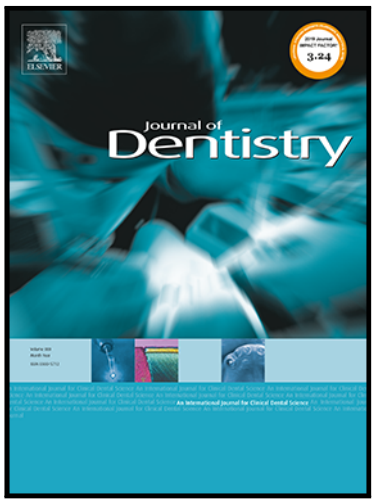

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The effect of different concentrations of fluoride in toothpastes with or without bioactive glass on artificial root caries

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Short title: fluoride toothpastes with or without bioactive glass in root caries

Abstract

Objective: To investigate the effect of different toothpastes either containing 5,000ppm-F, 1,450ppm-F or bioactive glass (BG) with 540ppm-F on artificial root carious lesions (ARCLs).

Method: The crowns of 23 extracted sound teeth were removed leaving their roots only. Subsequently, each root was divided into four parts. A total of 15 sound root dentine (SRD) was left untreated as baseline. The ARCLs were developed for the remaining roots using demineralisation solution (pH-4.8). 15-ARCLs samples were then left untreated. The rest of samples were divided into four groups (n=15 each) and treated with Group-1(BG with 540ppm-F); Group-2(5,000ppm-F); Group-3(1,450ppm-F) and Group-4(deionised water). 13-day pH-cycling included using demineralisation solution for 6hrs, then placing samples into remineralisation solution (pH-7) for 16hrs. Each sample was brushed with the assigned toothpaste twice a day during pH-cycling. Fluoride concentrations at each time point were measured using F-ISE, whilst calcium (Ca^{2+}) and phosphorus (P) ion release was determined using ICP-OES, KHN, XRD, ^{19}F-MAS-NMR analyses.

Results: KHN showed significant surface changes for each group (p<0.001). The uptake of Ca^{2+} occurred at days 1-2, phosphorus ion loss was high when compared to the uptake in all groups. XRD showed presence of sharp diffraction lines evidencing apatite formation for Groups 1-3. ^{19}F-MAS-NMR confirmed fluorapatite presence in Groups 1-3.

Conclusion: All toothpastes were promising in fluorapatite formation. BG with 540ppm-F toothpaste released more ions ($Ca^{2+}$and P) and reharden the artificial root carious lesions when compared to other groups. However, 1,450ppm-F toothpaste showed more fluoride-substituted apatite formation whilst 5,000ppm-F toothpaste had more fluorapatite formation.

Clinical Significance: Toothpaste containing BG with 540ppm-F, 5,000ppm-F and 1,450ppm-F toothpastes are likely to have a significant impact in reversing and arresting root caries. However, randomised controlled double-blinded clinical trials are required to translate these results into clinical practice.

Keywords: Demineralisation, Remineralisation, Cariology, Toothpaste, Fluoride, Bioactive glass

1. Introduction

Root caries is one of the major causes of tooth loss in aging population [1]. Toothpastes containing fluoride are considered as cost-effective anti-caries agent due to their remineralisation effect which is attributed to fluoride-enhanced precipitation of calcium and phosphate in the form of fluorapatite [2, 3]. Therefore, more than 95% of population in developed countries use fluoridated toothpastes[4].

The high fluoride toothpaste (5,000ppm-F) was reported to reverse 51% of leathery primary root caries in comparison to toothpaste containing 1,100ppm-F [5, 6]. Subsequently, Ekstrand et al. (2008) evaluated toothpastes containing either 5,000 or 1,450ppm-F on patients >75 years old for eight months. The results showed that toothpaste containing 5,000ppm-F was effective in the management of root caries when compared to the 1,450ppm-F one. The fluoride bioavailability plays an important role in the management of dental caries. However, the bioavailability of fluoride after tooth brushing is limited to a short period since the fluoride level in saliva decreases rapidly due to salivary flow [7]. Fluoro calcium phospho-silicate glass, which is a novel low concentration fluoride-containing bioactive glass (F-bioactive glass) was incorporated into toothpastes to increase the bioavailability of fluoride in the oral cavity over 12hrs. The F-bioactive glasses are amorphous silicate glasses, that degrade in aqueous solutions [7]. These glasses serve as a source of calcium, phosphate and fluoride ions. Fluoride promotes remineralisation [8] and results in fluorapatite formation. Laboratory-based studies previously reported that fluoride ions released from the F-bioactive glass can be retained up to one week [9, 10].

Recent *in vitro* studies reported that the toothpaste containing BG with low fluoride was effective for the remineralisation of early artificial enamel carious lesions including the subsurface [11]. However, there is no evidence on root caries related to this toothpaste. Therefore, the aim of this laboratory-based study was to investigate the structural changes of artificial root carious lesions (ARCLs) according to formation of apatite using different toothpastes either containing bioactive glass with low fluoride or high sodium fluoride alone, using the Knoop hardness test (KHN), Fluoride-Ion selective

electrode (F-ISE), Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES), X-ray diffraction (XRD), ^{19}F Magic angle spinning-nuclear magnetic resonance spectroscopy (^{19}F-MAS-NMR).

2. Materials and Methods

2.1. Preparation of Root Dentine Samples

A total of 23 extracted sound teeth were collected from Dental Clinics (QMREC 2011/99) and stored in 0.1% thymol [12]. Subsequently, they were cleaned and polished with non-fluoridated prophylaxis paste (NUPRO-Dentsply, USA). The crowns of these teeth were removed by leaving their roots using 0.3mm thickness diamond disc under running water at 3000-rpm speed (Struers, Copenhagen, Denmark) and divided into four pieces (n=90). The root dentine samples were embedded into self-polymerising acrylic resin (Simplex-Rapid, Kemdent, UK) exposing labial/buccal/palatal/lingual root surfaces. They were then polished with 400-grit abrasive silicon carbide papers (Buehler, IL, USA) using micro-grinding system (Automatic Lapping and Polishing Unit, Kemet, UK).

2.2. Preparation of ARCLs

ARCLs (n=75) were then developed using demineralisation solution (pH-4.8) for five days at 37°C, whilst 15 samples were left untreated as sound root dentine (SRD) [13, 14].

2.3. The pH-cycling conditions

The pH-cycling conditions included exposure of the dentine to alternate demineralisation and remineralisation. The demineralisation solution consisted of 1.5mmol/L $CaCl_2$, 0.9mmol/L KH_2PO_4 and 50mmol/L acetic acid. The remineralisation solution contained 1.5mmol/L $CaCl_2$, 0.9mmol/L KH_2PO_4, 20mmol/L HEPES and 130mmol/L KCl. 5mmol/L NaN_3 was added to the demineralisation and remineralisation solutions to prevent microbial growth. The pH was adjusted at 4.8 and 7.0 using 1M KOH for demineralisation and remineralisation solutions respectively.

Each sample was immersed in 10mL of the demineralisation solution for a period of six hours followed by immersion in 10mL of the remineralisation solution for 16 hrs using new solutions for each cycle. This procedure was repeated for 13-days at 37°C.

2.4. The use of different toothpastes

60 samples with ARCLs were allocated into four different treatment groups. Each group (n=15) received one of the allocated treatments (Table-1). Each toothpaste was diluted by deionised water at a dilution of 1:3 to make a toothpaste slurry [13]. Tooth brushing was simulated using medium-bristle

toothbrush (Colgate Palmolive, UK) and slurries twice a day for 13-days. The brushing process was carried out using an electrically-powered toothbrushing machine (Boston Gear, Braintree, MA) designed to produce constant reciprocal movements (150g force for 10s) [15]. The samples were left for two minutes prior to rinsing with deionised water [16].

The mechanical toothbrushing with allocated toothpastes was chosen to simulate the clinical setting according to the recommended brushing time which was two minutes for the entire mouth [16]. It should be noted that toothpastes in this study had different relative enamel/dentine abrasive indices, however these RDAs were within acceptable level (ranging from 65 to 124). In addition, the deionised water had low abrasion effect. Therefore, the samples in control group were brushed using deionised water alone.

2.5. KHN

A total of 15 samples from each group were selected to measure the KHN following the development of ARCLs and after 13-days treatments with pH-cycling. Using the micro-hardness tester (Micromet II, Buehler, USA), each sample with an indentation load of 50g for 15s, using the Knoop indenter [17]. The minimum space of indents was at least 50µm apart to avoid interferences and crack propagation in the central area of each sample. The depth of KHN was up to 0.1mm. There were 10 indentations on each sample. An average of 10 indentations readings were then recorded. Subsequently, data of each experimental condition from 15 samples were averaged.

2.6. F-ISE

The F-ISE was carried out to measure fluoride ion concentration. The F-ISE (Nico2000 Ltd., UK) was calibrated by gradient fluoride concentration (serial dilutions of standard fluoride solution, 1000ppm, 100ppm, 10ppm, 1ppm, 0.1ppm) at room temperature. After calibration, fluoride ion concentrations were measured for all groups at each time point in the 10 ml demineralisation and remineralisation solutions using the same F-ISE. The ISE calibration equation was provided by converting the ISE reading (mV) into concentration of fluoride ions.

2.7. ICP-OES

The ICP-OES (Thermo Fisher ICP-OES 7400, Waltham, MA) was used to quantify the concentrations of calcium and phosphate ions. The demineralisation and remineralisation solutions were analysed to measure the concentration of calcium and phosphorus at each time point (at 422.673 and 213.618nm respectively). The calibration range was 0.5–100ppm.

2.8. XRD

The XRD analysis was performed to evaluate the crystallinity. A total of 15 samples for each group (SRD and ARCs groups, Groups 1-4) were investigated on PANalytical CubiX3 X-ray powder diffractometer (Malvern, Worcestershire, UK) under 45kV Cu Kα radiation using Ni filter. The diffraction intensities were measured by scanning in the range of 2θ 5–70° in standard reflection mode, with sample holders spinning on the stage during the scans.

2.9. ^{19}F-MAS-NMR

The ^{19}F-MAS-NMR enables fluorapatite to be distinguished from hydroxyapatite. ^{19}F-MAS-NMR were carried out using 600MHz Bruker spectrometer with the magnetic field of 14.1Tesla. The ^{19}F-MAS-NMR spectra were collected at the resonance frequency of 564.7MHz. The samples were run in 2.5mm zirconia rotor spinning at 22kHz using the standard double resonance Bruker probe with low fluorine background. 1M aqueous solution of sodium fluoride producing a sharp signal – 120ppm was used to reference at the ^{19}F chemical shift scale. This was run with 30s recycle delay with 1us pulse at 100W.

2.10. Statistical analysis

The data from the KHN hardness was analysed using the Kruskal-Wallis H nonparametric test. Mann-Whitney test was carried out to compare the SRD and ARCL samples with Groups 1-4 in relation to the KHN analysis. The differences in Ca, P and F ion release among each group were analysed using One-way analysis of variance (ANOVA) and Kruskal-Wallis H nonparametric tests. The differences between the groups in relation to Ca, P and F ions were tested by Tukey's multiple comparison range test and Mann-Whitney tests. A significance level of 0.05 was performed using the using SPSS 28.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. KHN

The mean micro-hardness (±SE) of root dentine after 13-days of pH-cycling are shown (Figure-1). The Kruskal-Wallis H test showed that there were significant differences among all groups, H(5)=496.434; p<0.001. In between test analyses using the Mann-Whitney test, the results revealed that the differences between Group-2 and Group-3 (U=10909; p=0.650); Group-4 and ARCLs (U=10784.5, p=0.535) were insignificant. However, there were significant differences in the KHN hardness for all the rest of inter-group comparisons (p<0.001). Following each toothpaste treatment,

there were significant increases in the KHN hardness in all toothpaste groups when compared to ARCLs group (p<0.001).

3.2. Fluoride concentration

Figures-2a and 2b show the concentration of fluoride during the 13-days pH-cycling. The fluoride concentrations for all groups (except Group-4) increased in each day after 13-day of pH-cycling. A remarkable increase in the fluoride concentration was also observed in the Group-2 after 11 days (7.8ppm). This was an outlier during the process, therefore, this point would not present the fluoride concentration in day-11. However, the concentration of fluoride for the Group-4 failed to be detected in the demineralisation solution (pH-4.8) except at days 5, 9, 12 and 13. In the remineralisation solution (pH-7.0), the concentration of fluoride for each group was high when compared to the fluoride concentration in the demineralisation solution. There was an increase in fluoride ion concentration from the allocated toothpastes in the remineralisation solution.

3.3. Calcium and phosphorus release

Based on the composition of both demineralisation and remineralisation solutions, there were 60ppm calcium and 27.9ppm phosphorus concentrations. Figures-2c and 2d showed the concentration profiles of calcium and phosphorus during the pH-cycling. Figure-2c (demineralisation) demonstrated that the loss of concentration of calcium in all groups increased from day 1 to 13. Table 2 showed that the cumulated average calcium and phosphorus ions in demineralisation, remineralisation and combined periods. Regarding the demineralisation process, the loss of phosphorus ions in Group-1 was high in comparison to Groups-2 and 3, whilst the uptake of phosphorus ions in Group-1 was high in comparison to Groups-2 and 3 following the remineralisation phase.

There were high concentrations of calcium ion release in Group-1 (81.87±4.08ppm) and phosphorus (31.67±1.01ppm) at day-11. In addition, the loss of calcium (69.35±2.87ppm) and phosphorus (30.58±0.92ppm) concentrations were low for Group-2 in the demineralisation solution (Figure-2c and 2d). Figure-2e showed that the uptake of calcium decreased from day-1 to 13 in the remineralisation solution. However, the greatest uptake in calcium (55.45±2.63ppm) and phosphorus (26.68±0.35ppm) concentrations were observed in Group-4 during the remineralisation process. The combined data (Figures-2g and 2h) provided information on the overall calcium and phosphorus ions uptake and loss. All groups showed the uptake of calcium ions from day-1 to day-2, while loss of calcium was observed

to increase with the time. However, the loss of phosphorus ions was high when compared to the uptake of phosphorus ions in all groups during the pH-cycling process.

3.4. XRD

The XRD pattern of each sample at SRD revealed two strong diffraction lines at 25.9° and 31.9° 2θ, all of which matched the JCPDS file for the fluorapatite (Figure-3). The strength of the diffraction peaks for apatite decreased in the ARCLs. The XRD patterns in Group-1 after treatment with pH-cycling immersion are illustrated in Figure-3. This pattern revealed a sharp diffraction line at 25.9° and a triple peak from 31.9° to 33.1° 2θ confirming the presence of more crystalline apatite.

The XRD patterns in Group-2 revealed a double peak from 31.9° to 33.1° 2θ showing more apatite formation. However, the lines became less pronounced following the use of toothpaste containing 5,000ppm-F when compared to the diffraction lines at the SRD. In addition, a double peak from 31.9° to 33.1° 2θ was also present in Group-3, which was the highest in all groups. The original diffraction lines became broader in Group-4 and there was also a double peak between 31.9° and 33.1° 2θ indicating both a poorly crystalline apatite and small amount of apatite.

3.5. ^{19}F-MAS-NMR

Figure-4 shows the ^{19}F-MAS-NMR spectra of each treatment group after 13-days pH-cycling. The spectra of the Group-2 and Group-3 indicated the dominant presence of the fluorine-19 signal at -103,6ppm and -104ppm respectively, which corresponded to the fluoride from the fluorapatite. In addition, there were double peak in the spectra of the Group-1 (-104.8ppm and -107.2ppm). The spectra of Group-1 at -104.8ppm corresponded to a partially fluoride substituted apatite. The spectra at -107.2ppm was close to the signal of fluoride in crystalline calcium fluoride (CaF_2). However, the ^{19}F-MAS-NMR spectra of Group-4 failed to show a signal. This meant no fluoride species was left in this treatment following the pH-cycling process.

4. Discussion

The KHN values in all toothpaste groups were high when compared to the control and ARCLs samples. This could be related to the formation of apatite crystals in ARCLs following the regular use of toothpastes for 13-days of pH cycling. However, the KHN values of each toothpaste group was less than the value for the SRD samples, which could explain the loss of calcium and phosphate ions observed in the ICP-OES (Figure-2g and 2h).

Toothpastes have the potential ability to promote the formation of apatite, however complete remineralisation of the lesion area is not achievable. The brushing procedure that was carried out throughout the study with rinsing would have reduced fluoride levels in the solution. Therefore, the formation of fluorapatite crystals within carious root dentine was challenging [18]. In addition, the previous study reported low penetration depths (up to 0.1mm in 1000g forces) [19]. In this study, the penetration depth was lower than 0.1mm with 50g forces. However, a comparison of KHN values at the same distance between SRD and toothpaste treated samples is unpredictable due to the loss of minerals. Interestingly, the highest KHN values were observed in Group-1. This could be related to the ability of bioglass within the toothpastes to release calcium and phosphate with fluoride ions to promote remineralisation. In this respect, it was reported that bioglass has the ability to act as a nucleating agent and re-attract calcium and phosphate ions forming hard with acid-resistant layer in a form of hydroxyl-like crystals on the tooth surface [20]. The bioactive bioglass (BG) used in this study forms fluorapatite and in the presence of apatite and collagen; the fluorapatite is likely to nucleate on these surfaces. In ARCLs group, the demineralised root dentine would probably have undergone histopathological changes, which resulted a decrease in hardness. There was no difference in KHN values between the ARCLs and deionised water groups. This could be attributed to the lack of additional fluoride ions to form apatite on the carious root dentine.

There were different kinetics of ion release in each toothpaste during the pH-cycling process. The maximum fluoride ion release was in Group-2 followed by Group-3 due to the soluble fluoride salt with a high concentration of fluoride. Group-1 had the lowest release, which based on the dissolution of the BG to deliver bioavailable fluoride, calcium and phosphate ions [21]. The ISE technique is unable to measure the concentration of fluoride <0.02ppm. There is no additional fluoride in demineralisation and remineralisation solutions to exclude the effect of the additional fluoride from these solutions [22]. Therefore, the detection of fluoride in the control group can only be derived from the tooth that uptakes fluoride i.e., from the toothpaste before extraction.

BG is able to release calcium attributed to dissolving the BG. This might explain the highest concentration of calcium for Group-1 in the demineralisation and remineralisation solutions [21]. The ability of BG to provide remineralising properties is due to the amorphous nature of the glass and its ability to dissolve. When BG from the toothpaste is in saliva, it reacts with the hydrogen ions (H_3O^+) that in turn causes the release of calcium and phosphate ions from the surface of the BG. This

process of degradation of the glass increases the pH, which enables the increased precipitation of calcium and phosphate ions from the BG which combines with fluoride ions to form fluorapatite. Phosphorus ions in the Group-2 were high when compared to other groups in the remineralisation solution. The tetrapotassium pyrophosphate in this toothpaste would contribute to the measured content of phosphorus. However, the pyrophosphate is unable to promote remineralisation in the absence of alkaline phosphatase.

Mohammed *et al.* (2013) reported the fluoride-substituted apatite formation at a concentration of 45ppm fluoride [23] whilst higher concentrations resulted in calcium fluoride formation. Fluoride is added to toothpastes at concentrations up to 15,000ppm with the aim of reducing caries [24] (although the concentration would be lower in the mouth due to the dilution effects of salivary flow). In this study, toothpastes were diluted by water and rinsed out after treatment, most of the fluoride ions would have been removed by this process. Therefore, the concentration of fluoride was less than 10ppm in all group, which could explain the fluoride-substituted apatite formation rather than calcium fluoride formation [23]. Farooq *et al.* (2015) reported that when the root carious lesions were remineralised, the mineral deposition was increased due to the formation of fluoride-substituted apatite [25]. It can be speculated that once the remineralisation occurred at the beginning of pH-cycling in this current study, the pores of the ARCL surface would have been blocked and fluoride-substituted apatite participation failed within the lesion.

The results from XRD supported the findings of ion release. Hydroxyapatite and fluorapatite diffraction patterns are challenging to distinguish. These two apatites can be identified by ^{19}F-MAS-NMR since hydroxyapatite contains no fluorine. The diffraction lines between 30° and 35° corresponding to 210, 211, 112, 202 were overlapped with fluorapatite and hydroxyapatite crystals except for the 300 diffraction (33.1° for fluorapatite, 32.9° for hydroxyapatite) [26]. Mneimne *et al.* (2011) reported that the low peak intensity indicated a small amount of apatite, and broad line was related to a small crystallite size [10].

Natural apatite in dentine also contains carbonate ions, which leads to strain in the crystal and more soluble crystals can be observed. The XRD pattern becomes broad, however on dissolution and reprecipitation, the carbonate ions are lost. As a consequence, the sharp diffraction lines were observed [27]. Compared with SRD, the diffraction lines in all toothpaste groups were narrow and well separated. The ^{19}F-MAS-NMR and XRD data confirmed that fluorapatite improved the process of

crystallinity and increased the crystallite size. This improvement might come from the driving force provided by the high affinity F-ions (compared to OH^- groups in the precursor solution) for the apatite crystal growth during precipitation [28]. Tulumbaci and Oba (2019) reported that the diffraction line in 1,450ppm toothpastes was sharp in comparison to the 5,000ppm one for enamel, however the crystal intensity and size were high in the 5,000ppm toothpaste [29]. This might indicate that more fluoride ions from toothpastes (after rinsing; 3ppm, 0.7ppm and 0.1ppm in Group-2,3,1 respectively) to interact with hydroxyapatite to form the fluorapatite. There were no CaF_2 ions in all groups, suggesting there is an optimum range of local fluoride concentrations to promote remineralisation [23]. However, the study samples analysed by XRD were not in the powder form. In addition, the XRD analysis can only detect the presence of crystals to a depth of approximately 45μm from the surface, which was unable to demonstrate the lack of remineralisation in the lesion subsurface.

The ^{19}F-MAS-NMR spectra confirmed the formation of fluorapatite with no precipitation of CaF_2 in Groups-2 and 3 [23]. The fluorapatite reference spectra showed a characteristic peak at -103ppm corresponding to the F-Ca(3) triangles in the apatite structure, whilst the CaF_2 reference exhibited a characteristic peak at -108ppm corresponding to the F-Ca(4) site. CaF_2 is insoluble [30]. Therefore, once CaF_2 precipitated or formed on the ARCLs, it would be detected in the ^{19}F-MAS-NMR spectra. The ^{19}F-MAS-NMR spectra obtained for Group-1 at -107.2ppm, which is close to the spectra of CaF_2. The signal had double peak in this spectrum, which demonstrated limited CaF_2. The spectrum in Group-1 was at -104.8ppm. However, Groups-1 to 4 came from the same tooth, therefore the SRD and ARC spectra are different from Group-4. ^{19}F-MAS-NMR signal between -101.0 and -107.0ppm is used to identify fluoride substitution in apatite. Gao et al (2016) showed ^{19}F-MAS-NMR chemical shift plotted against the F% in the apatite. The 100% highly fluoridated apatite (close to the stoichionetry of fluorapatite) presents at -103.5ppm chemical shift, whilst -106.7ppm chemical shift would indicate 20% fluoridated apatite or less [31]. The remaining spectra were all close to fluorapatite.

It should be noted that each technique used in this study demonstrated limitations however they complimented each other in relation to the structural changes within the ARCLs according to formation of apatite following the use of different toothpastes. In future, X-ray microtomography and Energy Dispersive X-Ray analysis would be useful to investigate the effect of these toothpastes without rinsing on subsurface of artificial/natural root carious lesions.

5. Conclusion

The results for all toothpastes were promising to form fluorapatite during the 13-day of pH-cycling. The BG with 540ppm-F toothpaste released more ions (Ca and P) and reharden the artificial root carious lesions when compared to other toothpastes. However, 1,450ppm-F toothpaste showed more formation of fluoride-substituted apatite whilst 5,000ppm-F toothpaste had more fluorapatite formation.

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Table 1. The allocated study groups

Groups	Toothpastes	Name Company	Ingredients
Group1	BG with 540ppm F	BiominF, Biomin®, UK	Glycerin, Silica, PEG400, Fluoro Calcium Phospho Silicate, Sodium Lauryl Sulphate, Titanium dioxide, Aroma, Carbomer, Potassium Acesulfame
Group2	5000ppm NaF	Duraphat®, Colgate, UK	Sodium Fluoride, Liquid Sorbitol (Non-crystallising), Silica, Silica (precipitated), Macrogol 600, Tetrapotassium Pyrophosphate, Xanthan Gum, Sodium Benzoate, Sodium Laurilsulfate, Spearmint Oil, Peppermint Oil, Carvone, Menthol, Anethol, Lemon Oil, Saccharin Sodium, Brilliant Blue FCF, Purified Water
Group3	1,450ppm NaF	Colgate-Advanced White, Colgate, UK	Sodium Fluoride, Aqua, Hydrated Silica, Sorbitol, PEG-12, Sodium bicarbonate, Aroma, Sodium lauryl sulfate, Xanthan gum, Cellulose gum, Sodium saccharin, Limonene, CI 74160, CI 77891
Group4	Deionised water	Avidity Science, UK	

Table 2. 13-day of pH-cycling cumulated group averages (and statistical data) from the demineralisation, remineralisation, and combined periods.

Groups	Mean±SE (Ca)	Statistics	Mean±SE (P)	Statistics	Mean±SE (F)	Statistics
Demineralisation						
Group1	74.41±1.13	ANOVA (p=.006), Group1 and 2 (p=0.004)	31.67±0.28	Kruskal-Wallis H (p<.001), Group1 and 2 (p=0.004), Group1 and 3 (p=0.002), Group2 and 4 (p=0.004), Group3 and Group4 (p=0.002)	0.09±0.01	Kruskal-Wallis H (p<.001), all intergroups (p<.001)
Group2	69.35±0.80		30.58±0.25		1.92±0.51	
Group3	70.75±1.12		30.57±0.23		0.44±0.06	
Group4	72.06±0.89		31.66±0.25		0.02±0.003	
Remineralisation						
Group1	57.27±1.00	Kruskal-Wallis H (p=0.139)	26.70±0.11	Kruskal-Wallis H (p<.001), Group1 and 2 (p=.002), Group1 and 3 (p=.029), Group2 and 4 (p=.002), Group3 and Group4 (p=.018)	0.12±0.01	Kruskal-Wallis H (p<.001), all intergroups (p<.001)
Group2	57.69±0.93		27.65±0.29		1.66±0.13	
Group3	57.36±0.95		27.10±0.11		0.56±0.06	
Group4	55.45±0.73		26.68±0.10		0.05±0.001	
Combined demineralisation and remineralisation						
Group1	65.84±1.05	ANOVA (p=0.277)	29.19±0.16	Kruskal-Wallis H (p=0.407)	0.22±0.02	Kruskal-Wallis H (p<.001), all intergroups (p<.001)
Group2	63.52±0.77		29.12±0.18		3.58±0.60	
Group3	64.05±1.02		28.84±0.14		1.00±0.12	
Group4	63.76±0.79		29.17±0.16		0.07±0.003	

Figure-1. Mean KHN value (SE) of root dentine following the application of different toothpastes and deionised water.

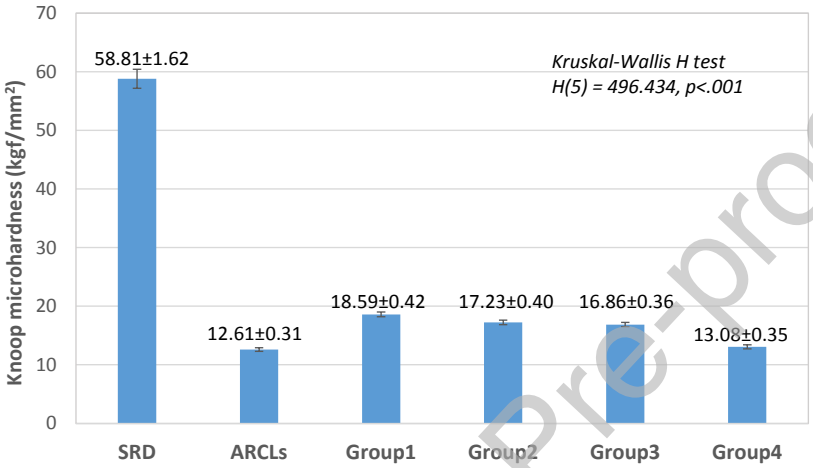
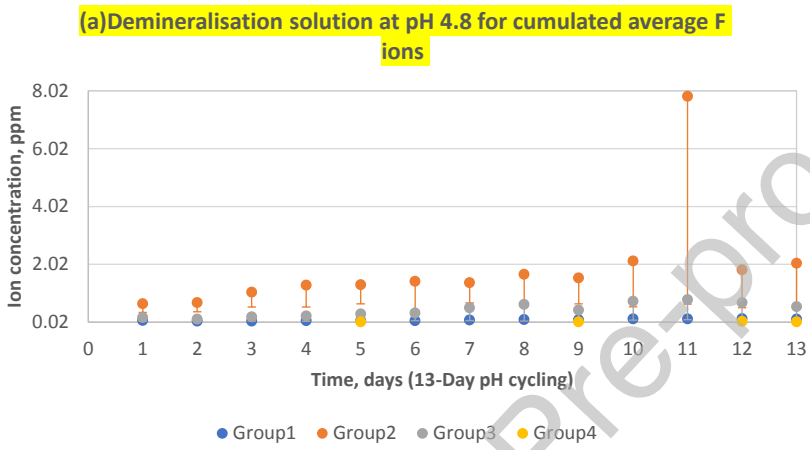
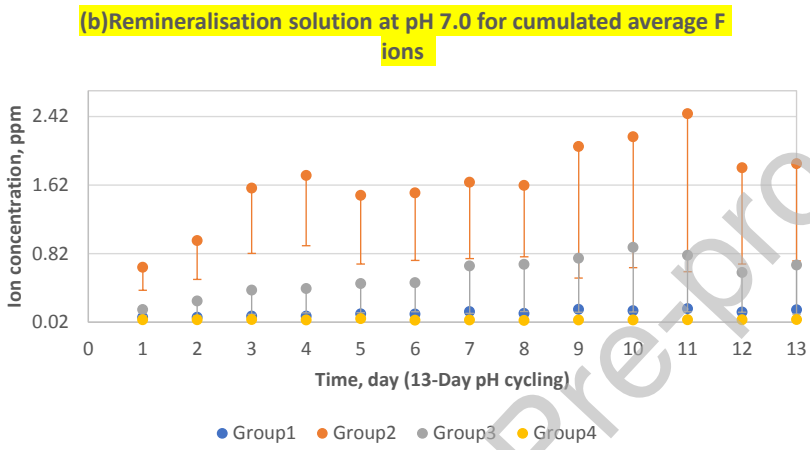
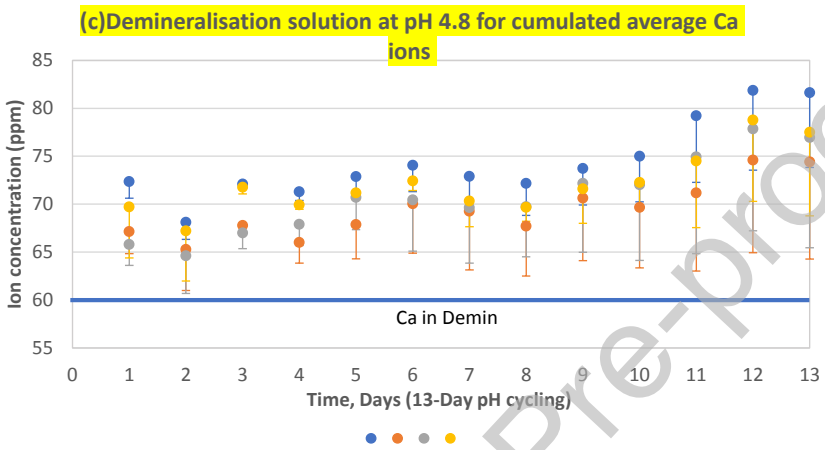
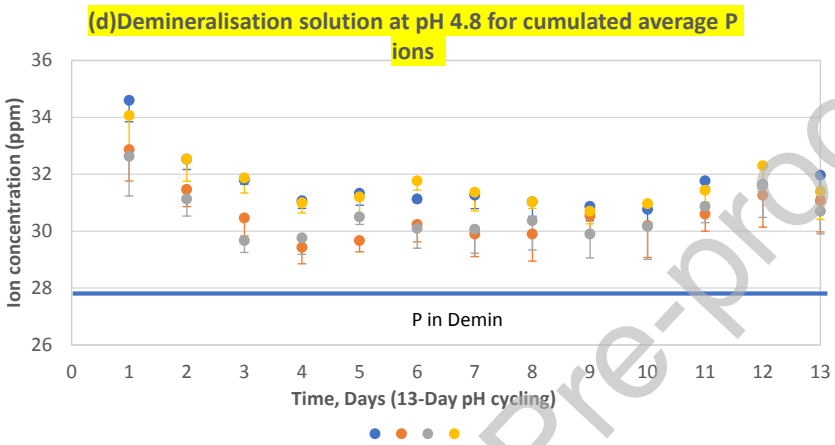


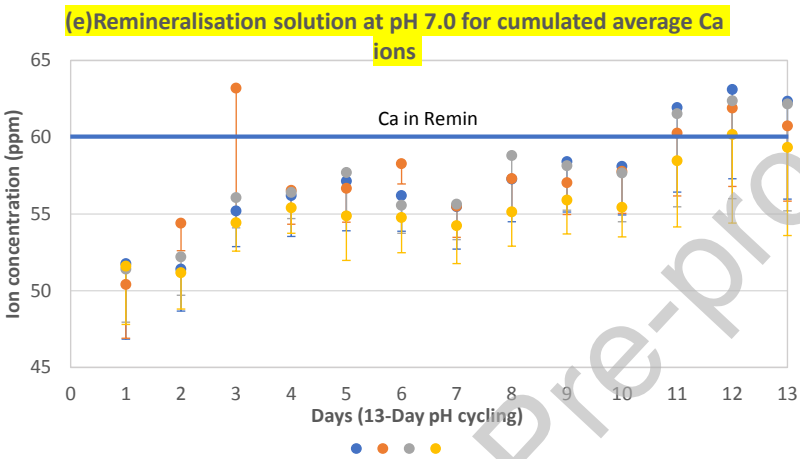
Figure-2 (a). The Fluoride ion release profile of each treatment group immersed in pH 4.8 solution (**b).** The Fluoride ion release profile of each treatment group immersed in pH-7.0 solution; Profile for **(c)**calcium and **(d)**phosphorus ion release in different toothpaste groups when immersed in pH 4.8 solution; Profile for **(e)**calcium and **(f)**phosphorus ion release in different toothpaste groups when immersed in pH 7.0 solution; Calculated profiles to **(c)** and **(e)** for **(g)**Calcium, **(d)** and **(f)** for **(h)**Phosphorus ion release in different toothpaste groups during the pH-cycling process.

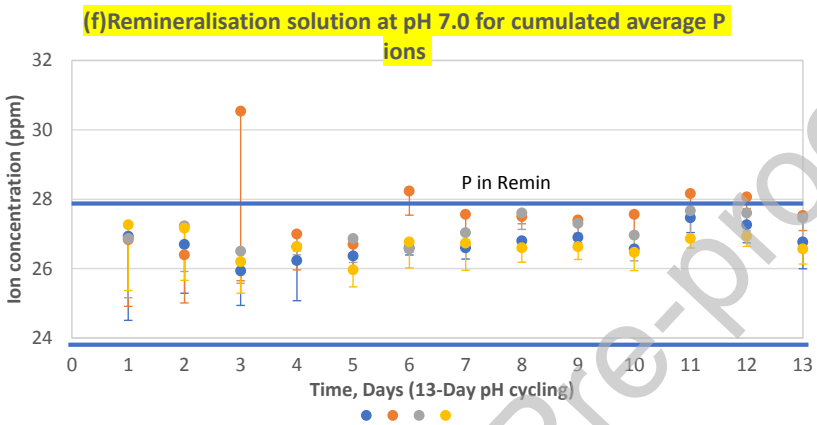


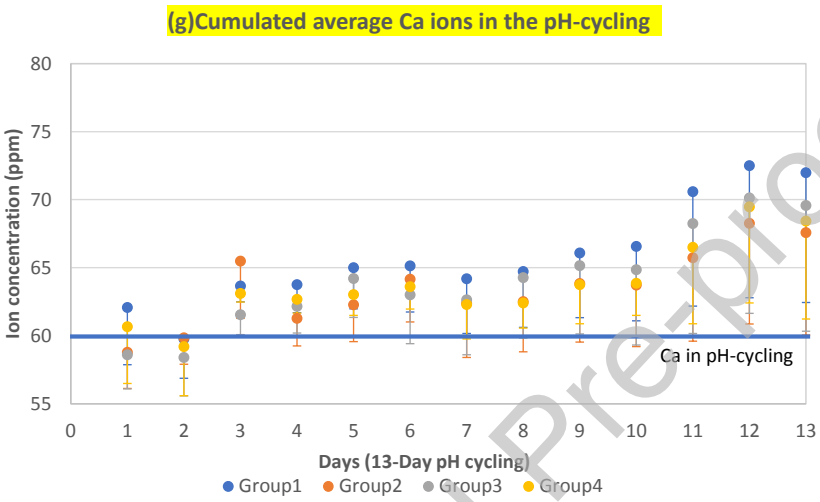












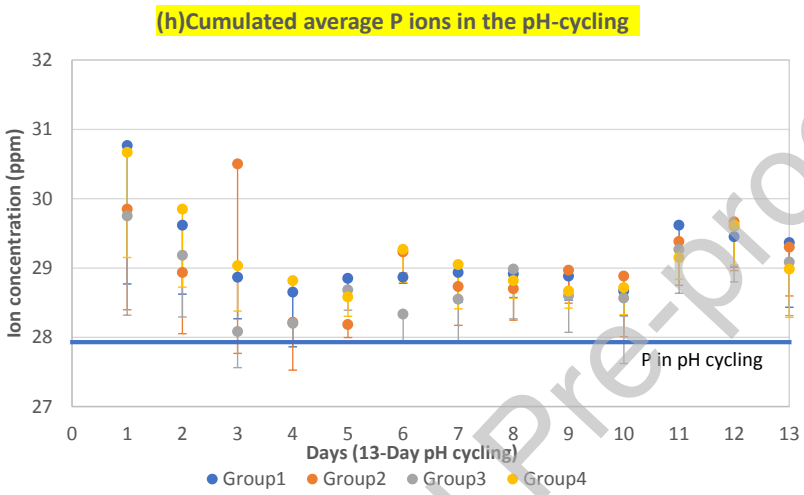


Figure-3. XRD of samples treated after 13-days pH-cycling: Sound root dentine; Artificial root carious lesions; BG with 540ppm fluoride; 5,000ppm fluoride; 1,450ppm fluoride; Deionised water. Included it the reference pattern for hydroxyapatite, note that this is almost identical to fluorapatite.

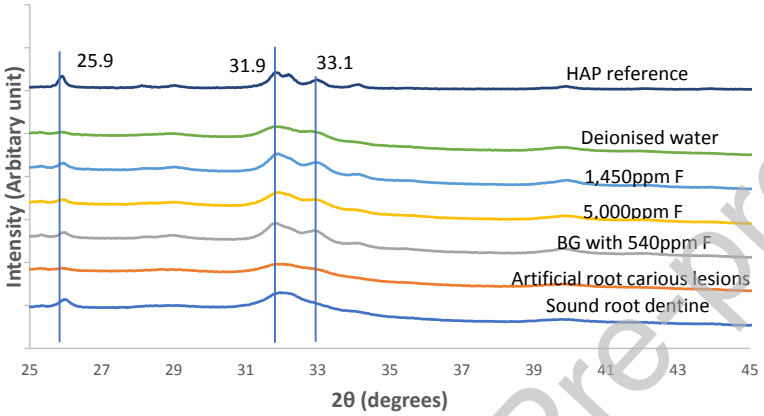


Figure-4. ^{19}F MAS NMR of samples treated after 13-days pH-cycling: BG with 540ppm-F; 5,000ppm-F 1,450ppm-F; Deionised water

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CRediT author statement

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

H. Chen and A. Baysan declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article. R. Hill has small number of shares in Biomin Technologies Ltd.