**Antibacterial, Remineralizing Zinc Oxide-Doped Phosphate-Based Glasses**

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**Abstract**

This study reports a novel melt quenched zinc oxide-doped phosphate-based glasses (Zn-PBGs) of varying CaO mol% designated as C11, C12, C13. The glass degradation rate, ions release, antibacterial activity against *S. mutans* and remineralization potential were investigated. Zn-PBGs showed one order of magnitude higher degradation rate than Zn-free PBG. The highest rate was observed for C11; Na+, Ca2+, Zn2+ and P5+ release followed the same trend. The higher the Zn2+ release, the greater the *S. mutans* growth inhibition. C11 showed significantly lower mineral loss from enamel than positive and negative controls. Zn-PBGs could be used to reverse enamel demineralization.

**Keywords:** phosphate-based glasses; zinc oxide; antibacterial; *S. mutans,* enamel remineralization.

**1. Introduction**

Global Burden of Disease Study in 2017 reported more than 500 million children with dental caries in primary dentition and 2.3 billion people with caries in permanent dentition [[1](#_ENREF_1)]. Dental caries usually starts with enamel demineralization which is very high among patients with fixed orthodontic appliances, poor oral hygiene or excessive consumption of sugary or acidic foods/drinks. More effective preventative measures are needed to target pathogenic bacterial strains and enhance hard tissue remineralization [[2](#_ENREF_2)].

Zinc was found intra-orally within plaque; at high concentration it inhibits plaque formation and metabolism. Zinc could be adsorbed onto the bacterial cell walls and inhibit acid production [[3](#_ENREF_3)]. *S. mutans*, the most common cariogenic bacteria, is then incapable of obtaining the nutrients required for its survival and growth [[4](#_ENREF_4)]. Zinc also shows good oral substantivity lasting up to 12 hours with 15-40% being retained intra orally after the use of mouth rinses and toothpastes [[5](#_ENREF_5)]. However, there is limited evidence that zinc can reduce dental caries clinically using conventional delivery methods. Phosphate based glasses (PBGs) have been used as effective delivery vehicle for many ions including zinc [[6](#_ENREF_6)]. The aim of the study is to prepare zinc-doped PBGs and evaluate their antibacterial effect on *S. mutans* and remineralizing action on demineralized enamel using in vitro biological and non-biological models.

**2. Experimental**

The glass composition, given in Table 1, were produced through the melt quenching process at 1100°C for 1hr using NaH2PO4, CaCO3, P2O5 and ZnO (Sigma, Gillingham, UK).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Glass code** | **Glass composition (mol%)** | | | | |
| Calcium oxide  (CaO) | Sodium oxide  (Na2O) | Phosphorous pentoxide  (P2O5) | Zinc oxide  (ZnO) |
| **C16** | 16 | 39 | 45 | 0 |
| **C11** | 11 | 41 | 45 | 3 |
| **C12** | 12 | 40 | 45 | 3 |
| **C13** | 13 | 39 | 45 | 3 |

***Table 1. Glass code and composition.***

Discs (n=3) from each composition were kept in 50ml of deionized water at room temperature. At various time points, discs were removed, blot dried and weighed. The weight loss/area was calculated. The medium was used for ion release analysis using A Spectro Ciros CCD Spectrometer (Boschstr, DE).

Antibacterial analyses were conducted using defined inoculum of *S. mutans* NCTC10449. Disc diffusion assay was performed on Iso-sensitest agar plate; the diameter of inhibition zones (mm) aroundglass discs (n=5) was measured. Liquid Broth Analyses were done by placing glass discs (n=5) into tubes containing 5 ml of the inoculated PBS and incubated at 37°C. At 2, 4, 6 and 24 hr, serial dilutions of the suspensions were carried; the log of colony forming units (CFUs)/ml was assessed.

De-/remineralization studies were performed using abiotic and biotic models*.* For the abiotic pH-cycling model, bovine enamel were subjected to 5 days of de-/remineralization cycles (6hr demineralization then18 hr/day remineralization) followed by 2-days remineralization [[7](#_ENREF_7)]. At each change of solution, the blocks were washed in deionized water. Samples were divided into 7 groups as in Figure 1. During this treatment, enamel blocks were incubated for 10 minutes in glass extracts, prepared by soaking each glass disc at 37◦C in 10 ml of ultrapure water for 72h. Deionized water and C16 glass extracts were used as negative controls while a 0.05% sodium fluoride mouth rinse (NaF) and a 0.2% chlorhexidine (CHX) were used as positive controls. For the biotic model, Constant Depth Film Fermenter (CDFF) was used to grow *S. mutans* biofilm [[8](#_ENREF_8)]. A 2% sucrose solution was also added for 30 minutes/8 times a day to simulate the sucrose spikes caused by meals and snacks. After 5 and 12 days, the enamel discs (n=15) were aseptically removed and dipped in deionized water to remove the non-adherent biofilm. They were then randomly divided into 5 groups (n=3 discs each) as in Figure 1.



***Figure 1. Diagrammatic representation and grouping of de-/remineralization studies carried out using both pH-cycling (a) and CDFF (b). NaF: sodium fluoride mouth rinse.***

Mineral loss (ΔZ, vol%.μm-1) ofenamel samples was micro-radiographed (HTA Photomask, San Jose, CA, USA), examined microscopically (Leica, Germany) and calculated using image analysis software (TMR 2000 V2.0.27.16, Inspektor Research Systems BV Amsterdam, The Netherland).

Statistical analysis was conducted using the Prism GraphPad software (San Diego, California, USA). *p* values <0.05 were considered statistically significant.

**3. Results and Discussion**

**3.1. Degradation and Ion Release**

The weight loss increased linearly with time; the degradation rate of Zn-PBGs was significantly (*p*<0.05) higher than the control glass (C16) - Figure 2 (a, b). It decreased linearly with increasing CaO content as previously observed [[9](#_ENREF_9)]. The degradation rate increased by one order of magnitude with decreasing CaO content; this could be attributed to the formation of P‐O‐Ca and P‐O− Ca2+−O‐P ionic cross-links in Q1 and Q2 glass units respectively. Replacing Ca2+ with Zn2+ which is smaller in diameter produces weaker bonds and the glass degrades more rapidly [[10](#_ENREF_10)]; the release of Na+, Ca2+, Zn2+ and P5+ followed the same trend as degradation [Figure 2 (c-f)].

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***Figure 2: Weight loss (a), degradation rate (b) and ion release (c-f) of different glasses***

**3.2. Antibacterial Analyses**

The zone of inhibition followed the same trend as the degradation and ion release data with C11 showing the highest inhibition (18±1.7, 16 ±1.6, 15 ±1.1 mm for C11, C12, C13 respectively). Viable cell counts in the liquid broth assay increased with increasing CaO. The control glass showed significantly higher viable cell counts (*p*<0.01) than Zn-PBGs which showed 1.2-1.7, 1-1.5, 1.2-1.9 and 0.4-0.5 log10 reduction in CFU after 2, 4, 6 and 24 hr respectively. Zn2+, Ca2+, Na+ and P5+ release is correlated well with the degradation data; this is reflected on the antibacterial action of these glasses. The role of zinc in inhibiting bacterial growth is still unclear. Generally, in its oxide form (ZnO), the reduced bacterial cell viability could be attributed to the production of reactive oxygen species (ROS) that could elevate membrane lipid peroxidation with the subsequent leakage of important cellular components as reducing sugar, DNA and protein [[11](#_ENREF_11)]. In its ionic form, zinc may inhibit acid production after being adsorbed onto the bacterial cell wall [[3](#_ENREF_3)]; cariogenic bacteria including *S. mutans* is incapable of getting the nutrients for growth [[4](#_ENREF_4)].

**3.3. De-/Remineralization Studies**

Transverse microradiography (TMR) analyses of the bovine enamel samples subjected to pH cycling revealed that the highest significant (*p*<0.05) mineral loss was observed for negative control (deionized water group) while the lowest for C11 followed by C13. All glasses-treated enamel showed significantly (*p*<0.05) lower mineral loss than the 0.05% NaF positive control – Figure 3 (a).

C11 showed the most optimal trends for degradation, ion release and antibacterial analyses; this dictated its use as an example of Zn-PBGs for the CDFF run with Zn-free control (C16). For a reliable model, only *S. mutans* was used to cause enamel demineralization [[12](#_ENREF_12)]. As observed, there was statistically significant mineral loss (*p*<0.05) in enamel samples exposed to C16 extract, 0.05% NaF and 0.2% CHX at day 12 compared with day 5. Enamel discs exposed to C11 extract showed no statistically significant difference in mineral loss (*p*>0.05) between day 5 and 12 – Figure 3 (b, c). This could be attributed to the highest Ca2+ and P5+ release observed with C11. In saliva, Ca2+ and P5+ act as buffering ions required to neutralize acids produced by bacteria so they can reverse enamel demineralization and enhance its remineralization [[13](#_ENREF_13)]. The enamel blocks were only treated for ten minutes after demineralization as this is more likely to be how the glasses would be used as a preventive measure for caries control.



***Figure 3: Mineral loss using pH cycling (a) and CDFF (b). TMR images (c) of demineralized enamel exposed to C11 extract and 0.05% NaF. CHX: 0.2% chlorhexidine solution.***

The gradual release of Zn2+ could prevent the build-up of biofilms in-between the use of fluoride toothpastes and mouth rinses for examples [[14](#_ENREF_14)] without developing any risk. In human, the LD50 for zinc is ~27g/day [[15](#_ENREF_15)]. A disc of C11 only contains ~2.8 mg of zinc; this might only cause a problem for infants and children who require daily intake of 2–3 and 5–9 mg/day respectively [[16](#_ENREF_16)].

**4. Conclusion**

The controlled delivery of Zn2+ from Zn-PBGs was effective against *S. mutans*. The released Ca2+ and P5+ were effective in inhibition of enamel demineralization. Zn-PBGs could be used to reduce dental caries incidence.

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