs for applications in medicine as the reaction rate is determined by a number of parameters including the chemical nature of the glass, treatment of the glass and the exposed surface area [17]. Regarding this study, strontium (Sr^{2+}) and zinc (Zn^{2+}) ions are included in the starting glass phase as they are regarded as having a positive effect on bone metabolism [18–20]. Both Al^{3+} and F^- have been excluded from the experimental glass composition (*BT 101*) as they are known to have cytotoxic effects in vivo. Also, Na^+ is thought to have a deleterious effect on the hydrolytic stability and mechanical properties of the cement as it competes with Ca^{2+} and Al^{3+} for carboxylate groups on the polyacid chains, and is therefore likely to inhibit the cross-linking process [4]. Al^{3+} and Ca^{2+} are the primary COO− cross-linking ions [21] and as such, the removal of Al^{3+} from the glass phase will significantly alter the properties. The authors have undertaken previous study which evaluated the effect of glass structure/composition on the handling, exotherm and mechanical properties of the same materials under investigations here [22]. This study aims to determine the biocompatibility, ion release and antibacterial efficacy of a SiO_2–ZnO–CaO–SrO based GPC and to compare this experimental GPC to two commercially available dental analogues, *Fuji IX* and *Ketac Molar*.

2 Materials and methods

2.1 Materials

BT 101-Experimental GPC. A 0.12CaO–0.04SrO–0.36ZnO–0.48SiO_2 glass (*BT 101*) was formulated by weighing out appropriate amounts of analytical grade reagents (Sigma-Aldrich, Dublin, Ireland) and ball milling (1 h). The mix was then oven dried (100 °C, 1 h) and fired (1500 °C, 1 h) in a platinum crucible and shock quenched into water. The resulting frit was dried, ground and sieved to retrieve a glass powder with a maximum particle size of 45 μm.

Fuji IX-GC Co. Japan (#0508291).

Ketac Molar-ESPE/3 Dental, MN, USA (#224927).

2.2 Cement reparation

BT 101-Cements were prepared by thoroughly mixing the glass powders (<45μm) with E9 (PAA-M*w*, 80,800, Advanced Healthcare Limited, Kent, UK) and distilled water on a glass plate. The cements were formulated at a Powder:Liquid (P:L) ratio of 2:1.5 with 50wt% additions of PAA, where 1 g of glass powder was mixed with 0.37 g E9 PAA and 0.37 mL water. Complete mixing was undertaken within 20 s.

Fuji IX (P:L—3.6:1.0) & *Ketac Molar* (P:L—4.5:1.0)—Appropriate quantities were used to fill moulds and preparation of cements was completed in accordance with the manufacturer's instructions. Each material was hand mixed using a clean glass plate and spatula.

2.3 Determination of radiopacity (*R*)

The radiopacity (*R*) of *BT 101*, *Fuji IX* and *Ketac Molar* was determined using the equivalent Al thickness method, per CEN ISO 4049. Disc-shaped specimens, approximately 12 mmϕ × 1.0 mm ($n = 3$). In the test, the experimental specimens, an Al step-wedge (Al, 98.96 mass%; Mg, 0.55 mass%; Fe, 0.48 mass% alloy; Kerr, MI, USA) (with thickness of between 1 and 10 mm), and a lead-stop (15.0 mm diameter and thickness 3.0 mm) were exposed together in an X-ray machine (Planmeca Prostyle Intra X-ray machine; Roselle, IL, USA) at 66 kV and 8 mA. Focus-to-sensor distance was 400 mm and the exposure time was 0.32 s. The images were taken on dental X-ray occlusal-size film (Kodak ultra-speed DF-50; Eastman Kodak, NY, USA). The optical density was measured using a transmission densitometer (DT1405; R Y Parry Ltd., Berks, UK). R was calculated from the linear regression of the logarithm of the optical density on the Al thickness for the step-wedge image.

2.4 Ion release analysis

Each cement, *BT 101, Fuji IX* and *Ketac Molar* (6 × 4 ϕ mm, where $n = 3$) was exposed to 10 mL of sterile de-ionised H_2O and rotated on an oscillating platform at 37 °C for 1, 7, 30 and 90 days. The ion release profile of each glass was measured using Inductively Coupled Plasma–Optical Emission Spectroscopy (ICP–OES) on a *Perkin-Elmer* Optima *3000DV* (Perkin-Elmer, MA, USA). ICP–OES calibration standards for Ca, Si, Zn and Sr (*BT 101*) and Al, Ca, P, Si, Na, La (*Fuji IX* and *Ketac Molar*) were prepared from a stock solution on a gravimetric basis. Three target calibration standards were prepared for each ion and de-ionized water was used as a control.

2.5 Biocompatibility testing

2.5.1 Simulated body fluid trial

Simulated body fluid (SBF) was produced in accordance with the procedure outlined by Kokubo et al. [23]. The composition of SBF is outlined in Table 1. The reagents were dissolved in order, from reagent 1–9, in 500 mL of purified water using a magnetic stirrer. The solution was maintained at 36.5 °C. 1 M-HCl was titrated to adjust the pH of the SBF to 7.4. Purified water was then used to adjust to volume of the solution up to 1 L. Cement discs ($n = 2$) were immersed in calculated concentrations of SBF as determined by Eq. 1 and were subsequently stored for 1, 7, 30 and 90 days in an incubator at 37 °C. 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 carry out chemical analysis of the surface of the cement discs. 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. Where *Sa* surface area and *Vs* volume of solution.

Table 1 Ionic composition of SBF

Order	Reagent	Amount
1	NaCl	7.996 g
2	$NaHCO_3$	0.350 g
3	KCl	0.224 g
4	$K_2HPO_4{\cdot}3H_2O$	0.228 g
5	$MgCl_2{\cdot}6H_2O$	0.305 g
6	1 M-HCl	40 ml
7	$CaCl_2$	0.278 g
8	Na_2SO_4	0.071 g
9	$NH_2C(CH_2OH)_3$	6.057 g

$$Vs = \frac{Sa}{10} \quad (1)$$

2.5.2 Cell culture analysis

The established cell line L-929 (American Type Culture collection CCL 1 fibroblast, NCTC clone 929) was used in this study as required by ISO10993 part 5 [3, 4]. Cells were maintained on a regular feeding regime in a cell culture incubator at 37 °C/5 % CO_2/95 % air atmosphere. Cells were seeded into 24 well plates at a density of 10,000 cells per well and incubated for 24 h prior to testing with the liquid extracts. The culture medium used was M199 media (Sigma-Aldrich, Ireland) supplemented with 10 % foetal bovine serum (Sigma-Aldrich, Ireland) and 1 % (2 mM) L-glutamine. The cytotoxicity of cement extracts were evaluated using the Methyl Tetrazolium (MTT) assay in 24 well plates. Aliquots (100 µL) of each sample (*BT 101, Fuji IX* and *Ketac Molar*) were added into wells containing L929 cells in culture medium (1 ml) in triplicate over 1, 7, 30 and 90 days. Each of the prepared plates were incubated for 24 h at 37 °C/5 % CO_2. The MTT assay reagent was then added in an amount equal to 10 % of the culture medium volume/well. The cultures were then re-incubated for a further 2 h (37 °C/5 % CO_2). Next, the cultures were removed from the incubator and the resultant formazan crystals were dissolved by adding an amount of MTT Solubilization Solution (10 % Triton X-100 in Acidic Isopropanol (0.1 *n* HCI)) equal to the original culture medium volume. Once the crystals were fully dissolved, the absorbance was measured at a wavelength of 570 nm. Aliquots (100 µL) of tissue culture water were used as controls, and cells were assumed to have metabolic activities of 100 %. Cement extracts totalled $n = 9$ per absorbance reading at each time period.

2.6 Antibacterial analysis

2.6.1 Agar disc-diffusion test

The antibacterial activity of each cement (*BT 101, Fuji IX* and *Ketac Molar*) was evaluated against *Escherichia coli* strain ATCC 8739 and *Staphylococcus epidermidis* strain ATCC 14990 using the agar diffusion method. Luria agar and broth and BHI agar and broth were used for each bacterium respectively, and each organism was grown aerobically at 37 °C. Preparation of the agar disc-diffusion plates involved seeding agar plates with a sterile swab dipped in a 1/50 dilution of the appropriate 16 h culture of bacteria. Each cement (2 × 12 ϕ mm, where $n = 3$) was 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 in a fumigation

hood using sterile swabs for inoculation of bacteria. Calipers were used to measure zones of inhibition at three different diameters for each disc and each sample was analysed in triplicate and mean zone sizes ± standard deviations were calculated. Inhibition zone sizes were calculated using Eq.2:

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

2.6.2 Agar analysis

Agar strips (3 mm × 4 mm × 5 mm) were prepared for investigation by X-ray Photoelectron Spectroscopy (XPS) 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, in a sterile petri dish and incubated at 37 °C in an air assisted oven for 24 h until dry. XPS was performed using a PHI Quantera SXM Scanning X-ray Microprobe to collect survey scans of each material to detect and changes in agar composition. The monochromatic X-ray source was obtained using an aluminium and magnesium whose Kα energies are 1486.6 and 1253.6 eV. The Analytical parameters include a 100 μm spot size, 25 W, 15 kV, 240 eV pass energy, 0.5 eV step size, 3 sweeps, and a binding energy range of 0–1100 eV.

2.6.3 Bacterial broth analysis

Cement samples (6 × 4 ϕmm, where $n = 3$, for each time period) were immersed in 1 ml cuvettes of sterile LB broth, and were inoculated with 10 μL (~1/100 dilution/mL) of bacterial culture containing *E. coli* over a time period of 24, 48 72 and 96 h in a sterile incubator at 37 °C. At each time period the broth was examined by UV–Visible light spectroscopy (Pharmacia biotech ultrospec 3000 UV–Visible light spectrophotometer) for the % transmission (%T) through the liquid medium. The cement samples (*BT 101*, *Fuji IX* and *Ketac Molar*) were compared against a growing population of *E. coli* at each time period. A sterile control (with sterile de-ionized H_2O used for 1/100 dilution/mL) was also analyzed at each time period which had a %T of approximately 92 %. At the final time period a 1/50 dilution of bacteria was used to plate standard agar plates and the bacterial colony growth was observed using an Olympus IX20-UCB Optical Fluorescent Microscope at 4× magnification.

2.7 Statistical analysis

One-way analysis of variance (ANOVA) was employed to analyse the cell culture and the antibacterial efficacy of the experimental materials in relation to (1) differences between material and (2) any changes present with respect to maturation. Comparison of relevant means was performed using the post hoc Bonferroni test. Differences between groups was deemed significant when $P \leq 0.05$.

3 Results and discussion

3.1 Radiopacity (*R*) and ion release

Characterization and structural comparison of each glass used for this study has been previously undertaken by the authors, including determination of the physical properties [22]. Initial testing regarding this work included determining the radiopacity (*R*) of each material. The radiopacity is an important characteristic as injectable materials are delivered subcutaneously under fluoroscopic guidance which results in minimally invasive surgery. The *R* of similar Zn–GPCs have previously been evaluated and compared to orthopaedic cements such as Simplex P (Stryker, MI, USA), and proved to be superior in radiopacity [24]. With respect to the materials studied here, BT 101 performed similarly to both *Fuji IX* and *Ketac Molar*. The *R* is presented in Table 2 and for *BT 101* *R* was determined to be 2.40 while the *R* of *Fuji IX* and *Ketac Molar* were 2.80 and 2.09 respectively. It is likely that the *R* of *BT 101* is predominantly due to Sr within the cement matrix. This is a positive attribute as *Fuji IX* contains both Al and Sr, while *Ketac Molar* contains both Al and La to produce similar *R* values [22]. Ion release studies were initially conducted on *BT 101* and are presented in Fig. 1, identifying the release of silica (Si), calcium (Ca), zinc (Zn) and strontium (Sr) over 1, 7, 30 and 90 days. With respect to Ca the release rate was found to increase with exposure time from 5 to 23 mg/L (1 and 90 days). Sr release was found to be very similar to Ca where Sr release increased from 4 to 23 mg/L (1 and 90 days). Zn release ranged from 5 to 13 mg/L (1 and 90 days) and Si presented the highest release rates with respect to maturation which ranged from 56 to 203 mg/L (1 and 90 days).

Ion release was conducted on *Ketac Molar* looking at aluminium (Al), Ca, phosphorus (P), lanthanum (La), Si and sodium (Na) and is presented in Fig. 2. Al release was relatively low at 1 day 0.2 mg/L, but spiked to 0.5–0.6 mg/L

Table 2 Radiopacity of *BT 101*, *Fuji IX* and *Ketac Molar*

	Radiopacity (R)	*SD*
BT 101	2.40	0.05
Fuji IX	2.80	0.00
Ketac Molar	2.09	0.08

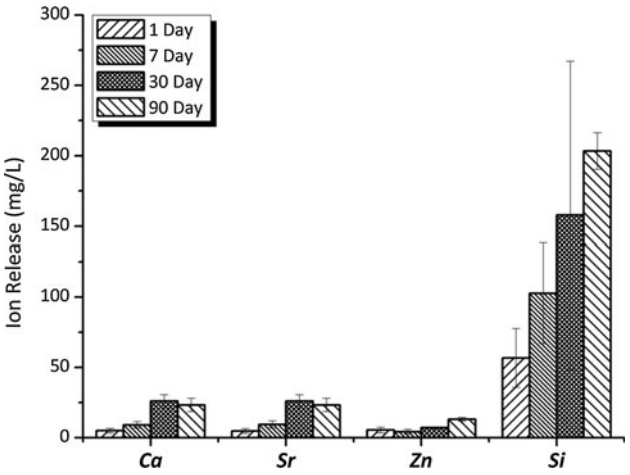


Fig. 1 Ion Release of *BT101* cement considering Ca, Sr, Zn Si

over the period of 7–90 days. Ca release ranged from 0.3 to 1.0 mg/L over the period of 1–90 days. P presented an incremental release rate which ranged from 0.2 to 0.7 mg/L over 1–90 days and La, which is the primary radiopacifier in *Ketac Molar* showed relatively low release rates which ranged from 0.02 to 0.05 mg/L which peaked at 30 days. Higher ion release rates were presented with both Si and Na. Si release ranged from 5 to 23 mg/L, and Na ranged from 15 to 76 mg/L over 1–90 days. The ion release profiles for *Fuji IX* is presented in Fig. 3. With respect to *Fuji IX* the Al release was significantly higher than *Ketac Molar*. Al release from *Fuji IX* ranged from 0.9 to 2.9 mg/L, Ca release ranged from 0.2 to 0.7 mg/L and peaked after 30 days. This is an unexpected result given that *Fuji IX* does not initially contain Ca as determined previously [22]. However, these minor Ca levels may be a contaminant in the incubation media, or traces of Ca, from the ICP testing of *Ketac Molar* and *BT 101*, are being detected by the instrument. *P* release ranged from 0.2 to 0.5 mg/L which was similar to the *P* release from *Ketac Molar*. Both Si and Na also presented higher rates of ion release which ranged between 6–26 and 22–60 mg/L over the period of 1–90 days respectively. Ion release studies on G338 (SiO_2–Al_2O–Na_2O–CaF–P_2O_5) GPCs by Czarnecka et al. determined similar values for Si (27 ppm), Na (151 ppm), Ca (1.11 ppm), however Al (41 ppm) and P (8.5 ppm) were found to be much higher. This study was also undertaken in water over a period of 1 week [25]. The relatively low Ca release in *Fuji IX* and *Ketac Molar* can be attributed to its role in the setting process. Ca-PAA complexes cause Ca to become bound within the matrix of the set cement, a hypothesis shared by Czarnecka et al. [25]. The lower Al release rates attributed to *Fuji IX* and *Ketac Molar* may also be attributed to extensive Al-PAA complex formation within the cement.

3.2 Biocompatibility evaluation

Biocompatibility evaluation was undertaken using simulated body fluid (SBF) testing and cell culture analysis in L929 mouse fibroblasts over a period of 1, 7, 30 and 90 days. Figure 4a, b show the SBF testing of *BT 101* at 1 day and 90 days. After 1 day in SBF, CaP precipitation was observed along with dehydration fractures on the cement surface. Quantitative EDX demonstrated the presence of Si (6 wt%), Zn (37 wt%), Ca (14 wt%) and Sr 4 wt%), which are components present within the cement. *P* was also detected (5 wt%) at 1 day. Precipitation increased up to 90 day where complete coverage of the cement surface by CaP can be observed. The concentration of each element can also be seen to increase and is incorporated into the new surface layer. Si (11 wt%) increase is likely due to soluble Si from the surface being incorporated into the surface layer during precipitation as high concentrations of Si can be observed to be released at 90 days, as presented in the ion release data. Also Zn (42 wt%), Ca (16 wt%) and Sr (15 wt%) are also observed to increase which is also likely due to the released ions being incorporated into the surface layer during the initial stages of CaP deposition. Previous TEM studies by the authors determined that the CaP surface precipitation layer is amorphous in structure by selected area diffraction, and that the release of Zn in particular results in inhibiting the crystallization of the CaP into crystalline hydroxyapatite [26, 27]. This hypothesis is further supported by the literature which states that Zn is 1000 times more effective at inhibiting crystallization than magnesium [28]. However the amorphous CaP surface layer presented here may not be too dissimilar to the initial amorphous calcium phosphate (ACP) which is formed during the initial stages of bone formation. It is also evident from Fig. 4b that the P concentration has increased considerably since 1 day immersion in SBF and since *BT 101* does not initially contain P, the P concentration is precipitating out of solution and depositing on the cement surface.

Figure 4c, d shows the SEM images of *Fuji IX* at 1 and 90 days. It is evident that after 1 day there is no CaP precipitation on the cement surface. EDX revealed the presence of Na, Al, Si, Ca, Sr and P on the surface of the cement discs. Ca and P are present in the glass phase used to formulate these cements which can explain their presence. After 90 days immersion in SBF there was also no CaP precipitants evident on the cement surface. Although P levels showed a slight increase after 90 days, it is difficult to determine whether the P is leaching from the cement or if P from the SBF is depositing on the surface. Figure 4e, f present the 1 day and 90 day *Ketac Molar* after immersion in SBF. There was relatively little difference between *Ketac Molar* and *Fuji IX* as no CaP surface layer was

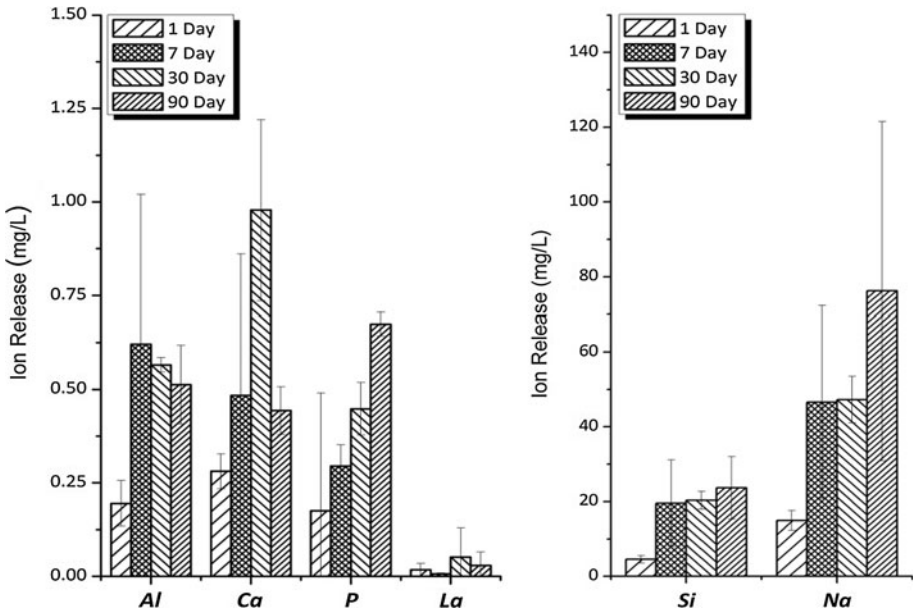


Fig. 2 Ion Release of *Ketac Molar* considering Al, Ca, P, La, Si and Na

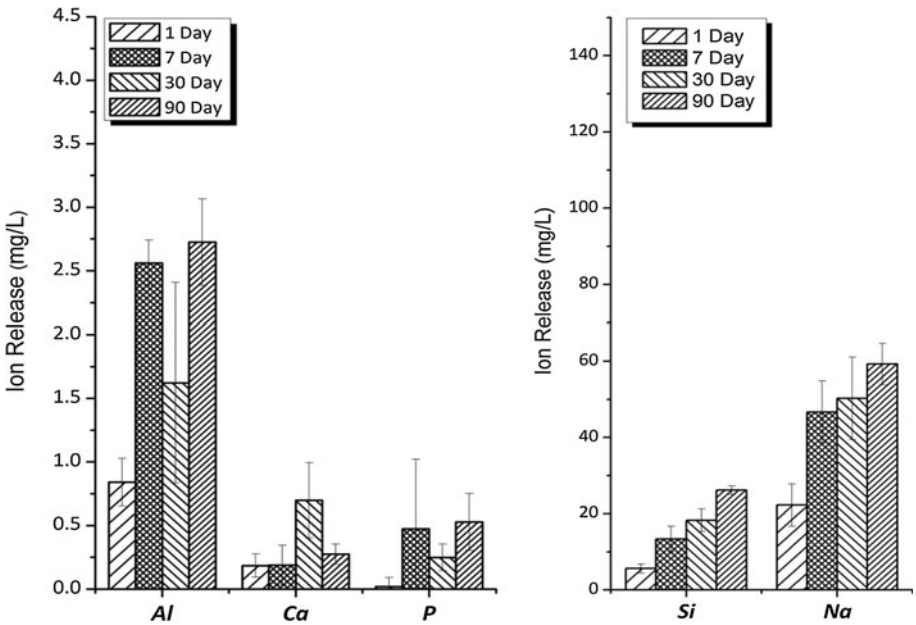
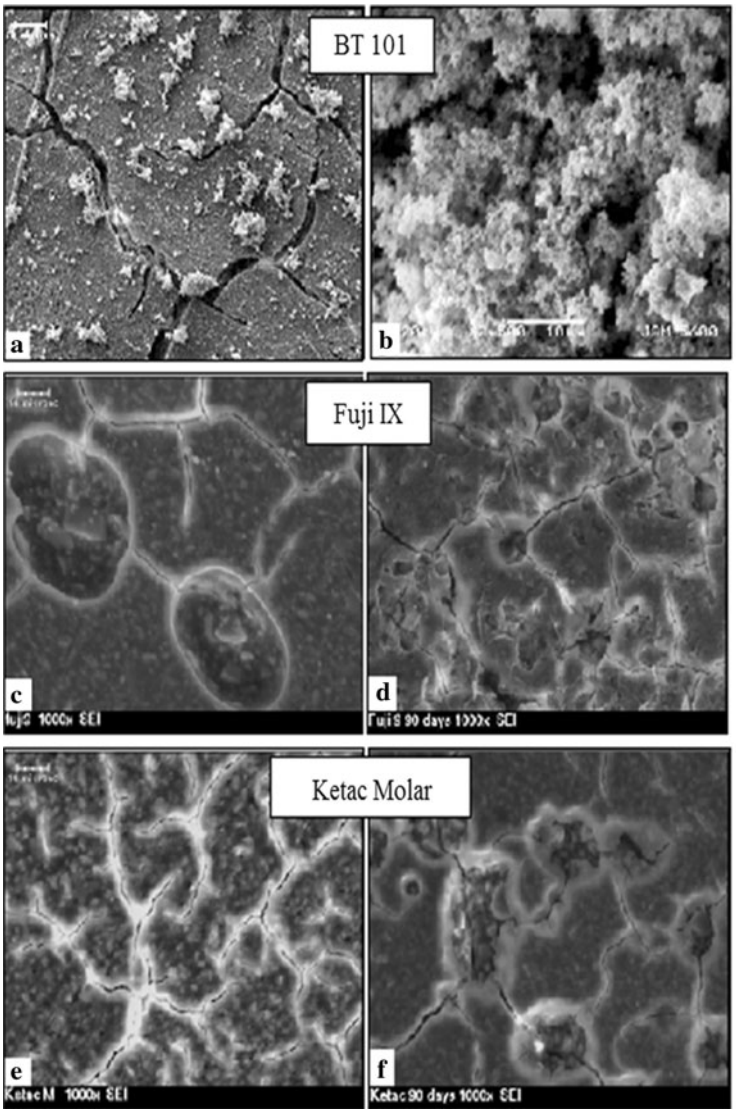


Fig. 3 Ion Release of *Fuji IX* considering Al, Ca, P, Si and Na

evident under SEM, the major difference being the presence of Sr in *Fuji IX* and La in *Ketac Molar* which, in both materials, constituted the highest concentration detected by EDX. The levels of each individual element detected show little deviation from the 1 day samples, which may be attributed to the relatively low concentrations of ions being released from both *Fuji IX* and *Ketac Molar*, in particular Ca (0.7 and 1 mg/L) and Si (23 and 26 mg/L). It is known that the solubility of Si and the subsequent formation of Si–OH^- on the cement surface is critical to the precipitation of CaP. Also, Ca release from the cement significantly aid in the precipitation of PO_4^{3-} and CO_3^{2-} resulting in an amorphous carbonate hydroxyapatite surface layer [23, 29].

Cytotoxicity testing was also conducted in order to determine the cell viability of each material with respect to maturation. Figure 5 shows the cytocompatibility of each of the materials condition medium tested over 1, 7, 30 and 90 days and were compared to a healthy growing population of fibroblast cells. Regarding *BT 101*, cell viability changed relatively little over time where the cell viability reduced to 59 % (1 day), 64 % (7 days), 51 % (30 days) and 59 % (90 days). There was no significant change in cell viability with respect to immersion time, $P = 0.065$–1.000. Regarding *Fuji IX* the cell viability was similar to *BT 101* where viability ranged from 56 % (1 day), 63 % (7 day), 48 % (30 days) and 61 %



a. b.

	1 Day	90 Days
Si	6.4	10.5
Zn	37.6	41.7
Ca	14.1	16.3
Sr	3.9	14.7
P	4.6	15.1

c. d.

	1 Day	90 Days
Na	2.4	3.7
Al	22.3	22.4
Si	21.3	20.8
Ca	14.3	17.6
Sr	28.6	24.2
P	6.5	11.1

e. f.

	1 Day	90 Days
Na	2.8	3.1
Al	18.3	18.7
Si	18.0	14.6
Ca	18.0	20.7
La	37.6	37.2
P	3.2	5.4

Fig. 4 SBF results of *BT 101, Fuji IX* and *Ketac Molar* after 1–90 days with corresponding EDX (**a**–**f**)

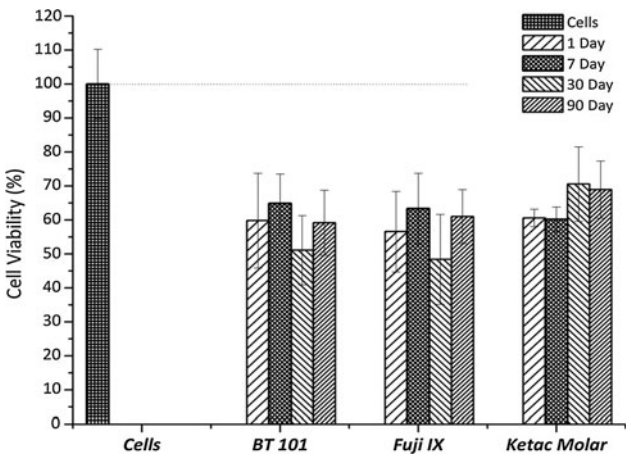


Fig. 5 Cell viability analysis of *BT 101, Fuji IX* and *Ketac Molar* over 1, 7, 30 and 90 days

(90 days), $P = 0.128$–1.000. Similar to *Fuji IX* and *BT 101*, *Ketac Molar* presented similar values of 60 % (1 day, 7 days), 70 % (30 days) and 68 % (90 days), $P = 0.093$–1.000. This reduction in cell viability can be attributed to ion release from the cement which accumulates to levels that become toxic to cells.

With respect to *BT 101* it is likely that the release of Zn, Ca and Sr ($\sim$5–20 mg/L) primarily results in the decrease in cell viability as Si levels increase from 56 to 203 mg/L with no significant change in cell viability. Regarding *Fuji IX* relatively low concentration of Al, Ca, P and La were detected, <1 mg/L. Similarly for *Ketac Molar*, the release of Al, Ca and P were below 3 mg/L. In both commercial materials it is likely that the release of F may be a predominant or contributing factor to the reduction in cell viability. However further studies will be required to

determine the effect of F. Each material tested (*BT 101*, *Fuji IX* and *Ketac Molar*), at each time period was found to be significantly reduced when compared to the control cell population, $P < 0.0001$.

3.3 Antibacterial analysis

Antibacterial analysis was conducted in order to compare the materials antibacterial efficiency in both agar medium and in an aqueous environment. Preliminary testing was conducted in *E. coli* and *S. epidermidis* and the results are presented in Fig. 6. It was determined that *BT 101* produced inhibition zones of 2.3 mm in *E. coli* and exhibited a bacteriostatic effect in *S. epidermidis*, however no conclusive inhibition zone could be determined. Both *Fuji IX* and *Ketac Molar* presented similar results where no bactericidal or bacteriostatic effect was identified in either bacterium.

The difference in the antibacterial effect of *BT 101* may be attributed to differences in ion release where Zn release may be responsible for the antibacterial effect. To investigate the antibacterial effect, *sections* of agar were extracted from the inhibition zone/area surrounding each cement disc and was analysed for changes in composition (by X-ray photoelectron spectroscopy) when compared to a sterile control agar sample. Figure 7 presents the composition of each material. Figure 7a shows the composition of the control sterile agar *section* which was found to contain Na, O, N, Ca, C, Cl and P. Figure 7b presents the composition of the agar containing *BT 101*. This *section* contains the elements present in the control agar *section* in addition to Sr and Zn which are not contained within the control agar. This indicates that Sr and Zn are the predominant ions being leached from the cement.

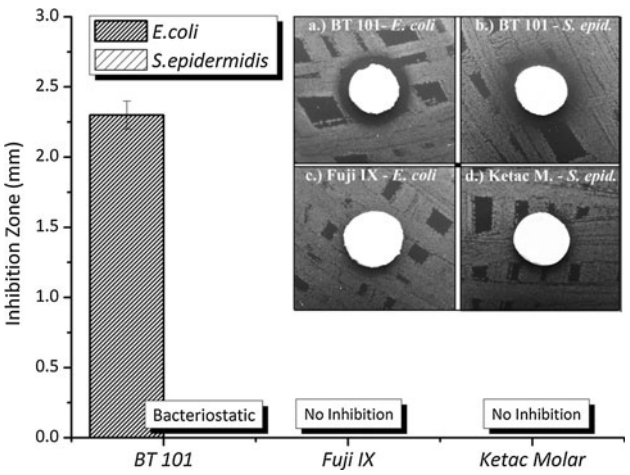


Fig. 6 Agar diffusion test considering *BT 101, Fuji IX* and *Ketac Molar*

Figure 7c, d shows the composition of the agar *sections* taken from *Fuji IX* and *Ketac Molar* respectively and show relatively little deviation from the control *section* with the exception that *Fuji IX* contains F and Sr, and *Ketac Molar* contains La. It is possible that the antibacterial efficacy of the materials is restricted by the highly viscous agar used in the agar diffusion test. Also extensive cross-linking within the cement matrix could restrict the diffusion and movement of ions through the media, thus further restricting any antibacterial potential. To further determine the antibacterial efficacy in a more fluid medium the broth dilution test was conducted. Figure 8 shows the results of the broth dilution test where a *growing bacterial broth* and *sterile broth* were used as comparative controls. The growing broth culture attained a %T of 5 % after 24 and 48 h ($P = 1.000$), and 7 % after 72 and 96 h ($P = 1.000$). The sterile broth retained a %T of between 93 and 96 % over 24–96 h. The %T of *BT 101* was found to be 40 % after 24 h and reduced slightly to 33 % after 48 h ($P = 0.002$). It further reduced to 28 and 26 % after 72 and 96 h ($P = 0.014$) respectively. The relatively high %T after 24 h can be attributed to ions leached from the cement eradicating bacteria in solution. The reduction in %T over time can be attributed to the exhaustion of antibacterial ions or precipitation/neutralization of the ions resulting in a reduced effect. Both *Fuji IX* and *Ketac Molar* experienced a similar trend where no significant difference was observed after 24 and 48 h ($P = 0.979$, 1.000), where the %T ranged from 4 to 7 %. At 72 h and 96 h both cements experienced a similar trend where they exhibited a %T of 7 % ($P = 1.000$) and 6 % ($P = 1.000$) respectively. The effects experienced by both *Fuji IX* and *Ketac Molar* were not significantly different from the control bacterial broth at any time period tested ($P = 1.000$), while *BT 101* was found to reach significance at each time period ($P = 0.000$). This supports earlier findings where little antibacterial effect was observed with *Fuji IX* and *Ketac Molar*.

This can also be seen in Fig. 8a–d where a clear reduction in bacterial colonies is evident. Quantitative assessment was conducted by determining the mean colony counts in CFU's/plate. The colonies from each plate were counted and the results are presented in Fig. 9 on a logarithmic scale. Initially it is evident that there is a high concentration of CFU's extracted from the growing control bacterial broth (2.4×10^4 CFU). *Fuji XI* and *Ketac Molar* presented slightly lower values at 1.5×10^4 CFU and 1.2×10^4 CFU respectively. A much lower distribution was determined for *BT 101* at 2.5×10^2 CFU's. The antibacterial effect demonstrated by *BT 101* can be predominantly attributed to Zn^{2+} release, however further studies will need to be conducted in order to determine if it is the direct action of Zn^{2+} on the bacterial cell or if

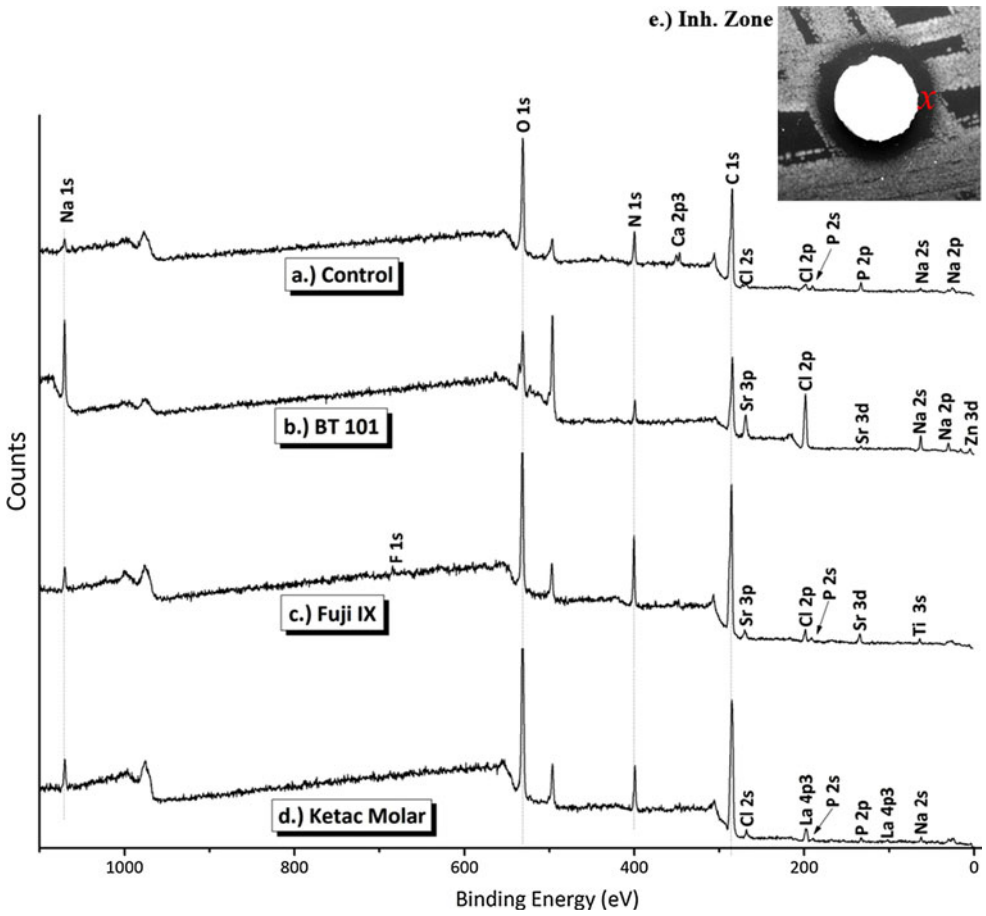


Fig. 7 Agar analysis by XPS considering **a** control agar, **b** *BT 101*, **c** *Fuji IX*, **d** *Ketac Molar* and **e** agar section used foe XPS

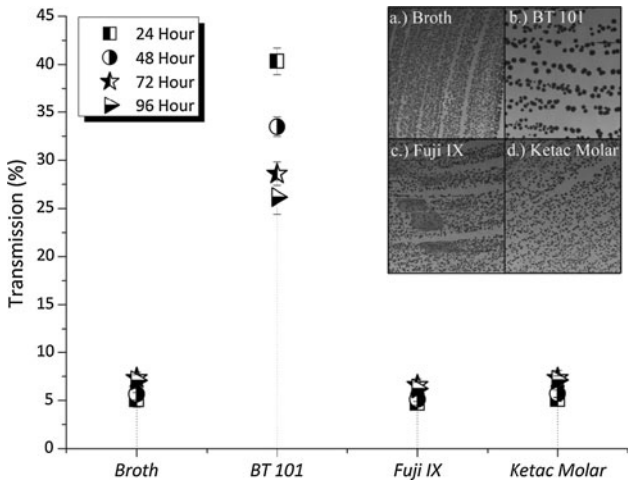


Fig. 8 UV/Vis analysis of bacterial broth considering **a** control bacterial broth, **b** *BT 101*, **c** *Fuji IX* and **d** *Ketac Molar*

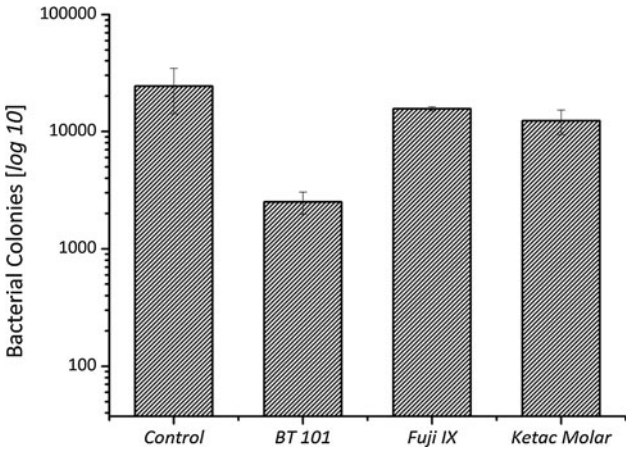


Fig. 9 Bacterial CFU present in GPCs broth after 24 h

changes in the surrounding pH presents an unfavourable environment for bacterial cell proliferation. The reduced concentration of CFU's presented by *Fuji IX* and *Ketac Molar* is likely due to F^- release, which is either not as bactericidal as Zn^{2+} or does not have as potent an effect on this particular bacteria.

4 Conclusion

This study was undertaken to determine the biocompatibility of a SiO_2–ZnO–CaO–SrO based glass polyalkenoate cement and to compare it to materials that have a similar setting chemistry, i.e. commercial GPC's *Fuji IX* and *Ketac Molar*. The experimental GPC (*BT 101*) was found to have a radiopacity approximately equal to both commercial

GPCs with the exclusion of Al from the starting glass. Ion release rates present high Si release from *BT 101*, which is known to aid in the precipitation of CaP surface layer which was found to be present on the surface of *BT 101*. Surface precipitation was not evident for either commercial GPC. Cell viability was approximately similar for each material, however, the antibacterial potential of *BT 101* was greater than either *Fuji IX* or *Ketac Molar*, in particular, liquid extracts were found to greatly reduce the concentration of bacterial colony forming units. The results from this study suggest that this experimental GPC (*BT 101*) may provide a beneficial therapeutic effect if applied to orthopaedics.

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