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LETTER

TEM analysis of apatite surface layers observed on zinc based glass polyalkenoate cements

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Glass polyalkenoate cements (GPCs) are acid base cements formed by the reaction of an aqueous solution of polyalkenoic acid, usually polyacrylic acid (PAA) with an acid degradable aluminosilicate glass. The result of the reaction is cement consisting of reacted and unreacted glass particles embedded in a polysalt matrix. In addition to these conventional GPCs, aluminium-free glass polyalkenoate cements based on zinc silicate glasses (Zn-GPCs) exhibit significant potential as bone cements for several reasons. Primarily, they are formulated without the inclusion of aluminium (Al) [1] in the glass phase and thus eliminate clinical complications arising from the release of the Al^{3+} ion from the cement in vivo. Such complications have, in the past, included aluminium-induced encephalopathy [2–5] and defective mineralization of cancellous bone [6]. Secondly, Zn-GPCs set without a significant evolution of heat, when compared with commercial bone cements such as Spineplex® (Stryker, Limerick, Ireland). Finally, these materials can be tailored to release clinically beneficial ions into the surrounding tissues [7]. In addition to Zn, these cements have been synthesized to contain strontium (Sr) [8, 9]. Both Sr and Zn inhibit osteoclastic turnover and promote osteoblastic turnover, resulting in increased bone strength and decreased fracture risk [10–14].

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Conventional GPCs bond directly to hydroxyapatite (HA), the mineral phase of tooth and bone, via the adsorption of carboxylate groups of the polyacid chains into the HA structure [15]. This indicates all conventional GPCs are capable of bonding to the living bone. However, it has been postulated that an essential requirement for a material to bond to living bone lies in its ability to form a bone-like crystalline apatite layer at its surface in vivo and that such a system can be replicated using simulated body fluid (SBF) [16]. Kamitakahara et al. recently used SBF to evaluate the bone bonding ability of conventional GPCs and found that PAA inhibited the formation of an apatite layer at the surface of GPCs and thus concluded that they were unlikely to bond to bone in vivo [17].

Given these conflicting viewpoints, the authors have previously evaluated the ability of Zn-GPCs to form a bone-like apatite layer in vitro using SBF [1, 9]. It was shown that calcium phosphate layers are produced on Zn-GPCs immersed in SBF within 24 h. However, thin film X-ray diffraction (TF-XRD) indicates no crystallinity within the surface layer, and chemical analysis using scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) of the surface layers is complicated by the collection of elemental signatures from the cement sample itself.

In order to eliminate these issues, this letter seeks to validate the amorphous nature and composition of the surface layers observed on Zn-GPCs after immersion in SBF using transmission electron microscopy.

One glass composition $0.08SrO/0.08CaO/0.36ZnO/0.48SiO_2$ (mol. fraction), was synthesized. Appropriate amounts of analytical grade strontium carbonate, calcium carbonate, zinc oxide and silicon dioxide (Sigma Aldrich, Dublin, Ireland), were weighed out in a plastic tub and mixed in a ball mill for 1 h, then dried (100 °C, 1 h). The

Table 1 Order and amounts of reagents used to prepare 1,000 mL of SBF

Order	Reagent	Quantity
1	NaCl	8.035 g
2	$NaHCO_3$	0.355 g
3	KCl	0.225 g
4	$K_2HPO_4{\cdot}3H_2O$	0.231 g
5	$MgCl_2{\cdot}6H_2O$	0.311 g
6	1.0 M-HCl	39 mL
7	$CaCl_2$	0.292 g
8	Na_2SO_4	0.072 g
9	Tris	6.118 g
10	1.0 M-HCl	0–5 mL

pre-fired glass batch was then transferred to a platinum crucible for firing (1,480 °C, 1 h). The glass melt was subsequently quenched into water and the resulting frit was dried, ground and sieved to retrieve a <45 μm glass powder. The glass was then annealed (645 °C, 3 h) for subsequent use in Zn-GPC specimen preparation.

One Zn-GPC (termed BT102) was prepared by mixing the glass with a 50 wt% aqueous solution of PAA; M_w = 80,800 (Advanced Healthcare, UK) on a clean glass slab with a dental spatula at a powder liquid ratio of 2:1.5.

SBF was produced in accordance with the literature [16]. The composition is illustrated in Table 1. Reagents were dissolved in sequential order (as per Table 1) into 500 mL of purified water (Reagecon, Shannon, Ireland) using a magnetic stirrer. The solution was maintained at 36.5 °C (±1.5 °C) using a water bath. One mole HCl was titrated to adjust the pH of the SBF to 7.40. Purified water was then added to adjust the total volume of liquid to one litre. Once prepared, the SBF was stored for 24 h (5 °C) to ensure that no precipitation occurred.

BT102 (n = 3, per incubation period) was prepared as described previously. Each specimen of cement was subsequently immersed in a volume of SBF such that the following equation was satisfied:

$$V_s = S_a/10 \quad (1)$$

where is V_s is the volume of SBF (mL) and S_a is the surface area of the specimen (mm^2).

Specimens were stored in plastic containers for 30 and 90 days. After the specified incubation times, the cements were removed from the SBF, gently rinsed in deionised water, placed on individually labelled sheets of filter paper and oven-dried (37 °C, 24 h).

TEM specimens were prepared by wiping a Formbar-backed carbon-coated copper grid (Agar Scientific, Stanstead, England) over the sample surface. Particles of the apatite layer was attached to the grid. Transmission electron microscopy was conducted using a JEOL JEM-2011 electron microscope operated at an accelerating voltage of 200 kV. Images were recorded using a Gatan DualVision 600*t* CCD camera attached to the microscope and were analysed using Gatan Digital Micrograph Version 3.11.1. The TEM was calibrated for diffraction and imaging mode using standard samples. Energy dispersive X-ray analysis was undertaken with a Princeton Gamma Tech Prism 1G system with a 10 mm^2 silicon detector attached to the TEM and the peaks were analysed with Imix 10.594 software. The resolution of the system was calibrated with manganese (Mn).

The rationale for the prolonged periods of immersion of BT102 in SBF was twofold. In the first instance, prolonged immersion ensured the development of a thick dense surface layer, such that TEM samples could be readily obtained. Secondly, it was contended that increased immersion time over conventional procedures may facilitate crystallization of the surface layer.

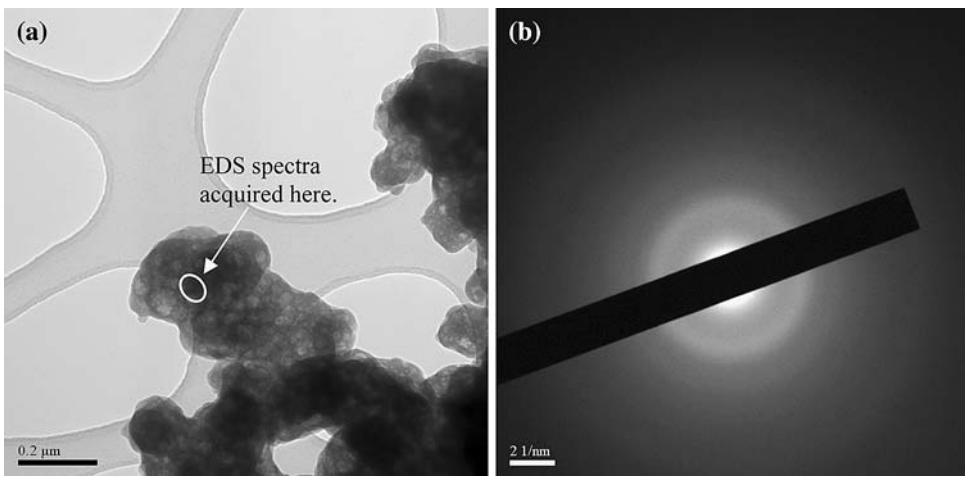

Fig. 1 (**a**) TEM micrograph of surface apatite layer after 30 days immersion in SBF. (**b**) Selected area diffraction pattern (from (**a**)) showing amorphous structure

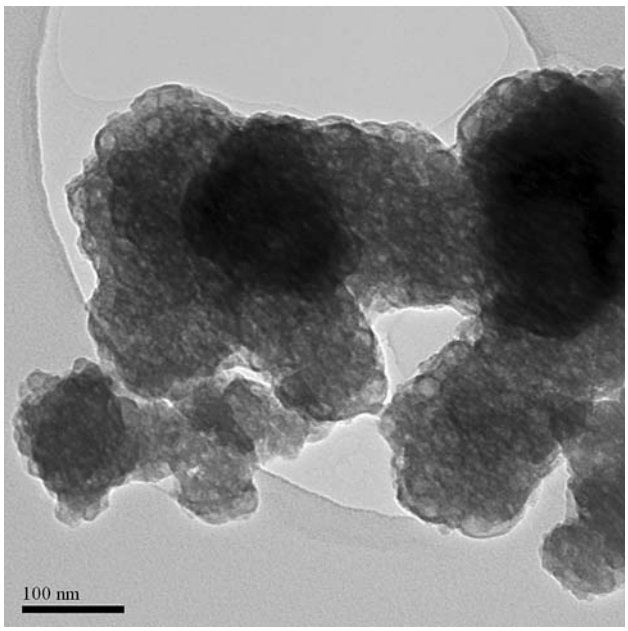

Fig. 2 TEM micrograph of surface apatite layer after 90 days immersion in SBF

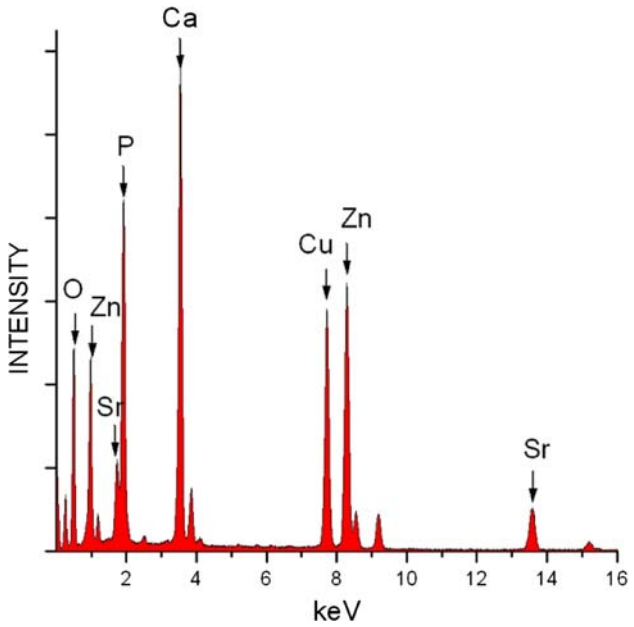

Fig. 3 TEM-EDS results for surface layer observed on cements after 30 days incubation in SBF

TEM analysis indicated a porous type structure, typified by the image shown in Figs. 1a and 2. Selected area diffraction analysis indicated an amorphous structure as indicated in Fig. 1b. The results indicated in Figs. 1 and 2 were found to be representative of the sample as a whole, after a number of different areas were analysed and varying selected area diffraction apertures used to record the diffraction pattern.

The compositional elements of the surface layer, as identified by EDS (primary elemental peaks illustrated in Fig. 3) indicate the presence of calcium (Ca), phosphorus (P), zinc (Zn) and strontium (Sr) within the amorphous structure. The presence of copper (Cu) is due to the Cu-grid on which the sample is placed for analysis. Critically, this composition, in particular the inclusion of Zn in the structure, explicates the lack of crystallinity associated with the formation of the apatite layers observed on Zn-GPCs after immersion in SBF [1, 9]. Zn-GPCs release clinically beneficial amounts of Zn^{2+} from the mantle of set cements for prolonged periods of time to inhibit bacterial colonization of the implant [7]. However, literature shows that Zn is effective at inhibiting the crystallization of HA [18]; one report states that Zn is 1,000 times more effective at inhibiting the crystallization kinetics of hydroxyapatite than magnesium (Mg) [19]. As such, the release of Zn^{2+} and its subsequent inclusion in the apatite layer favours the formation of amorphous apatite (Figs. 1 and 2), as opposed to the deposition of crystalline apatite on conventional GPCs examined under simulated in vivo conditions [20]. However, the presence of such an amorphous layer is comparable to the biological formation of HA in vivo, where prior to the formation of crystalline apatite, the predominant precursor has been cited as amorphous calcium phosphate (ACP) [21]. The ability of Zn-GPCs to produce amorphous apatite layer in vivo is related to zinc release and its subsequent uptake by the precipitating apatite layer. The apatite layer observed in this work is similar to that contended to occur in the natural mineralization process in bone [21] and indicates that these materials will likely bond directly to living bone tissue [16].

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