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A preliminary study of an aluminum-free glass polyalkenoate cement

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Glass polyalkenoate cements (GPCs) are formed by the reaction of an ion leachable alumino-silicate glass with an aqueous solution of poly (alkenoic acid). Water is used as the reaction medium. An acid-base reaction, occurs, whereby the acid attacks and degrades the glass structure, releasing metal cations which are then chelated by the carboxylate groups and serve to crosslink the polyacid chains. The final cement consists of residual glass particles embedded in a hydrogel polysalt matrix [1]. The setting reaction in GPCs is a continuous process evident by the increase in compressive strength of the cement with aging time [2–5].

GPCs can release clinically beneficial amounts of fluoride [6, 7] and have acceptable handling properties and aesthetics [8, 9] making them suitable as dental restoratives. However, the presence of aluminum in the glass phase of the cements limits orthopaedic applications as research has shown that aluminum ions released from glass polyalkenoate cements can result in defective bone mineralization [10] and as a consequence the ability of these cements to chemically bond to bone may be retarded. There are also concerns over the toxicity of aluminum [11]. An aluminum-free polycarboxylic acid based cement would be of considerable interest in the surgical field.

The aluminum ion is believed to play an integral role in the setting process of a GPC and the complete removal of aluminum is likely to hinder cement formation. However, zinc oxide is unusual in that it can act both as a network modifying oxide and as an intermediate oxide [12] in a similar fashion to alumina. This results in ternary systems based on zinc silicates (with the exception of those containing alumina) often having very extensive regions of glass formation. Zinc silicate glasses containing little or no alumina may therefore be suitable for forming polyalkenoate cements and the purpose of this work is to investigate cement formation between such a zinc silicate glass and a series of polyacrylic acids, with the ultimate objective of developing a new range of GPCs that have orthopaedic applications.

One glass composition was produced, $0.4SiO_2$ $0.32CaCO_3$ 0.28ZnO. This formulation corresponds to one of the eutectic points on the relevant ternary diagram [13], chosen from this system on the basis of ease of glass formation [14].

Appropriate amounts of analytical grade silica, zinc oxide and calcium carbonate were weighed out in a plastic tub and mixed in a ball mill for one hour, then dried in a vacuum oven (100 °C, 1 h) before being transferred to a mullite crucible for firing (1580 °C, 1 h). The melts were then shock quenched into demineralized water. The resulting glass frit was dried, ground in a gyro mill (Glen Creston, London, UK) and sieved. The fraction that passed through a 45 μm sieve was used for the subsequent research. The glass was found to be fully amorphous by X-ray powder diffraction.

The polyacrylic acids used to form cements were E5, E7 and E9 (Allied Colloids, Bradford, UK). These acids were freeze dried and ground to a maximum particle size of 45 μm. The molar masses of these acids have been determined previously [15] and are shown in Table I.

Cements were prepared by mixing 2.0 g of glass powder with 0.4 g of dried PAA and 0.6 g of double distilled water (10 wt% tartaric acid). Cements all had suitable working and setting times for restorative purposes. Hydrolytic stability was assessed by immersing the set specimen into 5 ml of water. Any softening of the specimen or marked cloudiness of water after 24 h immersion was taken as evidence of hydrolytic instability.

The flexural strength of the cements was determined by the method of Billington *et al.* [16]. The one day samples produced with the E5 and E7 acids were not hydrolytically stable enough for testing. The results are collated in Table II and expressed graphically in Fig. 1. Flexural strength of the cements increased with both maturation and PAA molecular weight.

TABLE I Molar mass details of the poly(acrylic) acids

Code	Mw	Mn	PD	Peak mol. wt.
E5	9270	3030	3.1	6810
E7	25 700	8140	3.2	19 100
E9	80 800	26 100	3.1	83 500

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TABLE II Flexural strengths of the cements at 1, 7 and 30 days

Cement	σ_f (1 day) (MPa)	σ_f (7 days) (MPa)	σ_f (30 days) (MPa)
A/E5	–	15.5	22.1
A/E7	–	20.3	26.5
A/E9	6.2	34.6	35.1

TABLE III Nano-indentation results from Fuji IX, SerenoCem, bovine bone and AE9

Material	Hardness (MPa)
Fuji IX	310
SerenoCem	322
Bovine bone	201
AE9	155

Nano indentation was employed to determine the surface hardness of the most promising zinc based GPC, the glass being mixed with acid E9 (AE9). These values were compared with those obtained from two commercial GPCs, Fuji IX (GC Corporation, Japan) and SerenoCem (Corinthian Medical Ltd, UK) and also bovine bone. Experiments were performed using a CSEM Nano-Hardness Tester (NUI Galway, Ireland) fitted with a Berkovich indenter. All samples were subjected to the same indentation cycle using a 1mN maximum force giving penetration depths in the range of 50 nm–500 nm and corresponding contact areas of the order of a few μm^2. Testing was undertaken at room temperature. Further details of the test procedure are available elsewhere [17]. Hardness values from the tests are collated in Table III and include results from at least two samples in both cases. The results have been calculated for contact with the solid surface and represent standard responses from the materials.

This research has suggested that replacing aluminum with zinc produces a network of similar integrity; the flexural strengths reported in this work are comparable to conventional GPCs that have been mixed using a similar powder : liquid ratio. The strengths also follow the same trends as would be seen for a conventional GPC, in that flexural strength increases with both maturation and PAA molecular weight. Hardness values of the zinc containing cement are considerably lower than either of the commercial materials tested, but only slightly lower than those of bovine bone.

A preliminary cyto-toxicity study was performed using eighteen discs of the most promising cement (AE9) alongside six of a commercial cement, SerenoCem, which has the following composition:

$$21.7SiO_2\ 11.9Al_2O_3\ 29.8AlPO_4\ 24.2CaCO_3\ 12.5CaF_2$$

The liquid phase is composed of acrylic acid, isopropanol, ammonium persulphate and deionized water. These discs were autoclaved prior to cell culture analysis. All of the samples were placed in tissue culture water (0.6 cm^2 surface area of sample/ml of extractant). Six samples of AE9 were placed in a static water bath (37 °C), six in a moving water bath (37 °C, 1000 rpm) and six in an oven (70 °C). Only one sample of the commercial material was placed under each of these conditions. Half of the samples were stored in these conditions for one day, the remainder for seven days. After this time the discs were removed, washed with distilled water, placed directly onto a mono-layer of L929 cells and incubated for 48 h in the presence of 5% CO_2. In order to observe the effect that zinc has had on the cells, 1 μl and 10 μl of a zinc solution were each added to 3 ml of cell media resulting in a concentration of 0.33 μg/ml and 3.32 μg/ml respectively. A direct assay was also performed using organo-tin stabilized polyvinyl chloride (PVC, Portex, Kent) as a positive control and phosphate buffered saline (PBS) as a negative. Cell viability was measured by staining the cells with a neutral red. The total protein was calculated by carrying out a Bicinchoninic acid (BCA) assay. Absorbance was read at 562 nm. The neutral red (NR) assay procedure is a cell survival/viability chemosensitivity assay, based on the ability of viable cells to incorporate and bind neutral red, a supravital dye. The BCA assay is a direct measurement of cellular protein enabling the protein concentration to be determined. The influence of the cements on cell viability of L929 cells following 48 h direct contact as a BCA assay and as a NR assay are shown in Figs. 2 and 3 respectively.

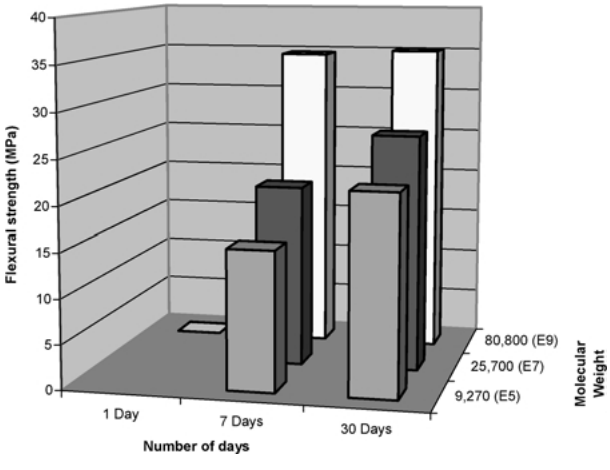

Figure 1 Flexural strength of the cements plotted with respect to maturation time and molecular weight.

A high concentration of zinc ions were released from the AE9, resulting in cytoxicity. These values ranged from 1.17 μg/ml to 28.67 μg/ml. As expected, in going from 1 day to 7 days, there is an increase in ion concentration as they are being constantly released from the cements. However, in comparing the 1 and 7 day values, the ion release rate has slowed down. For those cements analyzed at 37 °C at 1000 rpm, zinc concentrations are higher than those stored under static conditions. Agitation releases more ions from the cement into the extractant. Increasing the temperature also increases the rate of release with this condition leading to the highest zinc concentration values.

The SerenoCem, while still toxic, released significantly lower amounts of the active ion. Cell viability values of 65 and 74% were obtained for 1 and 7 days respectively. It is speculated that this cell death is due to the release of aluminum ions. Serenocem is based on a flouroaluminosilicate glass and it is the release of aluminum that is cyto-toxic.

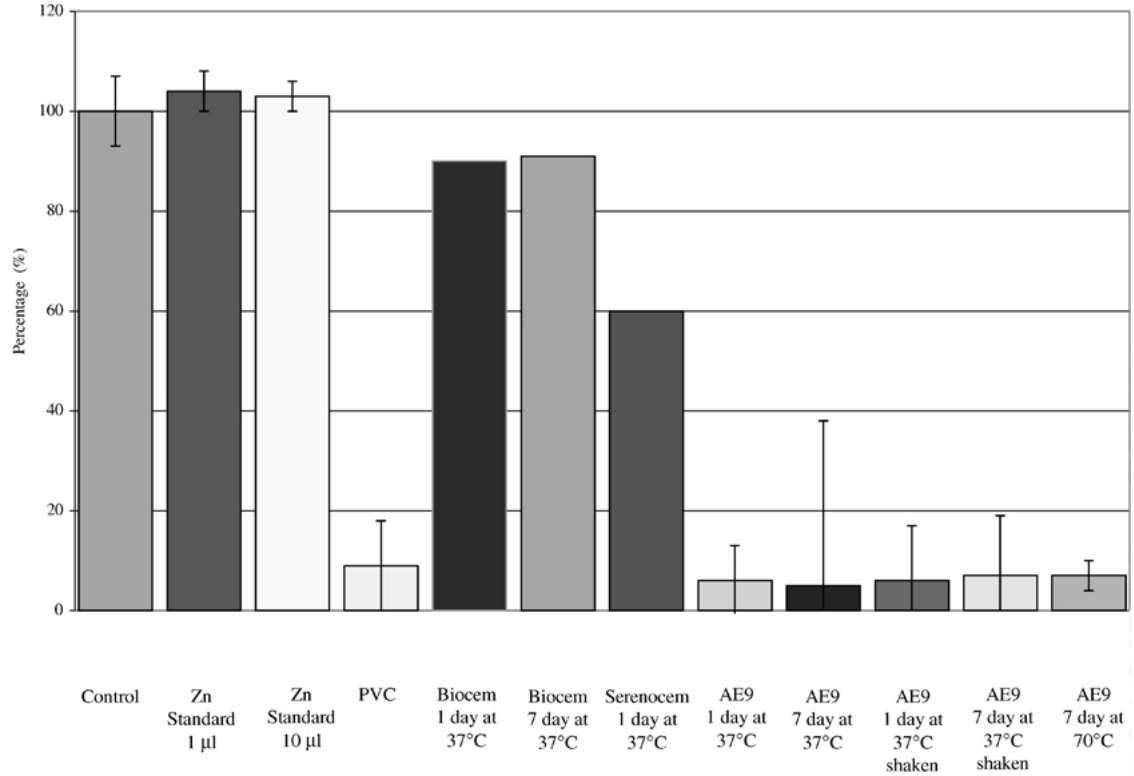

Figure 2 Influence of bone cements on the viability of L929 following 48-h direct contact (neutral red assay).

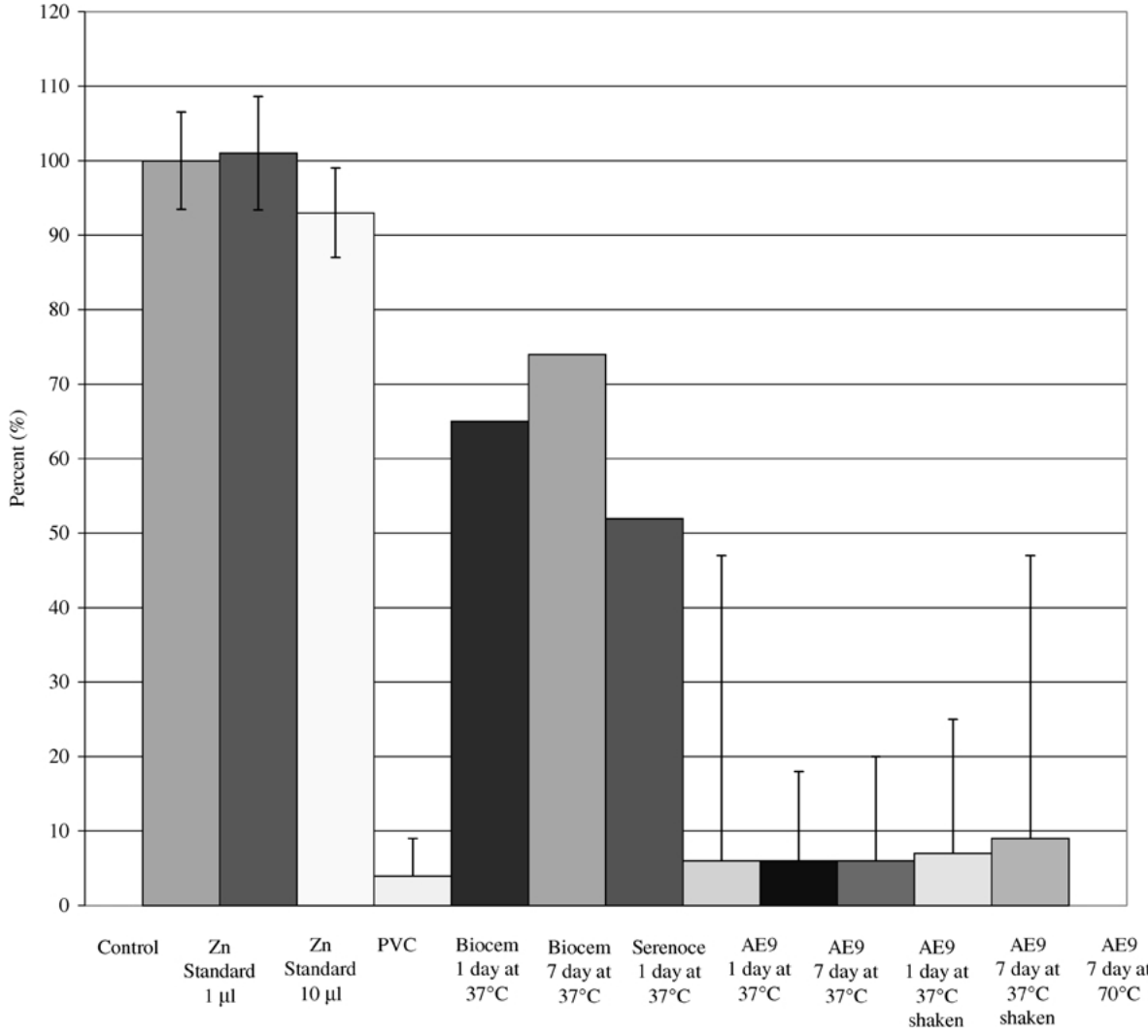

Figure 3 Influence of bone cements on the viability of L929 following 48-h direct contact (total protein assay).

There are two reasons why the Al-free GPC is appearing to be so toxic:

1. A low powder : liquid ratio similar to that used in the production of luting, rather than restorative, cements was employed in the formulation of the Al-free cements. This results in a less cohesive network and subsequently the release of more ions.
2. Cell culture methods used to determine cytotoxicity involve a closed system. GPCs often register as toxic materials when analyzed by cell culture, whereas *in vivo* testing proves that such materials are non-toxic. This implies that it is the test method, not the material that is at fault.

Comparing graphs of the influence of the cements on the cell viability it can be seen that there are slightly higher results being obtained for the NR assay. Neutral red readily penetrates cell membranes by non-ionic diffusion, accumulating intracellularly in lysosomes, where it binds with anionic sites in the lysosomal matrix. Specific dyes can give an inaccurate result due to the presence of certain ions. Positive ions can lead to an increase in the uptake of NR. Thus, the presence of Zn^{2+} in the extractant leads to an increase in lysosomal activity resulting in slightly higher viability values.

Transition metals are essential for life. Zn^{2+} is the second most prevalent trace element in the body and is present in large concentrations in the mammalian brain. Although Zn^{2+} is a cofactor for many enzymes in all tissues a unique feature of the brain is its vesicular localization in presynaptic terminals where its release is dependent on neural activity. Several lines of evidence support that, upon excessive synaptic Zn^{2+} release, its accumulation in postsynaptic neurons contributes to the selective neuronal loss that is associated with certain acute conditions, including epilepsy and transient global ischaemia. More speculatively, Zn^{2+} dishomeostasis might also contribute to some degenerative conditions, including Alzheimer's disease. Borovanský *et al.* [18] investigated the cytoxicity of zinc *in vitro* using B16 mouse melanoma lines, HeLa and I-221 epithelial cells. It was suggested that a prerequisite for the toxic action of zinc is entry into cells using channels that are shared with iron or calcium. They stated, "*in vitro* Zn^{2+} should be regarded as a dangerous cation."

This preliminary study has shown that cements can be formulated by an acid base reaction between poly acrylic acids and a calcium zinc silicate glass. The mechanical properties of these cements are slightly inferior to GPCs formulated from aluminum-containing glasses but may still have potential for biomedical applications. Unfortunately, the high zinc ion release has been shown to be highly toxic by cell culture methods, thus *in vivo* test procedures are required to fully understand the biocompatibility of these novel materials.

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