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Bioactivity of toothpaste containing bioactive glass in remineralizing media: effect of fluoride release from the enzymatic cleavage of monofluorophosphate.

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Abstract: *Objectives*. The aim was to introduce a new methodology to characterize toothpaste containing bioactive glass and to evaluate the effect of release of fluoride ions, by cleaving monofluorophosphate (MFP), on the mineral forming ability of Sensodyne Repair & Protect (SRP). which contains NovaMin™ (bioactive glass, 45S5 composition).

Methods. SRP, NovaMin particles, and placebo paste (PLA) which did not contain NovaMin, were immersed into a remineralization media (RS), which mimics the ionic strength of human saliva, for 3 days with different concentrations of alkaline phosphatase (ALP): 0, 25 and 75 $U.L^{-1}$. Ion concentration profiles and pH were monitored by ICP-OES and F^- ion selective electrode. Remaining solids were collected by freeze-drying and their surfaces analysed.

Results. Hydroxyapatite (HA) formed on the surface of BG alone (after 1 h) and in toothpaste (after 2 h), whereas PLA did not induce any precipitation. ALP cleaved MFP at different rates depending on the enzyme concentration. Increasing the concentration of ALP from 0 and 75 $U.L^{-1}$ reduced the time of HA formation from 2 h to 24 h. However, the presence of fluoride induced the precipitation of fluorapatite. No evidence of fluorite (CaF_2) was observed. The apatite formation ability of toothpaste can be assessed using the presented method.

1 Introduction

Dentine hypersensitivity is a common dental condition characterised by acute pain arising in response stimuli, typically thermal, chemical or tactile [1]. This discomfort is widely accepted to result from the movement of fluid located within the dentine tubules [2]. However, it is now well established that daily brushing using toothpaste containing calcium sodium phosphosilicate bioactive glass (BG), such as NovaMin™ (46.1 mol% SiO_2, 26.9 mol% CaO, 24.4 mol% Na_2O, 2.6 mol% P_2O_5), helps to reduce exposed dentine hypersensitivity [3–5]. The pain relief provided by BG relies on a sequential mechanism. The dentine is remineralised though the interaction of BG with saliva, which causes mineral deposition that, occludes open tubules. When exposed to physiological or simulated fluid, BG releases cations such as Ca^{2+} and Na^+ leaving an open silica-rich layer with high silanol content on the glass surface, which is known to favour precipitation of calcium phosphate on the glass, followed by conversion to hydroxyapatite crystal formation ($Ca_{10}(PO_4)_6(OH)_2$) [6, 7]. The ionic dissolution products can also trigger precipitation. Over the past decade, understanding and quantifying the occlusion mechanism through *in vitro* characterization has been of great interest with a particular attention on variation in dentine permeability and morphology after exposure to BG [8–13]. However, there is little published work in the literature on the effect of organic and fluoride additives in dentifrice on the bioactivity of BG. Fluoride sources are used in toothpastes and drinking water to encourage fluoroapatite formation, which is harder and more resistant to acid erosion than hydroxyapatite. Several fluo-

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ride sources are used. The fluoride source used in SRP is monofluorophosphate (MFP), which is remarkably stable at physiological temperature in neutral or slightly alkaline pH [14]. However, non-specific salivary phosphatases can hydrolyze monofluorophosphate, releasing phosphoric acid and free fluoride into solution which are known to influence the nucleation of hydroxyapatite onto bioactive glass surfaces [15–17]. Unfortunately, not all published research on the *in vitro* characterization of SRP in synthetic media specifically mentions the use of phosphatase. Thus, a method is needed to quantify whether changes in paste formulation affects the apatite forming ability of BG, when it is in a toothpaste matrix, compared to the BG alone, in conditions that mimic the ionic strength of human saliva. In addition, new glasses are being developed for toothpaste so a test is needed to allow comparison between glass compositions in toothpastes. The design of such a method could have a considerable impact on the development of the next generation of desensitizing dentifrices. Herein, the aims were to i) develop a bench top bioactivity test for bioactive glass containing toothpaste and compare the apatite formation ability of BG alone and in toothpaste ii) to investigate the effect of introducing alkaline phosphatase to the mineralising solution, which should increase fluoride release through the enzymatic cleavage of monofluorophosphate (MFP) in the SRP formulation.

2 Materials and methods

Modus Laboratories, Reading, UK, supplied remineralisation solution (RS), which is synthesised to mimic the ionic strength of human saliva. It comprised 30 mM of potassium chloride, 3 mM of calcium chloride dehydrate, 10 mM of potassium di-hydrogen orthophosphate, 13 mM of sodium chloride and 0.22 wt% of Mucin. The alkaline phosphatase was isolated from bovine intestinal mucosa with a nominal activity of 10 $U.mg^{-1}$ and provided by Sigma Aldrich, SRP was purchased in retail outlets and the placebo paste and BG powder provided by GlaxoSmithKline (Weybridge, UK).

2.1 Dissolution of bioactive glass particle powders

In the dissolution studies, a constant ratio of bioactive glass powder to solution volume was used, as defined by Jones *et al*: 150 ± 0.5 mg of BG powder was immersed in 100 mL of RS in a screw top polyethylene (PE) container and agitated at 120 rpm in an orbital shaker held at 37°C [18]. At the following time points: 1, 2, 4, 8, 24, 48, 72 h, the pH of the solution was recorded (OAKTON pH 11 series pH) and 1 mL of supernatant was removed and replaced by 1 mL fresh RS. The container was subsequently replaced in the incubator. Each sample was run in triplicate, and the 1 mL solutions were analysed by inductively coupled plasma optical emission spectroscopy (ICP-OES) after dilution in acid. The same protocol was applied to the RS alone as a control. Separate containers containing 150 mg of BG powder and 100 mL of solution were also prepared for surface analysis and were removed from the incubator at each time point. The BG powders were collected using filter paper (particle retention 5-13 μm), washed with distilled water followed by acetone and dried overnight at 40°C. The surface of the BG powders were then examined by Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM) and X-ray diffraction (XRD).

2.2 Dissolution of toothpaste containing bioactive glass

The reference commercially available toothpaste used in this study, SRP, contains 5 wt% of bioactive glass (BG). To maintain a consistent BG concentration of 1.50 $mg{\cdot}ml^{-1}$, 3 g of SRP were required for 100 mL of RS. The container was vigorously shaken by hand for 30 s and placed in an incubator held at 37°C and 120 rpm. The same protocol was applied to the RS alone as a control. A placebo of the toothpaste (PLA) containing all the normal components except the BG was run as an additional control. For each of the following time points: 1, 2, 4, 8, 24, 48 and 72 h, a container was removed and the solution was centrifuged at 5000 rpm for 5 min. The pH of the clear solution was measured and the supernatant was analysed by ICP-OES. The precipitate was frozen in liquid nitrogen after removal of all the supernatant and subsequently freeze-dried overnight (Scanvac Coolsafe -110°C, 4.10^{-4} mbar).

2.3 Effect of alkaline phosphatase on dissolution in RS

Alkaline phosphatase (ALP) was added to RS media to simulate the role of enzymes in the oral environment. Three concentrations were chosen based on clinical data acquired from healthy adult's saliva, 25 $U.L^{-1}$, and patients suffering from gingivitis, 75 $U.L^{-1}$ [19]. SRP and PLA control were immersed in these solutions following the procedure

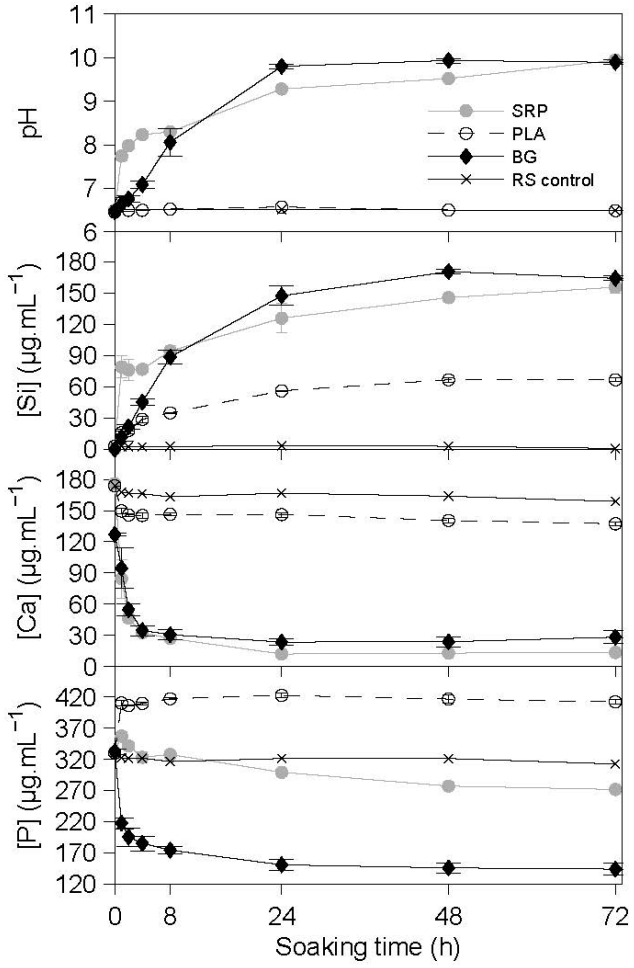

Figure 1: Silicon, calcium and phosphorus concentration profiles as well as pH upon 3 d immersion of SRP, PLA and BG in ALP-free RS

and sample characterization described in Section 2.2 with the addition of the measurement of fluoride in solution.

2.4 Ion concentration profile

2.4.1 ICP-OES

Concentrations in solution were measured with a Thermo Scientific iCAP 6300 Duo inductively coupled plasma - optical emission spectrometer (ICP-OES) with auto sampler. Sample solutions were prepared by diluting the samples by a factor of 10 with analytical grade 2 M HNO_3. Mixed standards of silicon, phosphorous, calcium, sodium and potassium were prepared at 0, 2, 5, 20 and 40 $\mu g{\cdot}mL^{-1}$ for the calibration curve. Silicon and phosphorus were measured in the axial direction of the plasma flame whereas calcium, sodium and potassium were measured in the radial direction.

2.4.2 Fluoride concentration

Fluoride concentration was measured using a fluoride ion-selective electrode (Orion, type 9409BN). The probe was calibrated using a one to one, RS doped with sodium fluoride to TISAB II buffer solution. Final concentration of fluoride varied from 1.10^{-6} to 1.10^{-2} M. The measurements were performed after twice diluting the dissolution media with TISAB II.

2.5 Surface analysis

2.5.1 Fourier transform infrared spectroscopy

The solids obtained after immersion of the BG alone, PLA and BG containing toothpaste were characterised in attenuated total reflection mode over a wavenumbers from 4000 to 500 cm^{-1} (Nicolet iS10 fitted with a Specac MK11 Golden gate single reflection ATR module).

2.5.2 Scanning electron microscopy

Field emission gun scanning electron microscopy (FEG-SEM) was performed on a Leo 1525 with Gemini column fitted equipped with an Energy-dispersive X-ray (EDX) module using a gun voltage of 5 kV for secondary electron imaging and 15 kV for spectroscopy with a working distance of 6-13 mm. Samples for scanning electron microscopy were prepared by mounting BG powders on double sided carbon tape and coating with chromium.

2.5.3 X-Ray Diffraction

The diffraction was measured with a Bruker D2 desktop XRD between 10° and 60°2θ, with a 0.03° step size and a total counting time of 25 min. The radiation source was a Ni filtered CuKα. Powdered samples were placed on an amorphous silicon disk.

2.6 Statistics

Student's t test was conducted using Matlab R2013b to compare the ion concentrations in solution of BG powder and SRP at a given timepoint. Each material type was assumed to have unequal variance and a sample size of n = 3. The level of statistical significance was set at $p < 0.05$.

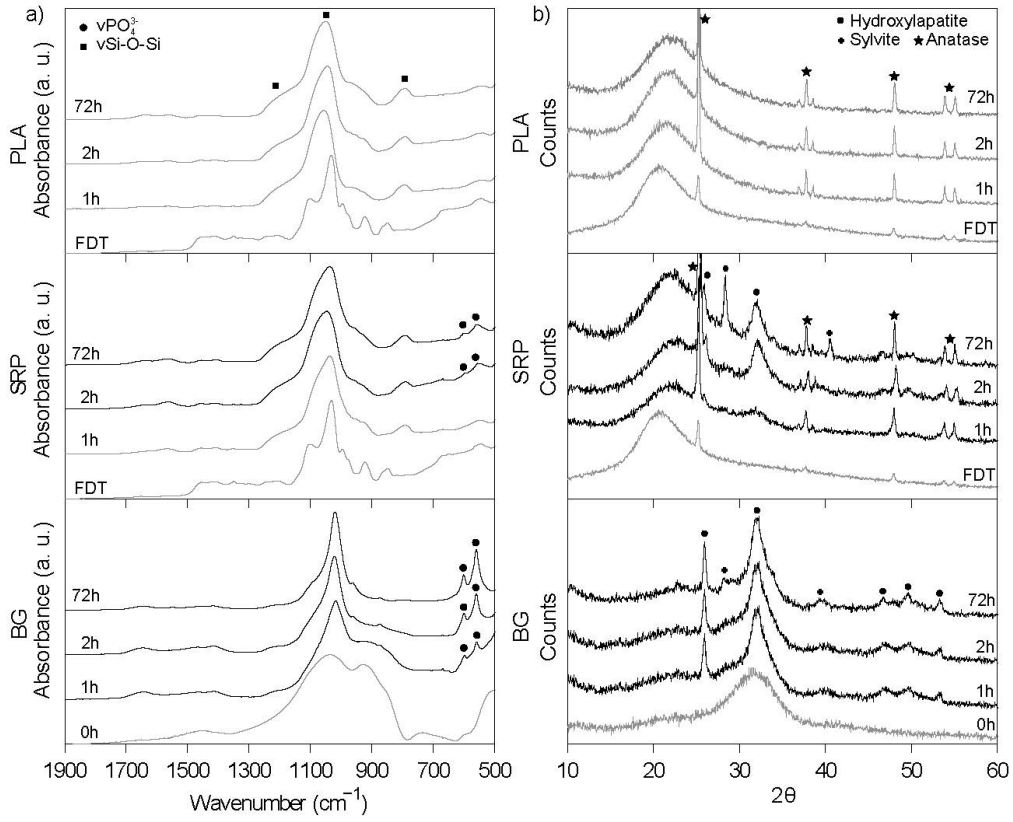

Figure 2: Surface characterisation by a) FTIR-ATR and b) XRD for solids from SRP, PLA and BG immersion in (ALP-free) RS, initial (neat BG or freeze dried paste) and after 1, 2 and 72 h.

3 Results

3.1 Test Validity

The first aim of the study was to evaluate whether it is possible to detect bioactivity with BG containing toothpaste in an *in vitro* model. SRP, BG powder and a placebo paste (PLA) containing no BG were immersed in RS over a period of three days and changes in the solutions and particles were monitored.

3.1.1 Ion concentration profiles

Upon immersion of SRP and BG into RS, the pH increased from 6.5 to 9.6 (Figure 1) over the first 24 h, due to the release of cations (*e.g.* Na^+ and Ca^{2+}) from the BG. No variation of pH occurred with PLA or the control RS over the length of test. The dissolution of the BG in the SRP and BG alone induced an increase in the concentration of silicon in RS against control (Figure 1). RS containing BG reached a Si concentration of 147 $\mu g \cdot mL^{-1}$ over the first 24 h, which further increased by 11.5% during the two remaining days, reaching a concentration of 164.2 $\mu g \cdot mL^{-1}$ which was statistically equivalent to the silica released with SRP ($p = 0.36$). However, silicon concentration also increased with PLA due to the partial solubilisation of the hydrated silica present in the paste, which is a common additive in many toothpastes. Therefore, the silicon release with SRP is a combination of dissolution of hydrated silica and BG. Thus, the distinct Si contribution of the BG in SRP was estimated to be 88.64 $\mu g \cdot mL^{-1}$ after 3 days of immersion.

Calcium concentration was strongly affected by BG and SRP addition to RS, as both materials caused a rapid decrease of Ca in solution, decreasing from 173.8 $\mu g \cdot mL^{-1}$ to 13.3 $\mu g \cdot mL^{-1}$ within the first 8 hours. PLA also caused a slight decrease in calcium to 137.2 $\mu g \cdot mL^{-1}$ in the first hour and then remained constant for the rest of the 72 h. However, the phosphate concentration followed a different trend. The phosphorus concentration in RS increased for the PLA control from 321 $\mu g \cdot mL^{-1}$ to 422 $\mu g \cdot mL^{-1}$ which was assigned to the solubilisation of sodium monofluo-

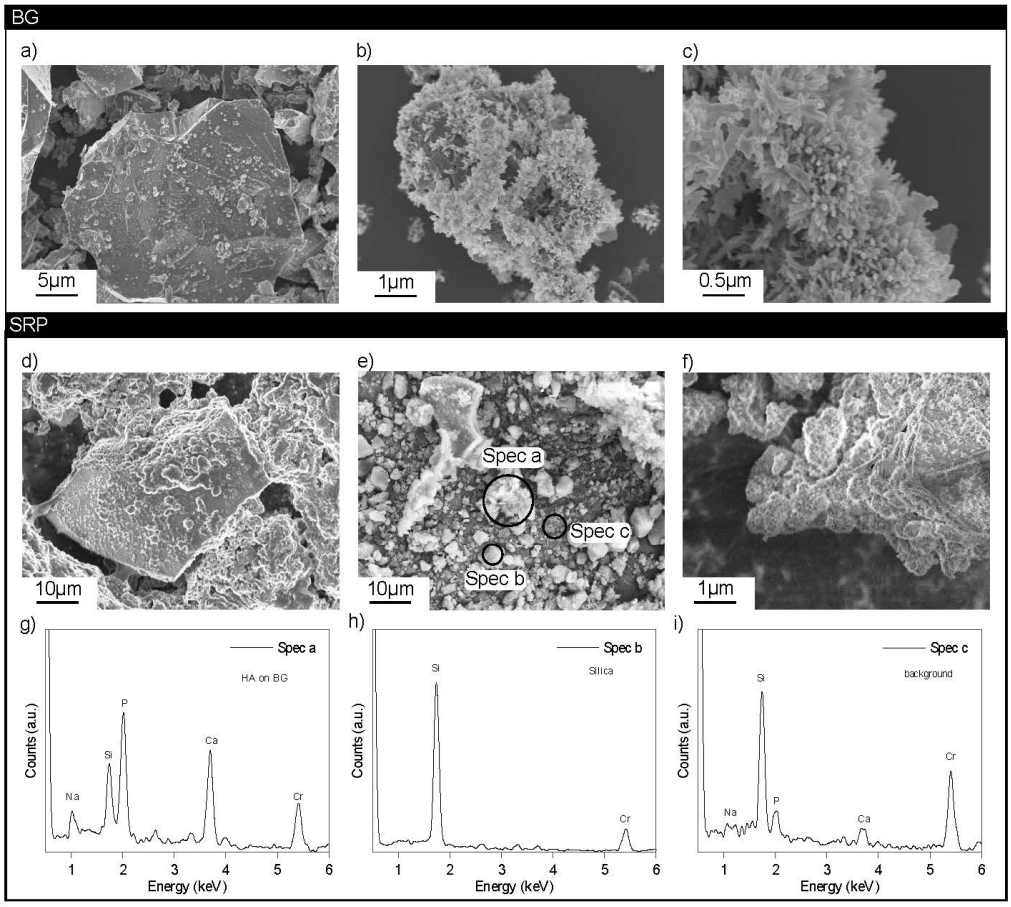

Figure 3: a), b) and c), SEM images of BG before and after 2 h of immersion in RS at different magnification, respectively. d), e) and f), SEM pictures and g), h) and i) EDX spectra of SRP before and after immersion in RS at different magnification, respectively.

rophosphate from the toothpaste. However, it is important to note here that the solubilisation of MFP does not necessarily lead to the release of free fluoride and that ICP is an elemental analysis where phosphonate and phosphate groups are indistinguishable. The phosphorus level in RS control remained constant throughout the experiment. SRP had a small, gradual effect on the phosphate concentration as it decreased roughly linearly throughout the study; reaching 271.7 $\mu g \cdot mL^{-1}$ after 3 d. BG particles caused the greatest decrease in phosphate concentration, decreasing rapidly in the first 8 h which mirrored its Ca ion profile. Depletions in calcium and phosphorus from BG and SRP samples were found to be significantly different.

3.1.2 Surface Analysis

Solids from the immersion of BG, SRP and PLA were examined by FTIR and XRD. FTIR spectra show sharp bands at 565 and 605 cm^{-1}, characteristic of P-O bending, and bands at 1030 cm^{-1}, characteristic of P-O stretching, after 1 h for BG and 2 h for SRP (Figure 2a), which correspond to the PO_4^{3-} tetrahedral vibration in crystalline calcium phosphate. Figure 2b shows XRD patterns of the collected powders, which confirmed abundant hydroxyapatite (HA) formation on the surface of the BG with sharp peaks at $2\theta \approx 26^\circ$ and 32°. Positive matches were also found for the other peaks ($2\theta \approx 47^\circ, 50^\circ, 53^\circ$) with HA external references (ICSD 01-084-1998). XRD patterns from SRP also contained an amorphous halo for the additional silica included in the formulation, plus peaks for titanium dioxide (anatase). Only the hydrated silica and titanium dioxide were identified in the powder collected from PLA with no sign of HA precipitation after 3 days. Results obtained by FTIR and XRD were confirmed using EDX-SEM (Figure 3).

The surfaces of the bioactive glasses were found to be smooth and dense before immersion in media. However, large needle-like structure characteristic to HA were found

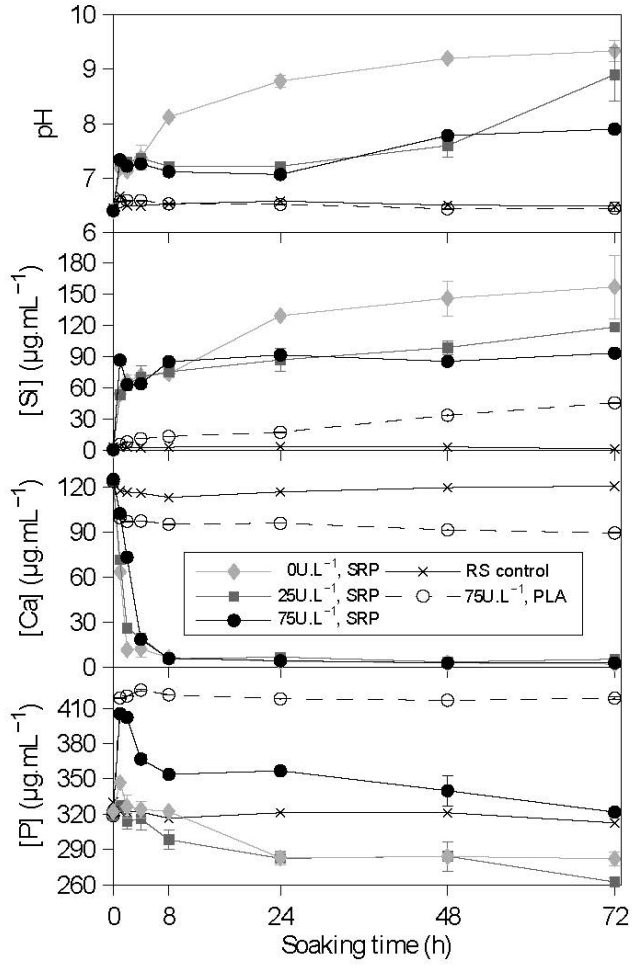

Figure 4: Silicon, calcium and phosphorus concentration profiles as well as pH upon 3 days immersion of SRP and PLA in RS, which contained ALP at 0, 25 and 75 $U.L^{-1}$.

to cover almost the entire surface of BG particles after 1 h immersion in RS when the particles were immersed alone, or after 2 h immersion for the particles in the toothpaste. Calcium and phosphorus were also found on the particles by SEM-EDX. However, for the SRP sample, Ca and P were only detected at a significant level by EDX locally on the surface of particles that contains sodium, which confirmed that the precipitation was occurring onto 45S5 and not silica particles, as indicated by Figure 3g. In addition, silicon dioxide remained smooth and featureless over the length of the experiment as shown in Figure 3h.

3.2 Effect of the fluoride release onto the bioactivity SRP:

Monofluorophosphate (MFP) is the fluoride source in SRP. Alkaline phosphatase (ALP) was added to the media at 0, 25 and 75 $U.L^{-1}$ in order to release fluoride ions through the enzymatic cleavage of MFP and study the effect on the bioactivity of SRP and PLA.

3.2.1 Glass dissolution and fluoride release

Figure 4 shows the pH and ion concentration profiles over 3 days of dissolution in RS containing different concentrations of alkaline phosphatase (ALP). Only one PLA control (PLA with ALP at 75 $U.L^{-1}$) is shown as no significant differences in pH and Si, Ca and P release ($p > 0.75$) was found regardless of the concentration of ALP and at any time point with PLA. Dissolution of the BG contained in SRP was greatly affected by the release of fluoride and phosphoric acid from the enzymatic cleavage of MFP (Figure 4). When ALP was introduced in the media, pH increase due to SRP was less than when no ALP was added and it remained constant over the first 24 h of dissolution at a value of 7.2 regardless of ALP concentration ($p = 0.57$). Between 24 h and 3 days the pH increased up to 7.9 and 8.9 as [ALP] increased to 25 and 75 $U.L^{-1}$, respectively. Release of silicon from SRP was found to be slower as ALP concentration increased, reaching 92.9 $\mu g \cdot mL^{-1}$ (75 $U.L^{-1}$ [ALP]) and 117.9 $\mu g \cdot mL^{-1}$ (25 $U.L^{-1}$ [ALP]) at 3 days as compared with 209.0 $\mu g \cdot mL^{-1}$ for the pure RS without ALP (0 $U.L^{-1}$). Depletion in calcium and phosphorus concentration was also observed. No significant differences were observed in calcium concentration at 3 days ([Ca] = 5.2 $\mu g \cdot mL^{-1}$, $p = 0.23$) independent of ALP concentration. However, the absolute value of the initial rate for calcium decreased as ALP concentration increased, going from −47.5 $\mu g \cdot mL^{-1} \cdot h^{-1}$, for the negative control, to −23.4 $\mu g \cdot mL^{-1} \cdot h^{-1}$ for 75 $U.L^{-1}$ [ALP]. Very different trends were observed for the phosphorus ion profile depending on the ALP concentration. SRP immersed in ALP-free RS and with 25 $U.L^{-1}$ of ALP caused an initial decrease in phosphorus concentration whereas SRP in RS with 75 $U.L^{-1}$ produced a sharp initial increase followed by a decrease from 2 h of immersion. Phosphorus concentration for SRP in RS with 75 $U.L^{-1}$ of ALP was 13.8% greater at 3 d than in ALP-free RS (0 $U.L^{-1}$), whereas SRP immersed in RS with [ALP] = 25 $U.L^{-1}$ had phosphorus concentration 7.0% lower than to the negative control ($p = 0.0074$) reaching 266.2 $\mu g \cdot mL^{-1}$ at 3 d.

Fluoride was steadily released from PLA with an increasing rate and total release which was dependent on ALP concentration as shown in Figure 5. For instance, an initial release rate of 0.62 $\mu g \cdot mL^{1} \cdot h^{-1}$ ($R^2 = 0.99$) and 0.14 $\mu g \cdot mL^{-1} \cdot h^{-1}$ ($R^2 = 0.95$) was observed with ALP at 75 and 25 $U.L^{-1}$ reaching 23 $\mu g \cdot mL^{-1}$ and 13 $\mu g \cdot mL^{-1}$ at 3 days, respectively. Significant differences were found when mea-

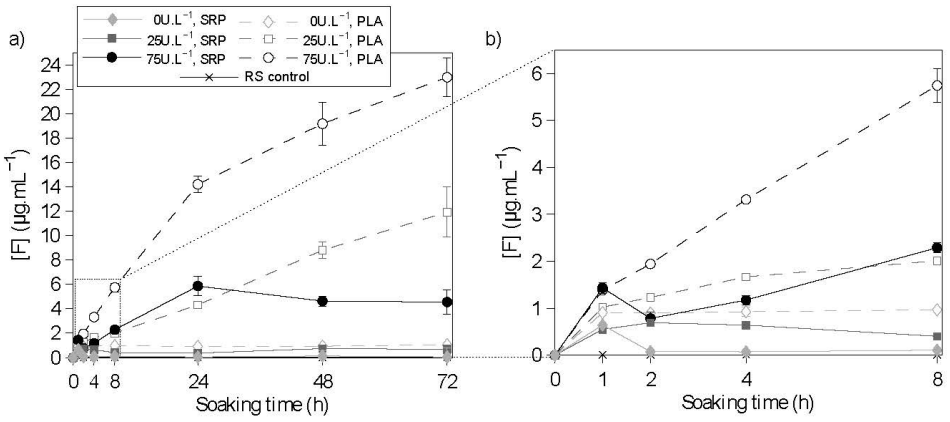

Figure 5: Concentration of fluoride ions in RS upon immersion of SRP and PLA at different ALP concentration over a) 3 days and b) an expanded view of the first 8 h.

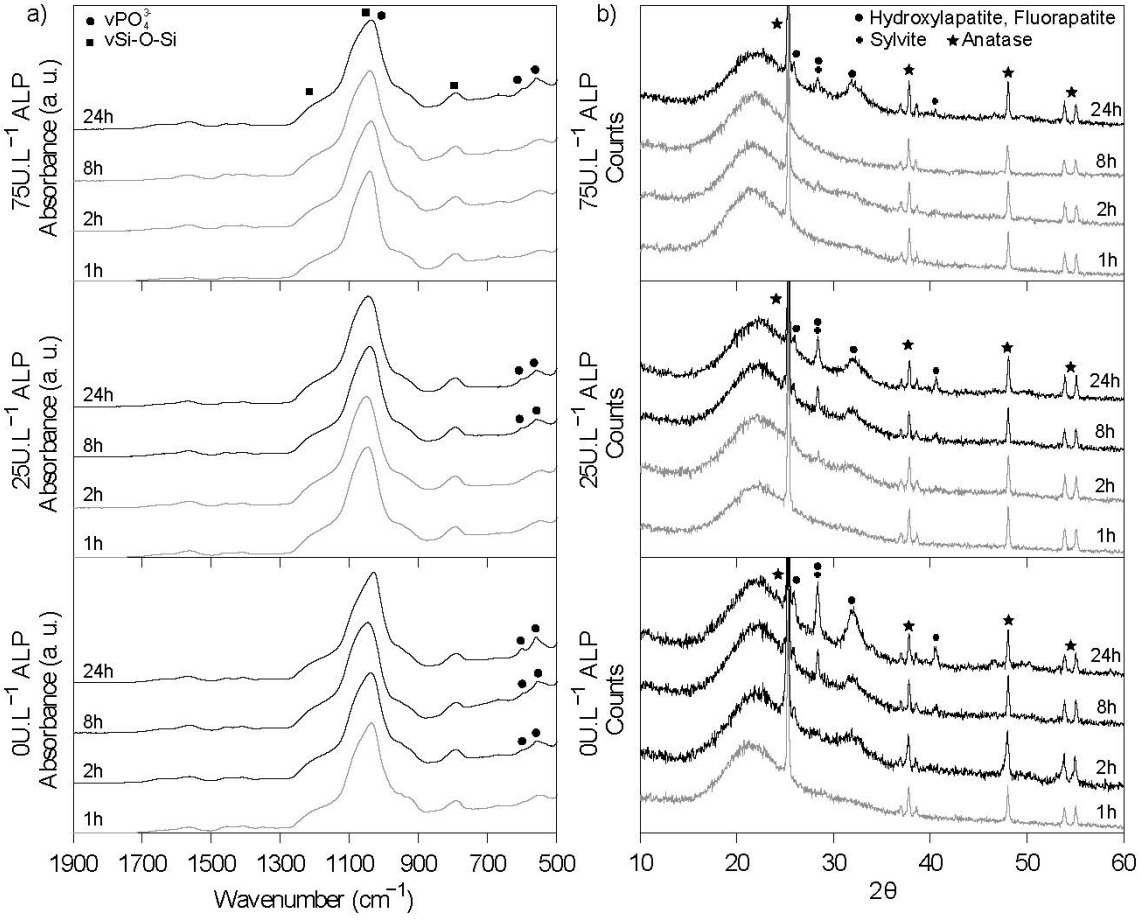

Figure 6: Surface characterisation by a) FTIR-ATR and b) XRD of the solids remaining after immersion of SRP in RS containing 0, 25 and 75 U.L^{-1} ALP for at 1 h, 2 h, 8 h and 24 h.

suring the fluoride concentrations with SRP. At 25 U.L^{-1} of ALP, a slight increase was observed in the first 2 h before decreasing to the limit of detection by 24 h. At 75 U.L^{-1} of ALP, a steady increase of the concentration of fluoride was monitored over the first 24 h up to 5.9 $\mu g \cdot mL^{-1}$, before plateauing until the end of the test.

3.2.2 Surface analysis following immersion in RS containing ALP

According to FTIR (Figure 6a), bands at 565 and 605 cm^{-1}, characteristic of P-O bending, and the band at 1030 cm^{-1}, characteristic of P-O stretching, were observed after SRP had been incubated for 8 h and 24 h for ALP at 25 $U.L^{-1}$ and 75 $U.L^{-1}$, respectively. However, XRD, which is more sensitive to crystalline species, suggested that crystalline calcium phosphate was present at 2 h with ALP at 25 $U.L^{-1}$ at a lower concentration than with 0 $U.L^{-1}$. No fluorite precipitates were observed. Thus, according to fluoride ions concentration profiles monitored for SRP and XRD references patterns (ICSD 01-084-1998 and 01-083-0557), it was assumed that a mix of fluorapatite and hydroxyapatite precipitated on the surface of BG.

4 Discussion

4.1 Test validity:

Bioactive glass particles on their own or in SRP were covered with hydroxyapatite after 1 and 2 h, respectively, upon immersion in remineralizing solution without ALP. Apatite deposition is in agreement with the Debye-Hückel theory, which describes the ionic activity of a dilute solution and suggests hydroxyapatite precipitation is favourable in RS [20]. The negative logarithm of the ionic activity product (IP) of the remineralization solution was calculated using the Debye-Hückel limiting law, $-\log_{RS}(IP) = 94.59$, which is inferior to the reference value of hydroxyapatite in water $-\log_{HA}(IP) = 117.0$ [21, 22]. This means that the stoichiometry of the media used in this work favours the precipitation of hydroxyapatite, which could be defined as a supersaturated solution with respect to hydroxyapatite [23]. Note here, that the value of IP obtained in this project was similar to the conventional simulated body fluid (c-SBF) introduced by Kokubo *et al*, extensively used to describe the *in vitro* dissolution behaviour of orthopaedic implants [24]. However, kinetics of precipitation of HA on BG in SBF and RS can't be compared due to their difference in ionic strength and protein content [25]. According to the ion concentration profiles, it seems that the bioactivity of the BG and SRP followed the established HA nucleation mechanism described by Hench [6]. The suggested mechanism is that when BG particles are exposed to physiological or synthetic body fluid, ion exchange occurs between cations (*e.g.* Ca^{2+}, Na^{+}) from the BG and H^{+} or H_3O^{+} from the media. Upon this exchange, a silica rich layer (Si-OH) is created at the surface of the bioactive glass, which is known to be a good deposition site for amorphous calcium-phosphate, which can crystallise to hydroxyapatite. Dissolution of the –Si-O-Si– bonds in the silicate network are more rapidly broken as pH increases. Precipitation of apatite is also more rapid at higher pH due to higher availability of OH^{-}. SRP was found to be slower at nucleating HA than BG. The toothpaste matrix is likely to encapsulate the BG particles and slow the dissolution/precipitation mechanism. Therefore, this delay in bioactivity could be attributed to a change in ionic mobility due to the large amount organic present in SRP and the possible interaction between the different ions in solution and surfactant that composed the dentifrice [26]. Despite the difference in kinetics between the neat BG and the toothpaste containing BG, the time taken to precipitate HA with SRP in this work was in agreement with the previous work carried out by Wang *et. al.* where highly permeable dentine was covered with HA within 24 h after treatment with SRP and incubation in similar remineralizing solution [9, 10].

4.2 Enzymatic cleavage of MFP and its effect on the bioactivity of SRP:

The addition of ALP in the media reduced the apatite forming ability of the BG. It induced the release of fluoride through the enzymatic hydrolysis of MFP as observed with the toothpaste without BG (PLA). However, it also released phosphoric acid. Fluoride release rates from PLA were in agreement with literature. According to Klimek *et al*, saliva from a healthy patient hydrolyses MFP at a rate of 0.147 $\mu g \cdot mL^{-1} \cdot h^{-1}$ and 0.43 $\mu g \cdot mL^{-1} \cdot h^{-1}$ for a patient having an approximate plaque index of 100% [27]. MFP hydrolysis rates from SRP were found to be 0.14 $\mu g \cdot mL^{-1} \cdot h^{-1}$ and 0.62 $\mu g \cdot mL^{-1} \cdot h^{-1}$ for [ALP] at 25 $U.L^{-1}$ and 75 $U.L^{-1}$, which are concentrations representative of healthy adults and patients suffering from gingivitis, respectively. The hydrolysis of MFP had a large effect on the dissolution and bioactivity of the toothpaste containing BG as compared to the dissolution made without ALP.

First, it seems that the release of phosphoric acid, along with the initial presence of potassium di-hydrogen orthophosphate increased the buffering capacity of the media, keeping the pH at approximately 7.2 ($H_2PO_4^{-}{}_{(aq)} + H_2O_{(l)} \rightleftharpoons HPO_4^{2-}{}_{(aq)} + H_3O^{+}{}_{(aq)}$, pKa = 7.21) over the first 24 h of dissolution regardless of the amount of ALP present in solution [28]. This slowed the dissolution of the BG and reduced rate of HA formation [29]. Despite slowing the HA formation, the release of phosphoric acid could be biolog-

ically beneficial. According to Bakry *et. al.*, an increase of phosphoric acid concentration at the surrounding of BG promotes the dentine-BG interaction through the formation of an intermediate acidic calcium phosphate crystal (brushite) layer, accelerating the occlusion of the exposed dentine [30].

Secondly, the apparent concentration of free fluoride was surprisingly different with samples containing BG compared to PLA. For PLA, the amount of fluoride released into the RS increased as ALP concentration increased. For SRP immersed in RS at 25 $U.L^{-1}$ of ALP, the fluoride concentration remained under 1 $\mu g{\cdot}mL^{-1}$ throughout the experiment, but it steadily increased over 3 days to reach 13 $\mu g{\cdot}mL^{-1}$ for PLA. This was surprising because ALP activity was previously found to increase as pH increases [31]. Therefore, a stronger release of fluoride was expected with SRP compared to PLA. This is a good indication that the fluoride released from MFP took part in the precipitation of apatite on the BG particles in SRP as as no fluorite (CaF_2) was detected using X-ray diffraction and knowing that soluble orthophosphate favors fluorapatite ($Ca_5(PO_4)_3F$) formation to the detriment of fluorite [32]. It is difficult to pick up differences in fluorapatite and hydroxyapatite with conventional XRD. The precipitation of fluorapatite is of a great importance to the repair of exposed dentine of sensitivity sufferers as it thought to reduce the solubility of a remineralized layer [33].

Based on the concentration profile of phosphorus and the surface analysis (Figure 4 and 6), it can also be assumed that the cleavage of MFP occurred in solution as no experimental evidence had been found of its absorption onto inorganic constituents of the toothpaste. There could therefore be differences in the mechanism *in vitro* compared to *in vivo* where MFP would be rapidly absorbed on the plaque after brushing and hydrolyze in the plaque fluid reducing the concentration of free fluoride in saliva [16, 34] Therefore, fluorapatite precipitation on BG particles observed in this *in vitro* model might be different *in vivo*.

5 Conclusion

The analytical techniques employed in these experiments can produce a more complete knowledge of the compositional effect on bioactivity of toothpastes containing remineralizing compounds and should prove useful in the development of more effective desensitizing toothpastes. The addition of MFP as the fluoride source to toothpaste and its degradation by ALP mediated the buffering capacity of the system, keeping pH low, thereby altering the kinetics of the system and slowing apatite formation in RS.

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