Title

Measurement of eroded dentine tubule patency and roughness following novel dab-on or brushing abrasion

Abstract

Objectives: To investigate the effect of dab-on or brushing of stannous-fluoride SnF2 or sodium-fluoride NaF dentifrice on eroded dentine tubule patency, surface and inter-tubular dentine roughness, using Confocal-Laser-Scanning-Microscopy (CLSM), Atomic-Force-Microscopy (AFM), Energy-Dispersive-X-ray-Spectroscopy (EDX), Scanning-Electron-Microscopy (SEM) and Contact-Profilometry (CP).

Methods: 75-polished human dentine samples were prepared and eroded in agitated 6% citric acid to expose patent tubules and 'initiate' DH. Samples were randomly allocated into 5 groups; artificial saliva control (1); electric tooth-brushing with NaF (2) or SnF2 (3), and dab-on application of NaF (4) or SnF2 (5). Samples underwent three cycles of interventions and acid challenges. Patent tubules, likely to cause DH clinically, were measured using validated biocomputational methods with CLSM images of dentine surfaces taken baseline and post-intervention. Randomised samples (n = 15, 20%) were investigated using AFM, EDX and SEM to study surface and sub-surface tubular occlusion. Dentine surface and inter-tubular roughness were measured using CP and AFM respectively.

Results: At baseline, mean tubule patency in all samples was 216 (SD 58) with no significant inter-group differences. Post-intervention, the mean patency was 220 (40) and 208 (35) in groups 1 and 2 respectively ($p > 0.06$), but decreased to 62 (41), 62 (21) and 63 (19) in groups 3, 4 and 5 respectively ($p < 0.0001$). Patency was confirmed using AFM, SEM and EDX. SnF2 interventions created greater sub-surface occlusion ($p < 0.01$), and CP surface roughness ($p = 0.015$). Significant negative correlation (-0.6) existed between CP surface roughness and tubule patency ($p = 0.009$).

Conclusions: Dab-on with NaF and SnF2 or brushing with SnF2 reduces DH in eroded dentine. Contacting surface roughness indicates risk of DH.

Clinical significance; Dab-on is a convenient supplementary method of dentifrice application to reduce DH; it beneficially avoids brushing post-erosion or overzealous brushing, enables re-establishment of an appropriate brushing regime post-DH and supports oral health. Significant modes of action of SnF2 in reducing DH are revealed. Finally, CP roughness measures provide indication of dentine lesions that may cause DH clinically.

Keywords

Biocomputational-method, Medical-imaging, Dab-on, Dentine-tubule, Dentine-hypersensitivity, Brushing

1 Introduction

Dentine hypersensitivity (DH) has been defined as a short, sharp pain arising from exposed dentine in response to stimuli - typically thermal, evaporative, tactile, osmotic or chemical – and which cannot be ascribed to any other dental defect or disease [1]. DH impairs oral health related quality of life [2]. One of the largest studies investigating DH in 3187 patients attending general dental practice reported a DH prevalence of 42% [3], but this proportion could be higher [4] in some populations. Cervical and occlusal tooth surfaces are often affected [4] with exposure of dentine due to tooth wear and/or gingival recession [4]. DH is becoming a greater problem, in particular due to increases in tooth wear from erosive diets, and has potential to affect more people as teeth are retained longer [5]. The timing and duration of dietary acid intake is relevant to DH presence [6][7]. In order to investigate the efficacy of methods to manage DH in the laboratory, the presence of certain sized patent dentine tubules are indicative of dentine lesions that clinically are likely to cause DH [5]. *In-situ* studies show brushing using sodium fluoride (NaF) dentifrice with a soft tooth brush and erosion, have been linked with tubule patency [8]. Laboratory studies have also shown that higher brushing forces (with a soft tooth brush and a NaF dentifrice) initiate DH itself without erosion by removing the smear layer and opening patent tubules that would lead to DH clinically [9].

As well as reduction in the amount of erosive acidic and reduction of prolonged or overzealous brushing [10], various approaches exist for management using desensitising dentifrices, albeit with limited investigation of their respective mode of actions *in-vitro*. Management approaches often use brushing application of dentifrices designed to occlude patent dentine tubules and some dentifrices may offer resistance to erosive challenge [8]. Occlusion might occur either at or below the dentine surface (‘sub-surface’ occlusion) [11]. Stannous fluoride (SnF2) has been shown to occlude dentine tubules [12] and offer resistance to acid challenge [13]. It may offer benefits in reducing erosive dentine wear [14]. However, much of the laboratory work investigating dentine tubule patency and SnF2 has been conducted using surface Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectroscopy (EDX) only [11]. Aside from tooth brushing of dentifrice, another method of application is dab-on. Dab-on may offer a supplementary and convenient method to maximize dentifrice application to tooth surfaces throughout the day, without need for additional brushing. Two clinical studies have investigated dab-on application of a strontium acetate dentifrice and shown favorable results in reducing DH [15][16]. Dab-on was achieved by massaging the dentifrice onto the area of DH for one minute [15][16]. However, these studies were clinical trials, and there are no laboratory studies to investigate the mode of action of dab-on applications in reducing DH to the author’s knowledge. One laboratory study did investigate the effects of dab-on application of strontium acetate in erosive tooth wear [17]. It found no benefit of SnF2 application to eroded dentine in reducing dentine surface loss, but it did not investigate the effects on DH [17].

A variety of methods have been developed *in-vitro* to measure dentine wear and tubule patency. Validated methods image and analyze dentine tubule patency using Confocal Laser Scanning

Microscopy (CLSM) or tandem scanning microscopy and software algorithms [18]. Such methods minimize dentine preparation and increase the sensitivity of measurement of patent tubules that have potential to cause DH clinically [18]. They also offer possibilities to measure dentine tubular patency at baseline and again after various interventions [9][18]. Furthermore, other measures of surface roughness have shown purpose in differentiating the nature of tooth wear patterns [19]. However, previous work using Non-Contacting Laser Confocal Profilometry found no correlation between surface roughness and the degree of tubule patency within a worn dentine lesion [10]. This may be due to the nature of non-contacting measurements. The relationship of Contacting Profilometry (CP) surface measures and tubule patency has not been investigated to the author's knowledge and may offer use in identifying dentine wear lesions that may cause DH clinically. Other higher resolution imaging modalities such as Atomic Force Microscopy (AFM) may also be used to investigate tubular patency and inter-tubular surface roughness in minimally prepared wet dentine [20][21].

This study aimed to investigate the effect of dab-on or electric toothbrush (with soft head) application of SnF2 or NaF dentifrice, on dentine tubule patency and surface roughness using CLSM, SEM, EDX, CP and AFM. The null hypotheses were that there was no effect of brushing or dab-on of either dentifrice on dentine tubule patency and that there was no correlation of surface roughness and tubule patency.

2 Methods

2.1 Sample and solution preparation

Ethical approval (TR467) was granted from the Tayside Biorepository Dental Tissue Access Committee (University of Dundee). Caries free human permanent teeth were obtained. Teeth were disinfected in sodium hypochlorite for a minimum of 72 h and sectioned at low speed just below the cement enamel junction using a Microslice 2 precision slicing machine (Malvern instrument 1989 No1). Tissue preparation followed previous published protocols [17]. The tooth was sectioned again at low speed approximately 2-3 mm below the last section to produce a coronal dentine portion. This tissue portion was then sectioned longitudinally to leave buccal and palatal/lingual dentine halves.

The dentine halves were embedded in self-curing bis-acryl composite (protemp4, 3M ESPE, Neuss, Germany) using a custom-made putty silicone mold to make samples. Orientation of the dentine halves occurred such that oral surfaces were uppermost at the sample surface, and dentine tubules perpendicular to the oral surface, as described previously [22]. The samples were polished flat at low speed with a water-cooled rotating polishing machine (WG2, Longitech LTD, Glasgow, Scotland) in calcined aluminium oxide slurry and washed in copious deionised water to produce areas of flatness tolerance 0.4 μm. The latter was measured with CLSM (Leica TCS SP8 MP, Leica Microsystems, Milton Keynes, UK) using a 488 nm laser light and HC PL APO CS 40x/0.85 DRY objective lens.

All polished specimens were immersed in 6% citric acid pH 2.06 for 2 minutes at room temperature, with gentle agitation of 30 revolutions per minute to cause erosion (Stuart GYRO-Rocker,

STR9, UK). Erosion removed smear layer, which has extensive effects on step height measures [23] and also served to exposure patent dentine tubules. Samples were further inspected using CLSM (Leica TCS SP8 MP, Leica Microsystems, Milton Keynes, UK) using a 488 nm laser light and HC PL APO CS 40x/0.85 DRY objective lens. Nineteen samples were excluded due to poor sample orientation and no patent tubules, or cracks. Included samples were taped to create reference areas. Then, samples were washed with copious distilled water and stored in phosphate buffering saline solution pH 7.0 until use.

Artificial Saliva (AS) was prepared and used within 24 hours following an established protocol and consisted of 10 ml of Potassium Chloride 30 mmol/l, HEPES (acid buffer) 20 mmol/l, Potassium Dihydrogen Ortho-Phosphate 4mmol/l, Calcium Chloride Dehydrate 0.7mmol/l, Magnesium Chloride 0.2 mmol/l and buffered to pH 7.0 using titrated Sodium Hydroxide [24]. For the acid, the titratable acidity following five repeat measurements of 20 ml of 6% citric acid solution was assessed with 0.1 mol sodium hydroxide using a calibrated bench top meter and electrode (Mettler-Toledo AG, 8603 Schwerzenbach, Switzerland). Mean titratable acidity was 155 ml and pH 2.00.

2.2 Experimental design

Sample size calculations were based on previously published work investigating dentine wear and dentine tubule patency [9][10][17], and calculations with an alpha level of 0.05, 80% power, and (for dentine tubule patency) mean 180 and standard deviation 50 [9][25]. The 75-dentine samples were randomly assigned to five groups (n = 15/group).

Group 1 was the control group; these samples did not undergo any dab-on or tooth brushing abrasion or exposure to dentifrice. Samples in group 1 were initially immersed in AS pH 7.0 for 2 min then rinsed in distilled water. Then, the samples were immersed in 6% citric acid pH 2.06 at room temperature for 2 min with a gentle agitation of 30 revolutions per minute using a 3D gyratory rocker (Stuart GYRO-Rocker, STR9, UK), followed by rinsing with copious distilled water.

The remaining groups 2-5 compared two dentifrice products and either electric tooth brushing or dab-on application of dentifrice on dentine eroded with 6% citric acid. Two dentifrice products were used; Crest® Decay prevention (with 0.32% NaF (1450 ppm F) control dentifrice) and Sensodyne® Rapid Relief (with SnF2 0.454%, NaF 0.072% (1450 ppm F) experimental dentifrice). Dentifrice slurries were freshly made before each use and consisted of 1-part dentifrice (330 ml) to 2-parts AS (660 ml), hand mixed for two minutes (Stuart magnetic stirrer SM1, Akribis Scientific Limited, Cheshire, UK) to ensure the uniformity of the mixture.

Samples from group 2 were immersed in the NaF dentifrice slurry and samples from group 3 were immersed in the SnF2 dentifrice slurry. Both groups were brushed for 2 minutes using separate electric toothbrushes (Oral-B® Pro2 2000 N Cross Action, Proctor and Gamble, Leicester, UK) with soft bristle round heads (Oral-B® Sensitive Clean replacement brush heads, Proctor and Gamble, Leicester, UK). Toothbrushes had calibrated force warnings at 200 g, therefore brushing forces were below 200 g. Samples were then rinsed with distilled water. This was followed by immersion in 6% citric acid for 2

min as described before with agitation at room temperature and rinsing with copious distilled water.

Samples from group 4 were immersed in NaF dentifrice slurry and samples from group 5 were immersed in SnF2 dentifrice slurry. Each sample surface was gently dabbed with a gloved (HS Gloves, Nitrile, Henry Schein®) index finger and gently massaged for 2 minutes, as per previous descriptions in the literature [15][16] and a previous laboratory study investigating dentine wear following dab-on [17]. The technique used was a gentle rotational force. The samples were then rinsed with distilled water, followed by 2 min immersion in 6% citric acid as described before.

The cycles of brushing or dabbing and/or erosion for all groups (including the control group) were repeated 3 times and the 2-minute erosive challenges were continuous. This was because work shows that the contact time of the acid to the tooth surface may have greatest influence on DH than the frequency (or number of cycles) of brushing or dietary acid [7]. The erosive challenge used in this study, measured by titratable acidity above, was also high. Brush heads, gloves, solutions and dentifrice slurries were replaced for each cycle, sample, and group to avoid cross contamination.

Samples were finally rinsed in sodium hypochlorite for 2 min, rinsed again with copious distilled water then stored in phosphate buffering saline solution pH 7.0. The subsequent experimental procedure occurred blinded.

2.3 CLSM and tubule patency

All samples were imaged at baseline and post intervention with CLSM (Leica TCS SP8 MP, Leica Microsystems, Milton Keynes, UK) using a 488 nm laser light and HC PL APO CS 40x/0.85 DRY objective lens. Gently air-dried samples were placed on a platform on the microscope for imaging. The light source was positioned over the centre of the imaging area. The adjacent protemp in the sample mount was marked to relocate the same imaging area post intervention. Images were stored as tiff files and a previously validated computer algorithm (run with Image J software, USA) was used to count the number of patent dentine tubules greater than 0.83 μm, which would likely cause DH clinically [18].

2.4 AFM

A total of 20% of samples were randomised for AFM. The protocol followed previous methods and instrumentation to investigate the dentine surface under slightly moist conditions [20].

The samples were left to minimally air-dry for twenty seconds prior to imaging. Samples were placed into the specimen area of the AFM (Digital Instruments Nanoscope III, Digital Instruments, Santa Barbara, CA, USA) and fields of view 50 x 50 μm were acquired from the centre of each experimental area. Three-dimensional images were stored and edited using AFM Software (Gwyddion 2.55, Brno, Czech Republic). The inter-tubular regions (areas in between dentine tubules) of dentine were selected from each image to perform roughness measurements using the software. This process was repeated 5 times per image and averaged.

2.5 CP

Dentine surface roughness was measured on 20% randomly selected samples. Samples were gently air dried and placed on the CP platform (Planer SF220 Surface Profiler, Planer Products Ltd., Sunbury on Thames, UK). The CP device used a diamond stylus of 20 μm tip diameter, moving along a straight line at 10 mm per min [26]. Average roughness (Ra) change per sample was obtained from five repeat measurements each taken of the intervention and baseline (taped) areas of each sample.

2.6 EDX and SEM

A total of 20% of samples were randomised for EDX and SEM. Samples were stored dry and fractured using a new scalpel blade per sample, following previously published protocols to investigate sub-surface dentine tubular patency [6]. The samples were then carbon coated and analyzed using EDX (Hitachi High Technologies) to evaluate the constituents of any dentine tubule occluding deposits (by weight). The EDX analysis involved backscattered imaging to allow spatial resolution of the analysis. The image capture time was set at 90 seconds, and spot analysis (around 2 to 3 μm) was used with spectra capture time of 90 seconds. The EDX analysis was performed at a comparable site on each sample, below the dentine surface, and was measurable using associated Wavelength Dispersive Spectroscopy Software (INCA wave, Oxford Instrument). EDX was also performed on each intervention dentifrice to confirm the nature of any occluding deposits in the samples.

The remaining half of each sample was scanned with SEM (Hitachi High Technologies) imaging. Samples were fixed to SEM pin stubs and gold sputter coated for SEM imaging. The SEM image was taken from the center of each sample. The secondary electron SEM images were captured with a magnification in the range from a few hundred up to a few tens of thousands. The accelerating voltage was set at 20 kV and filament current was 10 μA. On each SEM image, the depth of penetration of dentifrice (or amount of tubule occlusion sub-surface) was measured using image J software and a previously published computer algorithm (Image J software, USA) [11]. Mean depths of penetration were calculated for each group.

2.7 Statistical analysis

Data were analysed using a statistics package (IBM SPSS Statistics 2017, Armonk, NY, USA). Data were described using means, standard deviations and/or confidence intervals. The Data were normally distributed and were subject to a between interventions analysis of variance (ANOVA) and Tukey's post hoc testing. All statistical tests were completed with a 95% confidence interval. Pearson product-moment correlation coefficient was computed to assess the relationship between the number of patent tubules and the roughness of the dentine surface measured using AFM and CP.

3 Results

3.1 CLSM

Table 1 shows the mean patent tubules at baseline (following 2 min 6% citric acid erosive challenge) and at post interventions for all groups. At baseline, the mean patent tubules for all groups were 216 (SD 58) and there were no statistically significant differences between groups. Between baseline and post-interventions, the tubule patency for groups 3 (brushing with SnF2), 4 (dab-on NaF) and 5 (dab-on SnF2) decreased significantly ($p < 0.0001$). In particular, the mean numbers of patent tubules decreased from 238 (SD 52) to 62 (41) for group 3 (SnF2 brushing), from 200 (47) to 62 (21) for group 4 (NaF dab-on) and from 182 (47) to 63 (19) for group 5 (SnF2 dab-on). However, there were no significant changes in tubule patency between baseline and post-intervention for groups 1 (control) and 2 (NaF brushing), $p > 0.06$. At post intervention, there were statistically significantly less patent dentine tubules in groups 3 (SnF2 brushing), 4 (NaF dab-on) and 5 (SnF2 NaF dab-on) compared with both groups 1 (control) and 2 (NaF brushing), $p < 0.0001$.

Representative CLSM images for each group at baseline and post intervention are shown in Fig 1. At baseline, patent dentine tubules are visible across dentine surfaces in all groups. At post intervention, patent dentine tubules are also visible in groups 1 and 2, in similarity to at baseline. However, at post-intervention, there are fewer clearly visible patent tubules in groups 3, 4 and 5. In these groups, the dentine surfaces appear to be occluded.

3.2 AFM

Fig 2 shows AFM images from the dentine surface of representative images from each group. Group 1 (control) and group 2 (NaF brushing) show presence of patent dentine tubules. Group 2 shows a patent dentine tubule, with an adjacent uneven surface and wall. Group 3 (SnF2 brushing) show regions of dentine between occluded dentine tubules, with uneven surface or walls and some strips present. Group 4 (NaF dab-on) shows less clearly visible patent dentine tubules, with strips at the surface. Group 5 (SnF2 dab-on) showed occlusion of dentine tubules and the presence of surface strips.

There were no significant differences in inter-tubular dentine roughness measured with AFM between groups ($p > 0.5$). In numerical order, the mean inter-tubular roughness (SD) for group 1 (control) was 0.16 μm (0.04), group 2 was 0.22 μm (0.14), group 4 was 0.26 μm (0.05), group 5 was 0.26 μm (0.08) and group 3 was 0.38 μm (0.09).

3.3 CP

There was an increase in dentine surface roughness for all intervention groups between baseline and experimental areas. In numerical order, the mean change in surface roughness (SD) for group 1 (control) was 0.24 μm (0.04), group 2 (NaF brushing) was 0.27 μm (0.04), group 4 (NaF dab-on) was 0.30 μm (0.05), group 5 (SnF2 dab-on) was 0.42 (0.10) and group 3 (SnF2 brushing) was 0.47 μm (0.06). There

were statistically significant increases in roughness from baseline to post intervention for group 3 and group 5 compared with control ($p = 0.015$).

3.4 Correlation between roughness and tubule patency

There was a significant moderate negative correlation (-0.6) between the dentine surface roughness measured with CP and the number of patent dentine tubules ($p = 0.009$). A weak negative correlation (-0.4) existed between the inter-tubular roughness measured with AFM and the number of patent dentine tubules, but this was not significant ($p = 0.06$).

A weak positive correlation (0.4) existed between inter-tubular dentine roughness measured with AFM and surface roughness measured with CP but was not significant ($p = 0.076$).

3.5 EDX and SEM

Fig 3 shows representative SEM and elemental analysis taken in cross sectioned dentine for each dentifrice group (groups 2-5). The EDX analysis of occluding deposits conformed to the EDX analyses performed on NaF and SnF2 dentifrices, shown in table 2. The microanalysis instrument used cannot detect fluorine therefore Tin (Sn) and Sodium (Na) are identified.

Following NaF brushing (group 2), the SEM show patent dentine tubules, with little evidence of deposit occluding the dentine tubules. EDX confirmed Na close to the dentine surface.

Following the SnF2 brushing (group 3), the SEM image shows a dentifrice deposit above the dentine surface and slightly occluding the tubules. EDX confirmed presence of Sn within the occluding deposit. The depth of penetration was a mean 5 μm (SD 2 μm).

Following dab-on of NaF dentifrice (group 4), the SEM image shows lack of visible deposit above the dentine surface. However, there are occlusions of the dentine tubules, present sub-surface, but close to the dentine surface as plugs. EDX confirmed presence of Na within occluding deposits. The depth of penetration was a mean 2 μm (SD 2 μm).

Following dab-on of SnF2 dentifrice (group 5), there is visible occluding deposit at the dentine surface and below the surface into the dentine tubules as plugs. EDX confirmed presence of Sn within occluding deposits. Depth of penetration was a mean 8 μm (SD 2 μm).

The depth of penetration for SnF2 (groups 3 and 5) was greater than for NaF (groups 2 and 4) ($p < 0.01$). There were no statistically significant differences in depth of penetration between SnF brushing (group 3) or dab-on (group 5).

4 Discussion

This study showed that dab-on application of both SnF2 and NaF dentifrice and brushing application of SnF2 dentifrice all resulted in reduction of dentine tubule patency in eroded dentine. There was also a significant negative correlation found between surface roughness, measured with CP, and tubular patency. Therefore, we refute the null hypotheses.

A previously validated software algorithm was designed to measure dentine tubules that would likely cause DH clinically [18]. This software showed a significant reduction in the patency of dentine tubules that might cause DH clinically following dab-on applications of NaF or SnF2 and brushing of SnF2, in eroded dentine. This was confirmed using SEM images taken surface and sub-surface, that showed particulate deposits of dentifrice, and using higher resolution AFM. For SnF2 in particular, elemental analysis showed that the tin salt was able to crystallise out of solution and was detected up to 10 μm of the dentine surface following dab-on application. There were significantly more deposits of SnF2 sub-surface than NaF ($p = 0.01$). This study is unique in that the action of SnF2 is shown using CLSM, AFM, cross-sectional SEM and EDX to order to more fully understand its mode of action. Previous studies support the tubule occluding properties of SnF2 using surface SEM [13][12].

Interestingly, dab-on application (but not brushing) of NaF, resulted in a reduction in tubular patency measured, in similarity to the SnF2. This is in disagreement with previous studies, which showed increased patency of dentine tubules using SEM after dentine is brushed with the same NaF dentifrice and eroded [8]. However, in the present study, cross sectional SEM and EDX revealed sub-surface deposits of NaF dentifrice present in eroded dentine close to the dentine surface (within 2 μm, SD 2) following dab-on of NaF. Previous work has shown occluding deposits of dentifrice, otherwise soluble and not present at the dentine surface, can result in tubular plugs of dentine, sub-surface, and therefore reduce DH [11]. As reported previously, it is possible that silica itself, contained within NaF dentifrice, is a component of the dentifrice that remains within the dentine and is resistant to the acid challenges [27]. In the case of the present study, the technique of dabbing NaF dentifrice contributed itself to a reduction in dentine tubule patency. In contrast, in the NaF brushing group, dentine tubule patency did not decrease after interventions and there were no dentifrice deposits detected sub-surface in eroded dentine. Therefore, prolonged dabbing/massaging (up to 2 minutes) against eroded dentine with NaF appears to be beneficial in reducing patent tubules that might initiate DH and less likely to wear away surface deposits as reported elsewhere [17]. Further advantages of dabbing are that, in addition to a good oral hygiene and normal brushing regime, it might be undertaken more conveniently throughout the day than dentifrice application with a toothbrush. This offers benefits where brushing may be painful due to DH, in order to reduce tubule patency and enable re-establishment of an appropriate brushing regime going forwards. Likewise, it might be useful as a supplementary method of dentifrice application that avoids further or perhaps overzealous brushing applications in some clinical situations especially following erosion. Avoidance of brushing as a method of dentifrice application following erosion is necessary [17]. One difficulty with dab-on applications was standardizing the force applied to the dentine surface, which is variable. Nonetheless, this is the first laboratory study to investigate the potential and action of dentifrice application using dab-on applications to manage DH and support oral health following erosion.

The AFM revealed ‘walls’ or uneven surfaces, caused by mechanical preparations of the sample and dentifrice application, in the brushing groups. Some further ‘strips’ were also evident in both

dentifrice dab-on groups and also the SnF2 brushing group, due to contamination of the dentine surface with particulate deposits. Walls, drafts and/or strips have been described on dentine due to mechanical preparation [28]. Although this may interfere with imaging, their presence was also indicative of small particulate deposits remaining at or close to the surface. This agreed with the SEM, EDX, and CLSM observations, which showed reduction in tubular patency due to particulate dentifrice deposits at or near to the dentine surface in both dab-on groups as well as the SnF2 brushing group.

Sub-surface deposits of dentifrice were formed and detected (at various depths) using EDX following dab-on applications of both the SnF2 and NaF and following brushing application of SnF2. Previous *in-situ* work revealed dentifrice deposits up to 7 μm (SD 2 μm, range 1-9 μm) sub-surface in dentine (eroded with 6% citric acid) brushed with either 8% strontium acetate or 8% arginine desensitising dentifrices and subjected to grapefruit juice acid challenges [11]. These sub-surface deposits are likely caused by etching of the walls of the dentine tubules to the depth of dentifrice penetration as the remaining organic framework acts as nucleation sites for deposit formation [11]. The present *in-vitro* study with SnF2 produced no remarkable differences to the clinical study; sub-surface deposits were at 5 μm (SD 2) following brushing and 8 μm (SD 2) following dab-on applications. However, in contrast to the *in-situ* study, the length of acid challenge used in the present study was greater and the 6% citric acid has been shown as a stronger erosive challenge than lemon or lime juice and grapefruit [29]. Despite this, the change in erosive challenge did not appear to have a substantial difference in depth of dentifrice deposit sub-surface. This is likely related to limitations in the height of organic framework following prolonged acid challenge. Interestingly, greater penetration of sub-surface deposit was observed following dab-on as opposed to brushing with SnF2. Although this difference was not significant, it may relate to greater exposure of uneroded dentine following SnF2 brushing to eroded dentine as observed in another study [17]. Similarly, in agreement with previous work [17], surface roughness measured with CP was significantly greater following both dab-on and brushing applications of SnF2 compared with control group. This was supported by the inter-tubular dentine roughness measured using AFM and both CP and AFM showed the same order of roughness per group post-intervention. The highest roughness values were measured following brushing with SnF2 and lowest roughness values in the control group. Previous work on tooth wear attributes this finding to exposure of more uneroded dentine following brushing and inability to remove all eroded dentine following dab-on as well as the effects of the dentifrice at the tooth surface [17]. This further support an avoidance of brushing application of SnF2 post erosion.

The length of brushing (and dab-on) in this study was influenced by recommendations for the whole mouth [30], whereas an individual surface would receive a small proportion of the time. Therefore, for brushing, this study represents an oral hygiene regime over the equivalent of approximately 10-12 weeks, based on previous estimates [10]. For dab-on, clinical studies report single dab-on applications of a minimum of one minute in reducing DH [15][16].

Finally, surface roughness measured using CP, was significantly negatively related to the patency of dentine tubules. To the authors knowledge, only one other study to date has investigated correlation between surface roughness and tubular patency, but using non-contacting profilometry measures [10]. In that study limited correlation existed and it was discussed this was due to large variations in the dentifrices and the toothbrush or application regimes used on the dentine surface [10]. However, despite variations in application and dentifrices also existing in the current study, significant correlation did exist and dentine surfaces with fewer patent dentine tubules were likely to have greater surface roughness. The correlation is perhaps more likely due to the nature of the contacting measure in the present study and the presence of surface deposits on the dentine as opposed to measures of actual patent dentine tubules. This is because surface roughness, measured using CP, uses a 20 μm profilometer contacting tip, whereas patent dentine tubules (that would likely cause DH) are measured to a minimum of 0.8 μm [18]. The highest dentine surface roughness was recorded using the SnF2 groups and the EDX, SEM and AFM confirmed more particulate deposits recorded at or near to the dentine surface and less patent tubules in these groups. Taken together, these findings confirm that SnF2 has an effect on the surface nature of the dentine as reported previously [17][10] and discussed above. The results also show that surface roughness, measured with CP, provides a useful risk indicator of tooth surfaces that might cause DH. This offers use for research in investigating the efficacy of new technologies. Clinically, this might be achieved using high accuracy silicone impressions and contacting roughness measures taken from these to indicate changes in DH over time.

There were no significant correlations of inter-tubular dentine roughness measured using AFM and either the surface roughness of dentine measured using CP, or the mean patent tubules calculated from the CLSM and software algorithm. This might have been due to different sample preparations. However, the samples were minimally dried for CLSM, AFM and CP and, moreover, work has shown that dehydration of the dentine itself does not significantly affect roughness [20]. An alternative explanation is that AFM measures were taken from a relatively small inter-tubular area of the sample and are likely to be less representative of dentine surface roughness across the sample. Similarly, wider deviations in roughness measurements were reported using AFM, in contrast to CP. Despite these problems with AFM and although AFM imaging is relatively slow, it was useful in investigating individual dentine tubules and inter-tubular regions.

5 Conclusion

Gentle dab-on applications of either SnF2 or NaF dentifrice reduce the patency of dentine tubules and therefore reduce DH *in-vitro* in eroded dentine. The SnF2 dentifrice resulted in greater depths of sub-surface tubule occlusion following both tooth brushing and dab-on application. Dab-on is a useful method of reducing patent tubules that may otherwise cause DH clinically. It similarly offers use if brushing is sensitive in some areas of the tooth due to patent dentine tubules. This of course does not negate a good oral hygiene and tooth-brushing regime once DH reduces. Dab-on also offers a convenient

method of supplementary dentifrice application that avoids the need for additional toothbrushing. It may offer avoid (in some clinical situations) over brushing and abrasion. Brushing application post-acid challenge and overzealous brushing is not recommended.

Surface roughness measured with contacting profilometry is significantly negatively correlated with tubule patency in dentine lesions that are likely to lead to DH clinically. Contacting surface roughness measures taken from the dentine surface therefore provide an indication of the nature of a dentine surface lesion that may be at risk of DH.

6 References

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Table 1

Mean (SD) patent tubules measured at baseline and post intervention for all five groups; group 1 artificial saliva control, group 2 NaF brushing, group 3 Snf2 brushing, group 4 NaF dab-on and group 5 SnF2 dab-on application.

*Intra-group post-intervention values for groups 3, 4 and 5 significantly less versus baseline values ($p<0.0001$), and **inter-group post intervention values for groups 3, 4 and 5 significantly less versus post intervention values for groups 1 and 2 ($p<0.0001$).

Intervention (group)	Baseline patent tubules (SD)	Post intervention patent tubules (SD)
Control (1)	223 (64)	220 (40)
NaF brushing (2)	238 (64)	208 (35)
SnF2 brushing (3)	238 (52)	62 (41) *, **
NaF dab-on (4)	200 (47)	62 (21) *, **
SnF2 brushing (5)	182 (47)	63 (19) *, **

Table 2

EDX data taken from NaF dentifrice (groups 2 and 4) and SnF2 dentifrice (groups 3 and 5) C = Carbon, O = Oxygen, Na = Sodium, Si = Silica, P = Phosphorus, Ca = Calcium and Sn = Tin

	Element	Weight%
NaF dentifrice	C	32.46
	O	48.39
	Na	1.23
	Si	1.09
	P	4.00
	Ca	12.81
SnF2 dentifrice	C	35.08
	O	52.51
	Na	1.30
	Sn	4.26
	Si	1.29
	Ca	6.81

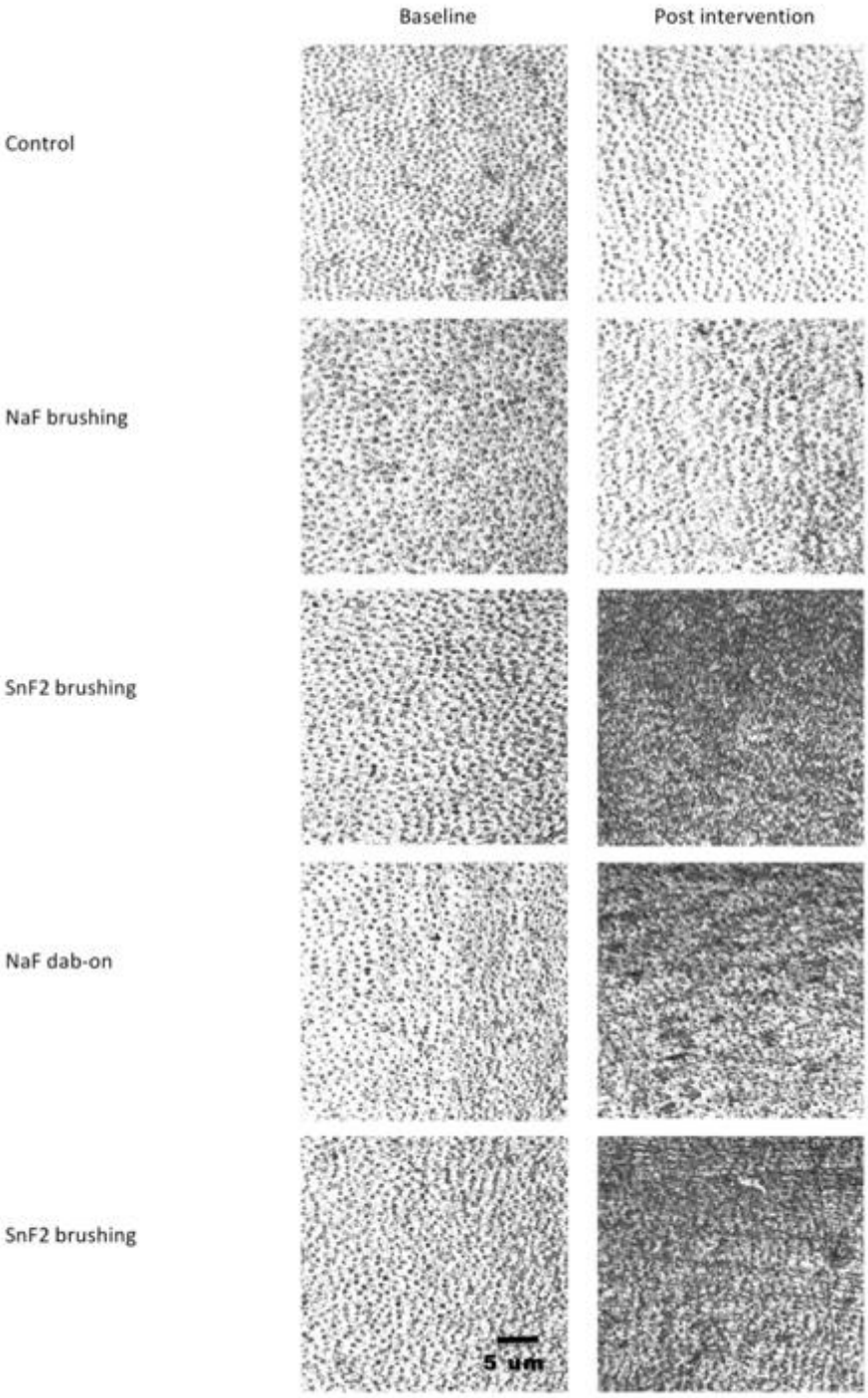

Figure 1

CLSM images of dentine surfaces at baseline and post intervention for control, NaF brushing, SnF2 brushing, NaF dab-on and SnF2 dab on applications

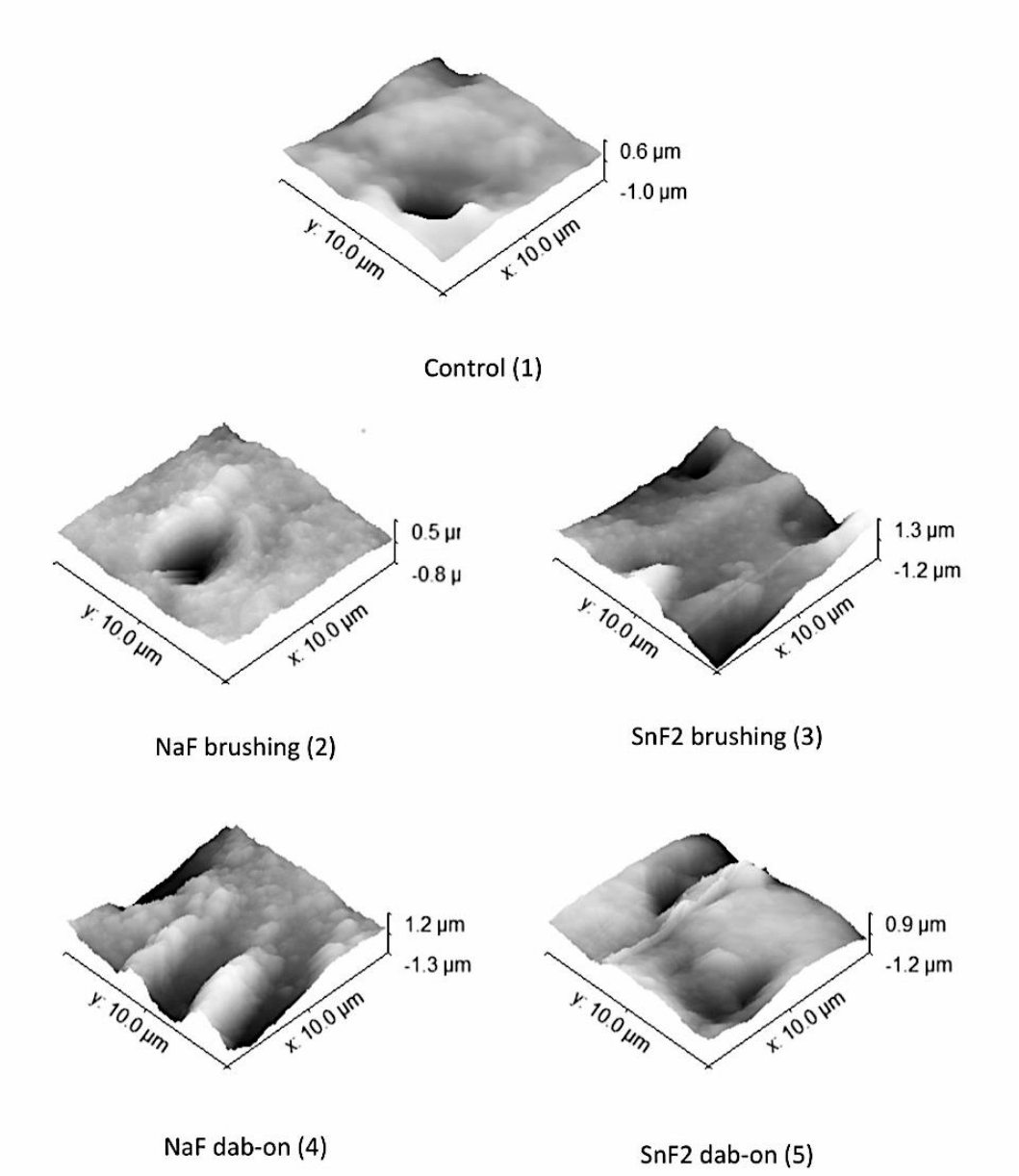

Figure 2

Three-dimensional images of dentine tubules and inter-tubular regions of the dentine surface obtained using AFM for all (groups); control (1), NaF brushing (2), SnF2 brushing (3), NaF dab-on (4), and SnF2 dab-on (5). Scan range 50 x 50 µm.

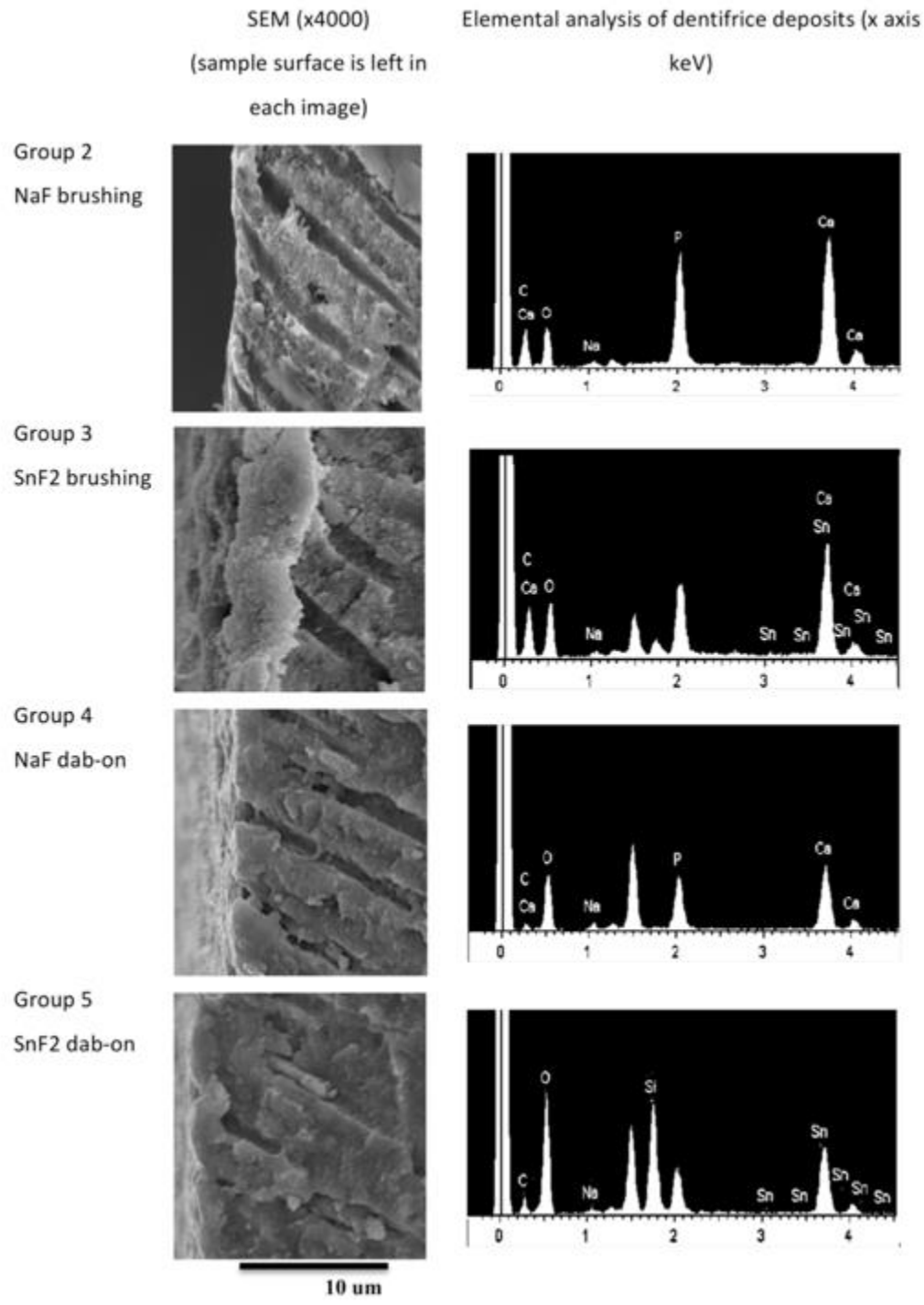

Figure 3

SEM image of dentine cross-section and EDX analysis within 10 μm sub-surface showing presence of C=Carbon, O= Oxygen, Na=Sodium, Si=Silica, P= Phosphorus, Ca= Calcium, Sn= Tin.