Multi-component bioactive glasses of varying fluoride content for treating dentin hypersensitivity

Eilis Lynch a, Delia S. Brauer b,*, Natalia Karpukhina b, David G. Gillam a, Robert G. Hill b

a Adult Oral Health, Institute of Dentistry, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, Turner Street, London E1 2AD, UK
b Dental Physical Sciences, Institute of Dentistry, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, Mile End Road, London E1 4NS, UK

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ABSTRACT

Objective. Dentin hypersensitivity (DH) is a commonly occurring dental condition, and bioactive glasses (BG) are used in dentifrice formulations for treating DH by forming a surface layer of hydroxycarbonate apatite (HCA) on the tooth, thereby occluding exposed dentinal tubules. Fluoride-containing BG, however, form fluorapatite, which is more stable toward acid attack, and provide a more sustainable option for treating DH.

Methods. Melt-derived multi-component BG (SiO2−P2O5−CaO−CaF2−SrO−SrF2−ZnO−Na2O−K2O) with increasing CaF2+SrF2 content (0–32.7 mol%) were prepared. Apatite formation, occlusion of dentinal tubules in dentin discs and ion release in Tris buffer were characterized in vitro over up to 7 days using X-ray diffraction, infrared spectroscopy, scanning electron microscopy and inductively coupled plasma emission spectroscopy.

Results. The fluoride-containing bioactive glasses formed apatite from as early as 6 h, while the fluoride-free control did not form apatite within 7 days. The glasses successfully occluded dentinal tubules by formation of apatite crystals and released ions such as fluoride, strontium and potassium.

Significance. Fluoride significantly improved apatite formation of the BG, allowing for treatment of DH by occlusion of dentinal tubules. The BG also released therapeutically active ions, such as strontium and fluoride for caries prevention, zinc for bactericidal properties and potassium, which is used as a desensitizing agent in dentifrices.

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1. Introduction

Dentin hypersensitivity (DH) is one of the most commonly occurring clinical dental conditions, and up to 69% of the UK population have reported experiencing some form of tooth sensitivity [1–4]. Although the etiology of DH is multi-factorial and not yet fully understood [5], it is attributed to the general increase in exposed root surfaces of the teeth from periodontal disease, toothbrush abrasion or cyclic loading fatigue of the thin enamel near the cemento-enamel junction [4]. The currently accepted theory for a DH mechanism is the hydrodynamic theory [6], which proposes that external stimuli such as cold, hot, tactile or osmotic pressure, when applied to exposed dentin, cause fluid movement within the dentinal tubules. This fluid movement stimulates mechanoreceptors near the base of the tubule and may, if certain physiological parameters are met, trigger a pain response. The hydrodynamic theory
is based on the understanding that open tubules allow fluid flow through the tubules [7], which results in pressure changes that excite the nerve endings in the dental pulp, and it is consistent with the observation that when DH is treated with a tubule-occluding agent, this will result in a reduction in DH [8,9].

Occlusion of exposed dentinal tubules is therefore a common approach for treating DH, and in several over-the-counter (OTC) dentifrices propose tubule occlusion as their mode of action. Strontium chloride is the active ingredient in the original Sensodyne® dentifrice (GlaxoSmithKline, London, UK) and was the first tubule occluding agent incorporated into a dentifrice; later products also contained strontium acetate [10]. Fluoride was first proposed as a desensitizing agent in 1941 [11] and has subsequently been used in dentifrices, gels, mouth rinses and varnishes. Recently, a bioactive glass (NovaMin®, developed by NovaMin Technology Inc., Alachua, FL, USA) based on the original 45S5 Bioglass® (US Biomaterials Corp., Jacksonville, FL, USA) composition has been incorporated as a remineralising ingredient in dentifrice formulations for treating DH by precipitating hydroxy carbonate apatite (HCA) onto the tooth surface and subsequently occluding the dentinal tubules [12–18]. However, concerns have been expressed over the long-term durability of HCA in the mouth, and formation of fluorapatite (FAP) rather than HCA is preferable, as it is more resistant to acid attack and would therefore dissolve less readily when teeth are exposed to acidic conditions (e.g. during consumption of fruit juice and carbonated beverages).

It was recently shown that fluoride-containing bioactive glasses form FAP rather than HCA in physiological solutions [19]. Here a series of bioactive glasses (SiO2–P2O5–CaO–CaF2–SrO–SrF2–ZnO–Na2O–K2O) were produced, which form FAP in physiological solutions, release strontium and fluoride for caries prevention, zinc for bacte- rical properties and potassium, which is currently used as a desensitizing agent in dentifrices. The aim was to investigate apatite formation, ion release and occlusion of dentinal tubules in vitro.

2. Experimental procedure

2.1. Glass synthesis and characterization

Fluoride (CaF2 and SrF2) was added to the glasses rather than substituting it for CaO or SrO, as this was shown to maintain glass solubility, bioactivity and network connectivity [19,20]. Glasses (Table 1) were prepared using a melt-quench route. Mixtures of analytical grade SiO2 (Prince Minerals Ltd., Stoke-on-Trent, UK), P2O5, CaCO3, SrCO3, Na2CO3, CaF2 and SrF2 (Sigma–Aldrich, Gillingham, UK) were melted in a platinum–rhodium crucible for 1 h at 1420 °C in an electric furnace (EHF 17/3, Lenton, Hope Valley, UK). A batch size of 100 g was used. After melting, the glasses were rapidly quenched into water to prevent crystallization. After drying, the glass frit was ground using a vibratory mill (Gyro mill, Glen Creston, London, UK) for 7 min and sieved using a 38 μm mesh analytical sieve (Endecotts Ltd., London, UK). The amorphous structure of the glasses was confirmed by powder X-ray diffraction (XRD; X’Pert PRO, PANalytical, Cambridge, UK).

The glass transition temperature (Tg) was determined using differential scanning calorimetry (DSC, Stanton Redcroft DSC1500, Rheometric Scientific, Epsom, UK). 50 mg of glass frit was analyzed in a platinum crucible using analytical grade alumina powder as reference with a heating rate of 10 K/min.

2.2. Tris buffer study

Tris buffer solution was prepared by dissolving 15.090 g tris(hydroxymethyl)aminomethane (Sigma–Aldrich) in 800 mL deionised water, adding 44.2 mL 1 M hydrochloric acid (Sigma–Aldrich), heating to 37 °C over night, adjusting the pH to 7.30 using 1 M hydrochloric acid using a pH meter (Oak- instrument, Nijkerk, Netherlands) and filling to a total volume of 2000 mL using deionised water. Tris buffer solution was kept at 37 °C.

50 mL of Tris buffer was pipetted into 150 mL PE bottles. pH was measured using a pH meter (Oakton Instruments) and 75 mg of glass powder (<38 μm) was dispersed in the Tris buffer solution. Samples were placed in an orbital shaker at 37 °C at an agitation rate of 60 rpm for 3, 6, 9, 24, 72 and 168 h. Tris buffer from the same batch without glass powder was used as control. After removing the samples from the shaker pH was measured and solutions were filtered through medium porosity filter paper (5 μm particle retention, VWR International, Lutterworth, UK) and kept at 4 °C. The filter paper was dried at 37 °C and the dried powders were analyzed using Fourier-transform infrared spectroscopy (FTIR; Spectrum GX, Perkin-Elmer, Waltham, MA, USA) and XRD.

Fluoride-release into Tris buffer was measured using a fluoride-selective electrode (Orion 9609BNWP with Orion pH/ISE meter 710, both Thermo Scientific, Waltham, MA, USA). Calibration was performed using standard solutions prepared using Tris buffer to account for ionic strength. For elemental analysis, solutions were acidified using 69% nitric acid and quantitatively analyzed by inductively coupled plasma–optical emission spectroscopy (ICP; Varian Vista-PRO, Varian Ltd., Oxford, UK).

2.3. Occlusion of dentinal tubules

Caries-free extracted maxillary and mandibular third molars used in this study were obtained from the tooth bank at the Royal London Dental hospital. Studies were performed in accordance with the guidelines by the Queen Mary Research Ethics Committee. In vitro occlusion of dentinal tubules was investigated using the dentin disc model [21]. The teeth were sectioned mesio-distally into discs approximately 1 mm thick using an internal edge annular diamond blade (Microslice annular blade, Ultratec, USA) mounted on a Microslice 2 saw (Malvern Instruments Ltd., UK) and halved. The discs were stored in sodium hypochlorite until required.

The dentin discs were etched with 6% citric acid for two minutes and rinsed with distilled water, prior to placement in a Tris buffer-filled container together with glass powder as outlined in Section 2.2. Controls were kept in Tris buffer without glass powder. Following storage in Tris buffer for up to 7 days the discs were allowed to dry in a dessicator for two days, attached to aluminum stubs with carbon conducting cement, carbon sputter-coated and viewed in a scanning
electron microscope (SEM, FEI Inspect F) using back-scattered electron mode at 20 kV. Untreated dentin discs were mounted, sputter coated with gold/palladium and subsequently viewed in a Cambridge stereoscan 908 SEM at a constant working distance of 10 mm. Variable accelerating voltages (10 or 15 kV) were selected to optimize image quality.

3. Results

3.1. Glass formation and properties

Glasses were optically clear after quenching and XRD patterns showed the typical amorphous halo confirming the amorphous state of the materials (not shown). 

3.2. pH

All glasses gave a pH rise in Tris buffer (Fig. 2a); the maximum pH rise was 0.5. There was no significant variation in pH with fluoride (CaF$_2$ + SrF$_2$) content in the glass (shown at 3, 24 and 168 h in Fig. 2b).

3.3. Apatite formation

The FTIR spectrum of glass F4 before immersion in Tris buffer (Fig. 3) showed two main bands at about 920 cm$^{-1}$ (Si–O–alkali$^+$ band) and 1030 cm$^{-1}$ (Si–O–Si stretch band). After immersion in Tris buffer, the intensity of the non-bridging oxygen (Si–O–alkali$^+$) band at 920 cm$^{-1}$ decreased over time, and disappeared at 72 h. At 72 and 168 h a small carbonate band at 870 cm$^{-1}$ and a broad feature at about 1440 cm$^{-1}$ appeared. At the same time, a new band appeared at about 790 cm$^{-1}$, due to Si–O–Si bond vibration between two adjacent SiO$_4$ tetrahedra. At 0 h, a single P–O vibration band was present at approximately 560 cm$^{-1}$, which later (at 72 and 168 h) had turned into a split band at 560 and 600 cm$^{-1}$. Due to noise in this area of the spectra, it is difficult to distinguish if the splitting had started to appear at earlier time points (e.g. 9 and 24 h). Presence of orthophosphate at later time points is confirmed by a clearly pronounced orthophosphate band at 1027 cm$^{-1}$ appearing at 72 and 168 h, superimposed on a Si–O–Si stretch band at 1030 cm$^{-1}$.

Changes in FTIR spectra over time for glass F17 were similar to those observed for glass F4; however, the non-bridging oxygen (Si–O–alkali$^+$) band at 920 cm$^{-1}$ disappeared at an earlier time point (between 3 and 6 h), the band at about 1030 cm$^{-1}$ increased but never markedly sharpened and the split band at 560 and 600 cm$^{-1}$ clearly was observed at 168 h only, but might be present at earlier time points, as noise affects interpretation.

XRD patterns of the glasses after immersion in Tris buffer (Fig. 4) were compared to reference patterns of HCA (JCPDS 19-272), fluorite (JCPDS 35-816), strontium fluoride (JCPDS 6-262) and calcium carbonate (JCPDS 5-586). At 6 h (Fig. 4a) glasses with no or low CaF$_2$+SrF$_2$ content (F0–F13) showed an amorphous halo only, similar to that observed for the untreated glasses. Glasses F17–F32 showed a slight asymmetry in the area between 30 and 35°, which is an area of the most pronounced apatite (Ap) peaks (JCPDS 19-272), but interpretation is difficult due to the low intensity of the features. Glasses F25 and F32 clearly showed three peaks at 27.6, 45.9 and 54.4°. CaF$_2$ is known to give peaks at 28.3, 47.0 and 55.7° and SrF$_2$ at 26.5, 44.1 and 52.3°. As the peaks observed for F25 and F32 were directly between those of CaF$_2$ and SrF$_2$, a possible solid-solution crystal phase (Sr$_x$CaO$_{1-x}$F$_2$) may have formed. The diffraction lines observed for F25 and F32 also showed significant shoulders, and one explanation is that Ca$_{1-x}$Sr$_x$F$_2$ with a larger proportion of calcium relative to strontium may have formed. Small peaks at the same positions seem to be present in glass F17, but interpretation is difficult due to low intensity.

At 168 h, XRD patterns showed significant changes compared to 6 h (Fig. 4b). Glasses F4–F13 showed presence of a single phase, as all present peaks correspond to Ap; the triple peak in the area between 30 and 35° was clearly pronounced for all three glasses. Glasses F17–F32 also showed triple peaks in this region, but of lower intensity. In addition, glasses F17–F32 showed sharp features in the area corresponding to a mixed calcium/strontium fluoride (27.6, 45.9 and 54.4°, cf. above).

3.4. Ion release

In general, ionic concentrations in solution increased with time due to glass degradation (shown in Fig. 5a and b for glasses F4 and F17). Phosphate concentrations increased at early time points, then decreased with time. Fluoride concentrations for glass F17 showed a maximum at 3 h, then decreased (Fig. 5b).

Ionic concentrations at early time points (6 h, Fig. 5c; 3 and 9 h not shown but gave similar results) did not vary significantly with CaF$_2$+SrF$_2$ content apart from fluoride, which increased linearly with CaF$_2$+SrF$_2$ content in the glass. The value for composition F13 could not be obtained at 6 h due to experimental error.

At 1 week (168 h, Fig. 5d), fluoride concentrations increased linearly with increasing CaF$_2$+SrF$_2$ content up to glass F13 (13.62 mol% CaF$_2$); for higher CaF$_2$+SrF$_2$ contents a plateau

<p>| Table 1 - Glass compositions (mol%) and total fluoride content (CaF$_2$ + SrF$_2$). Theoretical network connectivity is 2.36. |
|---------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|</p>
<table>
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<th>CaO (mol%)</th>
<th>CaF$_2$ (mol%)</th>
<th>SrO (mol%)</th>
<th>SrF$_2$ (mol%)</th>
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</table>

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Fig. 1 – Glass transition temperature vs. fluoride content for the glasses in this study (●) and by Brauer et al. [24] (○).

Fig. 2 – pH of Tris buffer solution after immersion of glass powders (a) vs time and (b) vs CaF$_2$+SrF$_2$ content in the glass; error ± 0.1.
was reached. At 168 h phosphate concentrations in solution dropped to close to zero for all compositions apart from the fluoride-free F0 glass. Concentrations of all other elements did not show significant variation with CaF₂+SrF₂ content in the glass.

3.5. Occlusion of dentinal tubules

After etching the dentin discs, open dentinal tubules were clearly visible (inset in Fig. 6). The time taken for Ap formation on dentin discs varied with glass composition, similar to that observed in Section 3.4. SEM images of the dentin discs demonstrate layers of bioactive glass particles on the dentin surface (Fig. 6a) and dentinal tubules partially occluded by Ap crystals (Fig. 6b).

4. Discussion

Occlusion of dentinal tubules is one approach currently used in the treatment of DH, and numerous in-office and OTC products use active ingredients that have been shown to occlude tubules in vitro. Although there are advantages to using OTC products in terms of both ease of availability and cost (to the consumer), one major disadvantage is that they may take up to 2–4 weeks in order to achieve any effectiveness in relieving the symptoms of DH. DH is related to the flow of fluids within the dentinal tubules, and according to Poiseuille’s law this fluid movement is directly proportional to the fourth power of the radius [22]. As a consequence any reduction in the radius of the tubule opening would be expected to reduce dentin permeability and as such should be effective in treating DH [23].

Bioactive glasses in remineralising dentifrices for treatment of DH have been previously shown to work by tubular occlusion through formation of HCA [17]. In vitro characterization of the Ap forming ability of novel bioactive glasses is therefore important when investigating their potential for use in remineralising dentifrices.

Phosphate in bioactive glasses is present as orthophosphate [24–26], and an increase in phosphate content (while adding additional Na₂O and CaO and maintaining a fixed

Fig. 3 – FTIR spectra of glasses (a) F4 and (b) F17 after immersion in Tris buffer over time.
Fig. 4 – XRD patterns of glasses immersed in Tris buffer for (a) 6 h and (b) 168 h. Crystal phases are apatite and a mixed Ca/SrF₂.

network connectivity) was previously shown to result in faster and more Ap formation [20]. These high phosphate content glasses are the basis of the present paper which investigates incorporating strontium, potassium, zinc and fluoride into a bioactive glass with a high phosphate content with the objectives of:

i. Forming Ap faster than currently available bioactive glasses.
ii. Forming FAp rather than HCA.
iii. Forming Ap with the minimal possible pH rise.
iv. Releasing fluoride and strontium ions for their caries inhibitory action.
v. Releasing potassium for its nerve desensitizing role.
vi. Releasing zinc for its antibacterial and anti-gingivitis action.

4.1. Apatite formation

The primary intended application for these new bioactive glasses is as a remineralising additive for dentifrices. Here, formation of FAp is preferred because it is much more chemically stable at lower pH values compared to HCA. The glass component of the dentifrice needs to perform its function and form FAp before it is washed away by salivary action. Ideally, it should form FAp in the mouth in <8 h, corresponding to an overnight period where salivary flow will be minimal.

Fluorides (such as sodium fluoride, ammonium fluoride or sodium monofluorophosphate) have been added to dentifrices; however, the soluble fluoride salt is likely to give a high fluoride concentration before the calcium concentration is increased by dissolution of the glass and therefore is more likely to result in formation of undesirable fluoroite (CaF₂), which may compete with or inhibit subsequent FAp formation. It might therefore be preferable to deliver Ca²⁺, PO₄³⁻ and F⁻ ions simultaneously in the appropriate amounts to form FAp from a single glass composition in order to avoid the possible formation of CaF₂.

In the present study, the ability of the glasses to form Ap was investigated in Tris buffer rather than in simulated body fluid (SBF). This represents a more rigorous test of bioactivity since, unlike SBF, Tris buffer is not saturated with calcium and
Fig. 5 – Elemental concentrations in Tris buffer (a and b) vs time for glasses F4 and F17 and (c and d) vs fluoride content (CaF$_2$+SrF$_2$) at 6 and 168 h. Fluoride concentrations increase linearly with CaF$_2$+SrF$_2$ content in the glass (c) for all compositions at 6 h ($R^2 = 0.988$) and (d) for up to 13.62 mol% CaF$_2$+SrF$_2$ at 168 h ($R^2 = 0.985$).

Fig. 6 – SEM images of dentin disc at 7 days in Tris buffer with glass powder F13 showing (a) glass particles on dentin surface, scale bar 100 μm, 1000 x magnification (inset shows dentin disc after etching with citric acid before storage in Tris buffer, scale bar 30 μm) and (b) dentinal tubules (white arrow) partially blocked by apatite crystals (black arrow), scale bar 5 μm, 16,000 x magnification.
phosphate. Concentrations of calcium and phosphate in saliva vary, and saliva is expected to be diluted after consumption of liquids and will no longer be saturated with regard to calcium and phosphate.

The glasses all formed Ap after immersion in Tris buffer for 168 h with the exception of the fluoride-free glass F0, which apparently formed no Ap. Glass F17 showed an asymmetry of the amorphous halo in XRD between 30 and 35° 2θ, which might indicate formation of FAp at early time points. However, the Ap formed in simulated physiological solutions has been shown to have crystals in the nanometre size range [19,27], which give rise to pronounced line broadening in the diffraction patterns. For this reason, XRD results at early time points in this study were not clear, and defined peaks for Ap were only observed at 72 h. This was confirmed by FTIR spectra, which showed clearly pronounced split phosphate bands at 72 h. The Ap formed by fluoride-containing bioactive glasses has previously been shown to be FAp [19,20], and the Ap formed by the present glasses therefore is likely to be FAp as well. However, the XRD patterns of FAp and HCA overlap, and therefore one cannot distinguish between these two phases based on XRD alone. High fluoride content glasses (F17–F32) showed formation of mixed calcium/strontium fluoride as early as 3 h, while formation of Ap occurred at significantly later stages (72 h).

It is worth noting that as CaF2+SrF2 was added to the glass composition the phosphate content of the glass decreased. If all the glasses degraded at the same rate and there was no influence of fluoride on Ap formation then one would expect a reduced rate of Ap formation, a reduced amount of Ap and an increased time for the formation of Ap. There was no evidence that either the amount or the rate of Ap formation decreased on going from the fluoride free glass F0 to the lowest fluoride content glass F4. Indeed the fluoride containing glass F4 formed Ap whereas F0 did not. This indicates that either incorporating fluoride increased the glass degradation rate and/or fluoride promoted Ap formation, probably by forming FAp with its lower solubility product rather than HCA.

In general, these results broadly mirror those found by Brauer et al. [19] but the formation of fluorides (Ca/SrF2) occurred at higher CaF2+SrF2 contents. The higher phosphate content of the present glasses (5 mol% vs 1 mol% P2O5) provided more phosphate with which to form Ap and there was consequently a larger demand for fluoride in order to form FAp. Ap formation was slower than observed by Mneimne et al. [20] for non-multi-component high phosphate content glasses, which can be explained by a higher network connectivity in the present study (2.36 vs 2.08). The network connectivity of bioactive glasses was shown to correlate well with their ability to form Ap [28,29].

4.2. pH and ion release

The bioactive glass component in a dentifrice should not raise the pH significantly above 8.0 when used at high loadings (up to 10% by weight) in the dentifrice. Bioactive glasses are well documented to give a pH rise in aqueous solutions [19,20,30], and in previous studies, fluoride was shown to reduce this pH rise [19,20]. In the present study, glasses gave a maximum pH of about 7.9; however, no relationship between pH and CaF2+SrF2 content was observed. It was previously postulated that the reduction in pH rise with fluoride content was due to ion exchange processes [19]. But despite a linear relationship between fluoride release and CaF2+SrF2 content in the glass (and no significant variation in all other ionic concentrations) the pH did not vary with CaF2+SrF2 content in the glass. This confirms that the influence of fluoride on the overall pH is less than the influence of the silicate matrix as suggested previously [31].

The caries inhibitory role of fluoride is well documented [32,33]. It works by reducing demineralisation (by forming FAp, which is less soluble than HCA, the mineral composition of enamel and dentin), enhancing remineralisation and inhibiting plaque bacteria [32]. In addition, fluoride has been suggested to act as a desensitizing agent [11]. Fluoride release from the glasses in the present study was shown to increase linearly with CaF2+SrF2 content in the glass at early time points, suggesting that initial fluoride release can be easily tailored by adjusting the CaF2+SrF2 content in the glass. At later time points, the high CaF2+SrF2 content glasses form calcium and strontium fluoride, which reduces the concentrations of fluoride in solution, so that the fluoride concentrations do not increase linearly with fluoride content in the glass.

The phosphate concentration was previously shown to be a limiting factor in Ap formation [20]. Ion release profiles in the present study showed a reduction in phosphate concentrations in solution over time, which was due to formation of Ap, during which phosphate was consumed. At 168 h, the glasses showed virtually no phosphate present in solution (again due to Ap formation); the only exception was glass F0. This agrees with the XRD and FTIR results (Sections 3.3 and 4.1) which showed no Ap formation for the fluoride-free glass F0 even at 1 week (168 h).

Strontium (in the form of strontium chloride or acetate) is the active ingredient in dentifrices for treating DH such as Sensodyne®. Strontium exhibits complete solid solution formation with calcium in apatites [34], and a mixed calcium/strontium Ap is more chemically stable than an all calcium Ap. In addition, strontium has a well documented anti-caries role [35–37], and it is thought to have a synergistic action with fluoride on caries inhibition [37]. Strontium release from bioactive glasses for use in dentifrices is therefore, like fluoride release, an attractive feature. Release profiles showed that strontium was released quickly, and strontium concentrations in solution did not vary significantly with either time or strontium content in the glass, suggesting that the glasses allowed for a relatively constant release of strontium ions over prolonged periods of time. Despite equal concentrations of strontium and calcium in the glasses, strontium concentrations in solution were consistently lower than those of calcium, suggesting different release kinetics for these two elements. The diffusion coefficient of Sr2+ could be lower than that of Ca2+ due to the larger ionic radius of Sr2+ (1.16 Å) compared to Ca2+ (0.94 Å). Another possible explanation could be calcium preferentially associating with the orthophosphate phase in the glass and strontium with the silicate phase. As the orthophosphate phase dissolves more readily than the silicate phase (due to the high network connectivity), Ca2+ concentrations in solution were higher than those of Sr2+.

Dentifrices containing 5% potassium nitrate have been used since 1980 [38], and potassium salts (e.g. potassium
chloride and citrate) are currently the most common active ingredient used in dentifrices for DH. Potassium ions are thought to block the synapse between nerve cells, reducing nerve excitation and the associated pain [39,40]. However, despite several publications reporting on the clinical efficacy of potassium-containing dentifrices in reducing DH [41–45], a recent meta-analysis (Cochrane review) on the effectiveness of potassium dentifrices by Poulsen et al. [46] was unable to support these findings. In the present study concentrations of potassium ions in solution were consistently lower than those of sodium. Again, this might be connected to differences in diffusion coefficient owing to differences in ionic radius (Na 1.02 Å compared to K 1.38 Å) or to how the cations are distributed between the orthophosphate and the silicate phase of the glass. If sodium preferentially associates with orthophosphate compared to potassium, sodium will be expected to be released faster as the orthophosphate phase dissolves more readily than the silicate phase, similar to what was observed for strontium and calcium (cf. above).

Zinc concentrations in solution were very low, which might be due to the low zinc content in the glasses (1 mol% ZnO). This suggests that in future glass compositions higher zinc contents should be chosen in order to achieve higher zinc concentrations in solution for antibacterial and anti-gingivitis action of the dentifrices.

In summary, the ion release kinetics showed no difference with glass composition, apart from the fluoride release, which increased linearly with CaF2+SrF2 content of the glasses. In addition, all fluoride-containing glasses formed Ap within one week, whereas only the fluoride-free glass F0 did not form Ap within this time period. This therefore suggests that fluoride is the key factor in Ap formation, and that addition of CaF2+SrF2 to a bioactive glass can significantly improve its mineralizing potential.

4.3. Occlusion of dentinal tubules and potential application in dentifrices

SEM micrographs of dentin discs treated with bioactive glasses showed a layer of bioactive glass particles on the dentin surface. At higher magnification (16,000×) it was also clearly visible that apatite crystals had formed on the dentin surface, resulting in complete or partial occlusion of dentinal tubules, illustrating the ability of the glasses to occlude the dentinal tubules. The main mechanism of action of bioactive glasses in dentifrices is the release of calcium, phosphate and, in the present study, both fluoride and strontium ions, which, together with a slight pH rise, result in supersaturation of the surrounding fluids (such as saliva in the oral cavity) with regard to Ap and, subsequently, precipitation of apatite crystals. Such a mechanism can enhance remineralisation processes within the oral cavity [32,47]. The SEM results in the present study also show that apart from a general enhancement of remineralisation, bioactive glasses can directly occlude dentinal tubules through Ap formation, and thus potentially reduce or prevent DH.

It should be noted, however, that the SEM images shown are at 1 week (168 h) in Tris buffer. While it may be assumed that the time taken to form Ap in saliva (which contains additional calcium and phosphate ions, albeit in varying concentrations, which should accelerate Ap formation) will be shorter, future studies will aim at increasing the reactivity of the glasses, to achieve tubule occlusion at faster rates.

The bioactive glasses in this study show a reduction in glass transition temperature with increasing CaF2+SrF2 content, an effect that has been observed previously [24]. Tg is determined by the bond strength in the glass, and it has also been shown to correlate with the hardness of glasses [48], which is likely to influence the abrasivity of a bioactive glass-containing dentifrice. A glass with a lower hardness than that of enamel (around 4 GPa [49]) is desirable, and variation of the fluoride content in the glass is an interesting option for adjusting the hardness and abrasivity of the glass.

The reported prevalence of DH increases with age [4]; however, an increase in the level of exposed dentin through gingival recession coupled with an increase in the level of tooth erosion (acid dissolution) through an acid-containing diet together with improvements in oral hygiene practices suggests that the prevalence of DH may further increase in the future. As the intake of food and drinks with erosive potential affects the tooth surface and subsequently may lead to the development of DH, it has been suggested that the age peak for DH (currently between the third and fourth decades [50]) may eventually shift to a younger age group [2]. Furthermore, populations appear to be keeping their teeth for longer in life, and an increase in exposed root dentin, either due to oral hygiene practices or repeated periodontal therapy, may also lead to an increase in the prevalence of DH. This clearly indicates a need for new, effective methods of treatment and prevention of DH. The use of dentifrices containing novel bioactive glasses is promising, as they can occlude exposed dentinal tubules via the formation of fluorapatite, but also release therapeutically active ions such as potassium, strontium, zinc and fluoride onto the exposed dentin surface.

Further in vitro studies should investigate the ability of these glasses to reduce fluid flow through dentinal tubules before and after an acid challenge and the ability of the glasses to remineralise carious lesions. Clinical studies will also be required to demonstrate that the promising laboratory studies to date can be translated into clinical benefits.

5. Conclusions

Novel multi-component bioactive glasses form apatite in physiological solutions and can successfully occlude exposed dentinal tubules. In addition (unlike conventional bioactive glasses such as Novamin® or Bioglass® 45S5), they release therapeutically active ions such as fluoride, strontium and potassium. This combined action of apatite formation and ion release makes these glasses attractive components for use in remineralising dentifrices, particularly for treating dentin hypersensitivity.

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REFERENCES


[36] Curzon MEJ, Spector PC, Iker HP. Association between strontium in drinking-water supplies and low caries


