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Research article

Novel Confocal-Laser-Scanning-Microscopy and conventional measures investigating eroded dentine following dentifrice dab-on and brushing abrasion

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ABSTRACT

Objectives: To validate novel non-contacting Confocal-Laser-Scanning-Microscopy (CLSM) methodology with conventional Contacting Profilometry (CP) measures investigating brushing or dab-on of stannous-fluoride dentifrice on early aggressive dentine erosion.

Methods: 75 polished human dentine samples were prepared and eroded in agitated 6% citric acid then randomly allocated into 5 intervention groups; artificial saliva control (1); controlled use of a pressure sensitive counter-rotating oscillatory powered toothbrush with sodium-fluoride NaF (2) or stannous-fluoride SnF_2 (3), and dab-on application of NaF (4) or SnF_2 (5). Samples underwent three cycles of intervention and 2-min agitated 6 % citric acid challenges. CLSM images were taken and 3D reconstructions produced of step height using a developed software algorithm. In addition, 20 % samples were randomised and profiled using CP to measure step height and surface roughness. Vickers's diamond micro-hardness testing was carried out on all samples.

Results: Comparing CLSM and CP; Pearson correlation was 0.77 and Intra-class correlation 0.81 ($p = 0.01$). There were no significant statistical differences in step height between groups using both CLSM and CP. From baseline, SnF_2 brushing (3) increased micro-hardness more than control (1) ($p = 0.03$), NaF (4) and SnF_2 dab-on (5) ($p \leq 0.001$), and increased surface roughness more than control ($p = 0.02$), NaF brushing (2) and NaF dab-on (4) ($p \leq 0.017$). Dab-on of SnF_2 (5) produced rougher surfaces than control (1) ($p = 0.014$) and reduced hardness compared with NaF brushing ($p = 0.04$).

Conclusions: Good agreement and correlation exists between CLSM and CP measures in dentine. There were no significant differences in surface loss after interventions between groups. Compared with control, SnF_2 application increased dentine surface roughness and SnF_2 controlled powered brushing application increased dentine hardness, likely caused by exposure of uneroded dentine.

Clinical significance: Isosurfaces produced using CLSM can be used to represent dentine step height loss. They show good correlation and agreement with conventional CP measures, following early aggressive erosion-abrasion cycles of dentine. The CLSM and computer algorithm therefore provides an accurate, standalone and non-contacting three-dimensional measurement of early dentine wear. Stannous-fluoride brushing, and dab-on application offer no benefits following early aggressive erosion in dentine. To reduce dentine wear, limiting erosive challenges and avoiding brushing post-erosion is advised.

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1. Introduction

Tooth wear is loss of tooth structure as a result of abrasion, erosion and/or attrition, often in combination. A recent European consensus describes severe tooth wear as substantial loss of tooth structure, often with dentine exposure and affecting more than 50 % of tooth height [1]. Whether this is pathological is often related to age i.e. wear processes occurring quickly, or wear that is deleterious to the aesthetics and function, or on-going wear that creates problems of increasing complexity to clinical management [1] and sensitivity [2]. The reported increase in severe tooth wear from young to older adults in western populations is an on-going problem especially considering an ageing populace and dentition [3, 4]. A more recent systematic review estimates the overall prevalence of tooth wear in children and adolescents worldwide to be 30.4% albeit with heterogeneity between studies influenced by varied diagnostic methods to assess aetiology [5]. A large European study reported 29% of 18–35 year olds affected by a distinct tooth wear defect affecting ≥50% of the tooth surface, with significant wear likely into dentine [6]. Buccal and occlusal tooth surfaces are commonly affected [7]. The causes of wear are often dietary erosive factors [2, 6]. Despite this, many western populations report increases in dietary soft drink consumption, for example fruit, still and juice drink consumption [8]. These drinks contain dietary ingredients such as citric acid. Prevention of advanced dentine wear is important as recommended in the latest management guidelines [1] and suggestions for primary dental care practice [9, 10]. Limiting the amount and erosive potential of consumed beverages (for example by reducing the frequency and amount of consumption of beverages of a lower pH and higher titratable acidity) is essential in reducing erosive tooth wear [2]. Other suggestions include, for example, stannous-fluoride dentifrice application on eroded dentine surfaces to limit wear [11].

A variety of methods exist for measurement of tooth wear. Quantitative measures are commonly conducted by either contacting (CP) or non-contacting profilometry (NCP). These in vitro methods aid a more detailed understanding of tooth wear aetiologies, for example through investigation of an erosion or combination of erosion-abrasion. Dentine is softer and wears faster compared to enamel [12] and therefore accurate methods of measurement are important to better understand the aetiology and management of dentine wear. However, due to dentine softness and the damage caused by stylus tips, conventional contacting methods such as CP may produce large data variation [13] and could over represent the degree of dentine wear in contrast to non-contacting methods of measurement [14].

NCP can be used for measuring surface planes using a laser scanning system [15]. However, inconsistencies in surface profiling can occur due to reflection from polished surfaces. Confocal Laser Scanning microscope (CLSM) methodology is a relatively recent non-contacting method and alternative to profilometry for measuring wear of tooth specimens [15]. CLSM combines visible light with a laser and is also widely available within both universities and industry. There are however no studies to validate CLSM in human dentine with conventional measures (CP). Other studies have investigated CP, NCP and/or CLSM, but in bovine [15] and human [13] enamel. The validity of bovine measures to human tooth wear is disputed [16].

This study therefore aims to investigate early changes specifically in human dentine following an aggressive erosive challenge, using a CLSM method and compare this with conventional CP measures, in order to validate CLSM as a non-contacting and convenient method of profiling the dentine surface. In doing so, the study also aims to investigate the early protective effects of a commercially available stannous fluoride dentifrice, tested and marketed for worn dentine, using dab-on or electric tooth brushing methods of application. There is limited work investigating stannous fluoride in early dentine wear. A sodium fluoride dentifrice is used as control. Microhardness data is also obtained as further verification of surface changes in addition to the CLSM and CP surface roughness data as suggested in the literature [12, 17].

In regard to method of dentifrice application, electric tooth-brushing was adopted as it has been shown as a cause of more tooth wear in western populations clinically [6]. In addition, to the author's knowledge, the effects of dab-on (as opposed to toothbrush) application of dentifrice have not been investigated in tooth wear. From a practical perspective, if dab-on application provides benefits to eroded dentine, it could provide a useful additional method of dentifrice application to eroded dentine sites, as instructed by a dental care professional. This is because dab-on can take place reasonably conveniently throughout the day, perhaps following a meal (in addition to the normal at home tooth brushing regime), without the need for a toothbrush.

Due to potential differences in the measurement of dentine between contacting and non-contacting methods, as discussed above, the null hypothesis was that no association or agreement of CP and CLSM step height measures exist in eroded dentine. In addition, that there were no effects of brushing or dab-on of a commercially available stannous fluoride dentifrice on early erosive dentine wear.

2. Materials and methods

2.1. Sample preparation

Dentine specimens were prepared from caries free human permanent teeth. Ethical approval (TR467) was obtained from the Tayside Biorepository Dental Tissue Access Committee at the University of Dundee. Teeth were disinfected in sodium hypochlorite for a minimum of 72 h following published protocols [18] and sectioned at low speed just below the cement enamel junction using a microslice 2 (Malvern instrument 1989 No1). The resultant coronal tooth portion was then sectioned longitudinally to leave buccal and palatal/lingual halves. The sectioned dentine specimens were embedded in self-curing bis-acryl composite (protemp4, 3M ESPE, Neuss, Germany) using a custom-made putty silicone mould to make samples. Dentine sections were oriented such that oral surfaces were uppermost at the sample surface. The samples were polished flat (to produce areas of flatness tolerance 0.4 μm measured with CLSM) at low speed (Longitech, Glasgow, Scotland) in calcined aluminium oxide slurry and washed in copious deionised water. Samples were further inspected, and seven samples were excluded due to cracks.

All polished specimens were immersed in 6 % citric acid pH 2.06 for 2 min at room temperature, with gentle agitation of 30 revolutions per minute (Stuart GYRO-Rocker, STR9, UK). This process eroded and also removed smear layer, which has extensive effects on step height measures [19]. The samples were taped with PVC adhesive tape covering half of the surface (reference area). Following this, samples were washed with copious distilled water, and stored in phosphate buffering saline solution pH 7.0 until use.

2.2. Experimental design

Dentine samples ($n = 75$) were randomly assigned to five groups ($n = 15$/group). Sample size calculations were based on previously published work investigating dentine and dentine wear [20, 21], and calculations with an alpha level of 0.05, 80 % power, and (for surface roughness) mean 0.17 μm and standard deviation 0.04 μm [22].

Group 1 was the control group; these samples did not undergo any toothbrush abrasion or exposure to dentifrice. Samples in this group were immersed in artificial saliva (AS) pH 7.0 for 2 min then rinsed in distilled water. Following this, 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 (Stuart GYRO-Rocker, STR9, UK), followed by rinsing with distilled water. This cycle was repeated 3 times. Samples were stored in phosphate buffering saline solution pH 7.0 and measured using the CLSM described below. The titratable acidity following five repeat measurements of 20 ml of 6% citric acid solution was assessed with 0.1mol sodium hydroxide using a calibrated bench top meter and electrode (Mettler-Toledo AG, 8603 Schwerzenbach, Switzerland). Mean Titratable acidity was 154.5 ml, and pH was 2.06.

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 (0.32 % Sodium fluoride (1450 ppm F) control dentifrice) and a Sensodyne® Rapid Relief (Stannous fluoride 0.454 %, Sodium fluoride 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. AS was prepared and used within 24 h following an established protocol and consisted of 10 ml of Potassium Chloride 30 mmol/l, Potassium Dihydrogen Ortho-Phosphate 4 mmol/l, Magnesium Chloride 0.2 mmol/l, Calcium Chloride Dehydrate 0.7 mmol/l and HEPES (acid buffer) 20 mmol/l and buffered to pH 7.0 using titrated Sodium Hydroxide [23].

Samples in group 2 were immersed in the control dentifrice slurry and samples in group 3 were immersed in the stannous fluoride experimental dentifrice slurry and brushed for 2 min using Oral-B® Pro2 2000 N Cross Action electric toothbrush with Oral-B® Sensitive clean soft bristle round head. These toothbrushes had a calibrated force warning at 200 g forces therefore brushing forces did not exceed this value. Samples were then rinsed with distilled water. This was followed by immersion in 6 % citric acid for 2 min as before with agitation at room temperature and rinsing with copious distilled water.

Samples from group 4 were immersed in control dentifrice slurry and samples from group 5 were immersed in experimental stannous fluoride dentifrice slurry. Each sample surface was gently dabbed with a nitrile gloved (Henry Schein®) index finger for 2 min, using a gentle rotational force. The samples were then rinsed with distilled water, followed by 2 min immersion in 6% citric acid.

These cycles of brushing or dabbing and erosion were repeated 3 times. Brush heads or gloves, solutions and dentifrice slurries were replaced for each sample, cycle and dentifrice to avoid cross contamination. Samples were finally rinsed in sodium hypochlorite, rinsed with distilled water then stored in phosphate buffering saline solution pH 7.0 and measured using the CLSM described below. The subsequent experimental procedure occurred blinded.

2.3. CLSM imaging

Following tape removal and gentle air-drying, each sample was scanned across the interface between experimental and reference (baseline) areas under CLSM (Leica SP8) using a 488 nm laser light and HC PL APO CS 40x/0.85 DRY objective lens. The focus was adjusted until the interface between experimental and reference areas were observed. For each sample, the z stack consisted of 101 images size 221.43 μm × 221.43 μm and step size 0.25 μm in a format 1024 × 1024 pixels, stored as a LIF file. Step height measurements (between experimental and reference areas) and 3D constructions were measured from LIF files using a computer algorithm, summarised in the appendix (Image J Software, Fiji, USA). This algorithm was designed to measure surface position using Image J's "Plot Z-axis profile" and Array maximum finder functions, reporting the difference in Z position between manually selected regions on experimental and baseline areas, either side of the step. An experienced operator undertook the manual selection. Prior to then plotting isosurfaces using MATLAB (Mathworks®, MA, USA), raw image data were processed using ImageJ; smoothing was applied using a 3D gaussian filter (sigma = 8 in XY, sigma = 2 in Z), images were then down sized 4 times in XY and 2 times in Z, and a macro was used to threshold the image data at the surface of the reflectance images and fill in the volume below the surface.

2.4. CP measurements

Following CLSM, randomly selected samples were obtained from each group (20%, n = 15; 3 samples per group) to measure step height using a contacting profilometer (Planer SF220 Surface Profiler, Planer Products Ltd., Sunbury on Thames, UK). The samples were gently air dried and placed on the CP platform. The CP device uses a diamond stylus of 20 μm tip diameter, moving along a straight line at 10 mm per minute [24]. Step height/dentine surface loss determination was measured as the difference between baseline/reference area and the deepest point on the trace. For each sample, this process was repeated 5 times to create a mean step height. In addition, the average roughness (Ra) change per sample was obtained from five repeat measurements each taken of the intervention and baseline (taped) areas of each sample.

2.5. Micro hardness measurements

Surface micro-hardness was measured for all the samples at reference and experimental areas using a TIV (Through Indenter Viewing) Vickers diamond hardness tester (GE Measurement & Control, Groby, UK). Each sample was subjected to 5 indentations in different regions on both reference and experimental areas under a 9.8 N load and a mean calculated.

2.6. 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. Graphs and linear regression plots were produced using spreadsheet software (Excel 2017, Microsoft, Redmond, WA, USA). Statistical differences between groups were analysed using ANOVA and Tukey's post hoc testing. All statistical tests were completed with a 95 % confidence interval. Intra-class Correlation Coefficient (ICC) was used to measure agreement between CLSM and CP methods. A Pearson Correlation test was also used to measure if there was a linear relationship between CLSM and CP step height measures. Both ICC and Pearson correlation are estimates of the magnitude of relationships between variables i.e. step height differences. However, ICC also includes measures of the reproducibility or reliability between CLSM and CP measures.

3. Results

3.1. Comparison of CLSM and CP

The data for all 75 samples measured with CLSM is shown in Table 1. Of the 15 samples measured with CP, positive step heights (dentine wear) were also measured. There were no statistically significant differences in step height recorded between groups for both CLSM and CP ($p \geq 0.3$).

Comparison of the two methods (n = 15) using Pearson Correlation showed a strong positive agreement of 0.77 ($p = 0.01$). Intra-class correlation also showed a good level of reliability between the two methods of 0.81 (95 % CI 0.26, 0.94) ($p < 0.0001$). A linear regression plot of the samples compared using both methods is shown in Figure 1 for 15 samples. The gradient of the linear fit of the two measures (CLSM versus CP) is 0.68; the step height measures with the CP are larger than the CLSM. The SD values are shown on the regression plot. The agreement is good, but there are wide variations in the standard deviations in the step heights in the dentine samples using both methods.

3.2. CLSM

Figure 2 shows 3D reproductions of the eroded dentine, at the interface of the step between experimental and reference areas. A gradual and similar step is visible in all images (z axis range between 15 and 5 μm), from baseline areas (reference) at the top left of each reconstruction,

Table 1. Step height measures on all samples (n = 75) using CLSM and software algorithm.

	Step height (μm)	Standard deviation (μm)
Control (group 1)	5.05	2.10
Sodium fluoride brushing (group 2)	4.75	2.54
Stannous fluoride brushing (group 3)	8.44	3.59
Sodium fluoride dab-on (group 4)	6.14	3.66
Stannous fluoride dab-on (group 5)	8.01	4.81

to experimental area (lowest step height/wear) at the bottom right of each reconstruction. The mean (95% CI) step height recorded using CLSM (n = 75) for group 1 (control) was 5.05 (3.34, 6.77) μm; group 2 (brushing NaF control dentifrice) 4.75 (3.34, 6.16) μm; group 3 (brushing with stannous fluoride dentifrice) 8.43 (5.79, 11.09) μm; group 4 (dab-on NaF control dentifrice) 6.14 (3.92, 8.35) μm; and group 5 (dab-on stannous fluoride dentifrice) 8.00 (4.50, 11.51) μm. The largest mean step heights were recorded in dentine samples either brushed or dabbed using stannous fluoride dentifrice. The smallest mean step heights were recorded in dentine samples in the control and NaF brushing control groups. However, there were no statistically significant differences recorded between groups for all samples (n = 75) measured using CLSM ($p \geq 0.1$).

3.3. Micro hardness

Micro hardness VHN (95% confidence intervals) increased significantly ($p < 0.001$) from 43.47 (41.50, 45.43) at baseline to 50.87 (47.85, 52.29) group 1 (control), 53.07 (50.79, 55.34) group 2 (brushing NaF control), 55.60 (52.63, 58.57) group 3 (brushing with stannous fluoride), 49.73 (45.60, 53.87) group 4 (dab-on control), and 47.87 (46.93, 48.80) group 5 (dab-on stannous fluoride). Figure 3 shows the mean VHN post experiment for each group and standard deviations.

Micro hardness was significantly higher post interventions for group 3 (brushing with stannous fluoride) than group 5 (dab-on stannous fluoride) $p < 0.0001$, group 4 (dab-on NaF control) $p = 0.001$, and group 1 (control) $p = 0.03$. In addition, micro hardness for group 2 (NaF brushing) was significantly higher than group 5 (dab-on stannous fluoride) $p = 0.04$.

3.4. Surface roughness

The increase in surface roughness post-experiment was significant in all intervention groups (n = 15 sample, 3 per group) ($p < 0.008$). The mean (95% confidence interval) roughness change from baseline to post intervention for group 1, 2, 3, 4 and 5 were 0.28 (0.25, 0.32) μm, 0.31 (0.22,0.39) μm, 0.51 (0.35, 0.66) μm, 0.30 (0.16,0.43) μm and 0.46 (0.14,0.79) μm. Figure 4 shows the mean roughness change and standard deviations for each group.

There were statistically significant increases in roughness from baseline to post intervention for group 3 (brushing with stannous fluoride) compared with group 1 (control) $p = 0.02$, group 2 (NaF brushing) $p = 0.017$ and group 4 (dab-on NaF) $p = 0.011$. There was also statistically significantly more roughness increase for group 5 (dab-on stannous fluoride) compared with 1 (control) $p = 0.014$.

4. Discussion

This novel study sought to investigate the measurement of tooth wear in dentine, by comparing a conventional contacting with a novel non-contacting method. The null hypothesis is refuted, as there was good agreement and correlation between the non-contacting CLSM and CP methods. In addition, there were changes in tooth wear measurements (in particular, for surface roughness and microhardness), following various dentifrice applications (dab-on and brushing) on eroded dentine, in contrast to the erosion only control group.

Previous work has compared these measurement methods in human enamel [13]. As well as reported differences in measurement created from contacting and non-contacting methodology, enamel and dentine vary in composition in addition to morphology [12]. Therefore, the processes involved in erosion and abrasion in enamel and dentine also vary [12]. Dentine is less homogenous and therefore likely to produce wider variations in measurement across the surface compared with

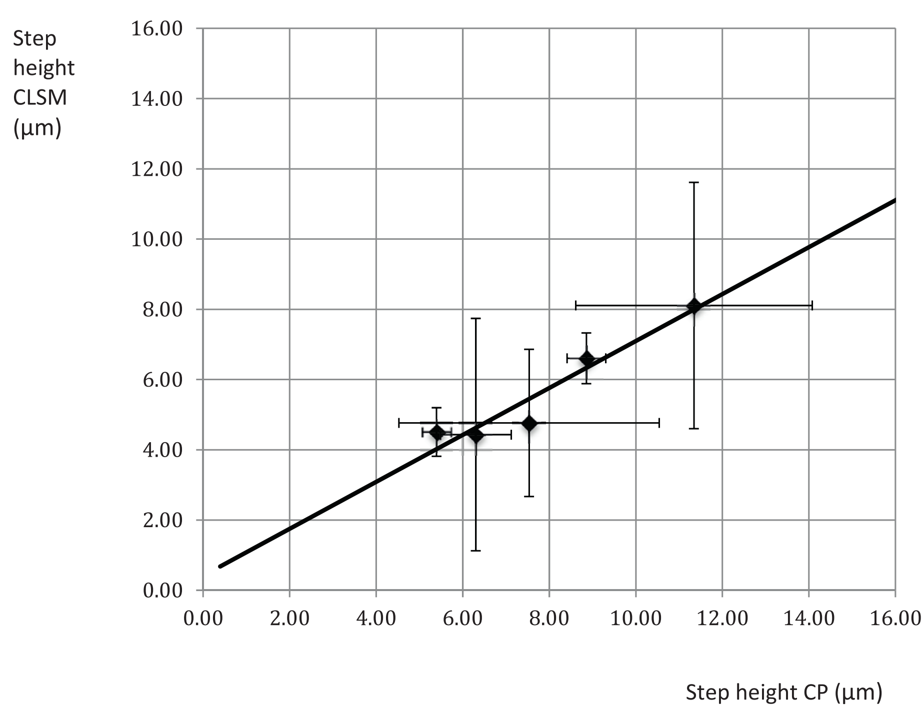

Figure 1. Linear regression analysis of step height (and SD) measured using CLSM versus CP for each group (n = 15). The line is a linear fit of the two quantities $y = m x + c$, where $m = 0.67$ and $c = 0.41$.

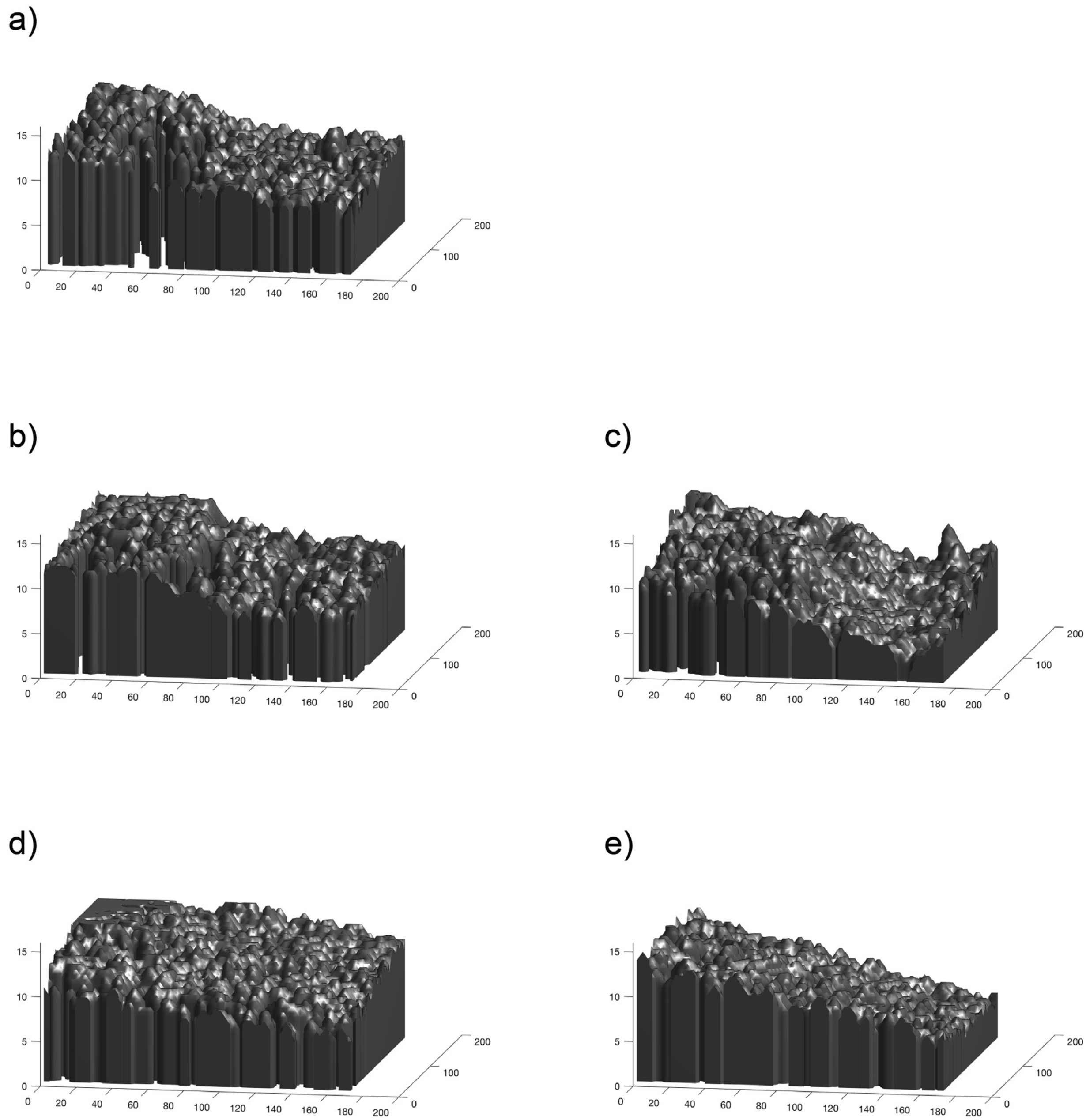

Figure 2. 3D reproductions (isosurfaces) of eroded dentine from CLSM data showing representative regions at the interface of the step between experimental (bottom right of each image) and reference (top left of each image) areas. Panels a) to e) are for 170 × 170 × 15 μm regions of image data from a) group 1 (control), b) group 2 (NaF control brushing), c) group 3 (stannous fluoride dentifrice brushing), d) group 4 (NaF control dab-on), and e) group 5 (stannous fluoride dentifrice dab-on). All axes are in unit of μm.

enamel [12]. Due to its contacting method of measurement, the step heights measured with CP were slightly larger than with CLSM. As anticipated, linear regression showed that for every 0.67 μm increase in step height measured with CLSM, the step height measured with CP increased by 1 μm. Similarly, it has been previously reported, that contacting profilometry may produce over-estimation of wear in dentine [12], with large ranges in step height measures recorded between 0.06 μm to 0.58 μm, in enamel, due to the effect of scratching by CP [13]. Despite both the variations in the surface of dentine as well as the difference in measurement between CP and CLSM methods, the present study in human dentine reports similar findings and good correlation and agreement between both measures, which were statistically significant ($p = 0.01$). This supports previous studies in both bovine [15] and human enamel [13], which each show good agreement or correlation between non-contacting and contacting profilometry methods for step height measurement. Samples were handled similarly in terms of moisture control to reduce variation [14] and all were subjected to an identical erosive challenge.

Alternatives to CLSM, such as Optical Coherence Tomography, may have clinical applications for tooth wear measures at 1310 nm [25], but their resolution is less than CLSM. Therefore, Optical Coherence Tomography has disadvantages for early erosive wear measurement. CLSM combines visible light microscope imaging with a laser, proving high-resolution, high contrast imaging. The lateral resolution is approximately 100 nm with no mechanical scanning required [26]. It can image at different planes, create three-dimensional reconstructions and be used for quantitative measurements.

Due to possible under-representation of the degree of mineral loss in dentine, it has been previously recommended that CLSM be combined with other methods such as micro-hardness or contacting profilometry

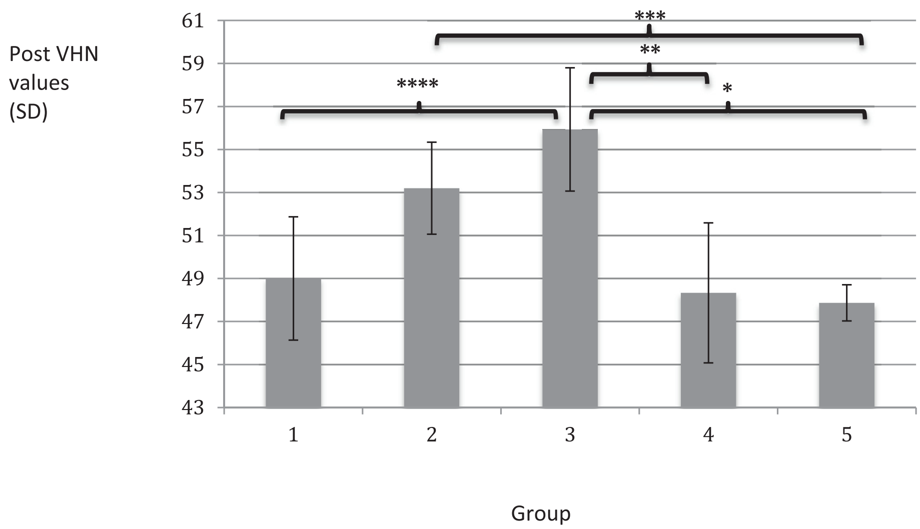

Figure 3. Mean VHN post-experiment (SD) for all 5 groups (group 1; control, group 2; NaF control brushing, group 3; stannous fluoride dentifrice brushing, group 4; NaF control dab-on, group 5; stannous fluoride dentifrice dab-on). Statistically significantly greater post-hardness in groups * 3 versus 5 ($p = < 0.0001$), ** 3 versus 4 ($p = 0.001$), *** 2 versus 5 ($p = 0.04$) and **** 3 versus 1 ($p = 0.03$).

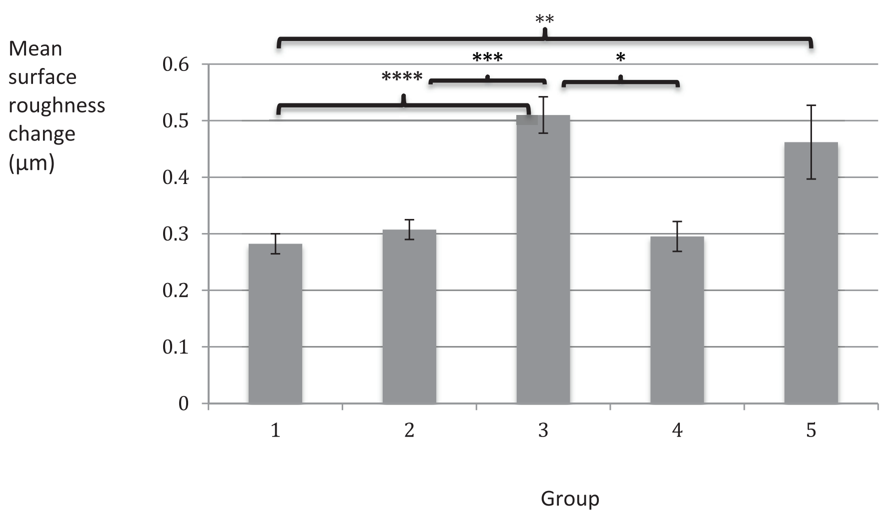

Figure 4. Mean surface roughness change (SD) for all 5 groups (group 1 control, group 2 NaF control brushing, group 3 stannous fluoride dentifrice brushing, group 4 NaF control dab-on, group 5 stannous fluoride dentifrice dab-on). Statistically significantly greater roughness changes in groups * 3 versus 4 ($p = 0.011$), ** 5 versus 1 ($p = 0.014$), *** 3 versus 2 ($p = 0.017$) and **** 3 versus 1 ($p = 0.020$).

measures, including step height and surface roughness, for early tooth wear measurement [12, 17] as verification of surface changes. Although further measures such as microhardness and surface roughness are useful, this study shows that CLSM is useful as a standalone tool for the measurement of dentine step height loss following tooth wear processes.

In regard to the wear process, this study involved an erosion regime of up to eight minutes with three two-minute dab-on or toothbrush abrasions. Agitation was used to increase the fluid dynamics of the acid challenge, which facilitates more tissue loss [19]. In addition, a 6% concentration of citric acid was used as it has been reported that juice squeezed from lemon and limes, may contain around 5% citric acid [27]. Therefore, this study represents a strong acid challenge to dentine. Previous work comparing contacting and non-contacting tooth wear measurements report longer erosion times with up to 5 h acid immersion, although in bovine as opposed to human enamel [15]. Other work in dentine has investigated six two-minute erosion challenges per day over nine days albeit with hydrochloric acid, which is a less erosive acid than citric acid [28]. The brushing or dab on application time of two minutes, was chosen based on public health recommendations for the whole mouth per day [29]. A single tooth surface would receive only a small proportion of seconds in one sitting and not the full two minutes; therefore the study represents an oral hygiene regime for 10–12 weeks, based on previous estimations [20]. Brushing occurred with controlled use of a counter-rotating oscillatory toothbrush, which had a pressure indicator warning at 200g. Brushing therefore occurred below a 200g force.

In regard to step height, there were no significant differences in measures between groups including the control group following aggressive erosion and three dab-on/controlled brushing cycles. Although not significant, the stannous fluoride group with brushing produced the greatest step height loss, an effect which the authors suppose may be exaggerated or become significant, with further cycles. Nonetheless, previous work supports the present study and has shown that larger toothbrush abrasion at forces (up to 400 g) had little effect on further wear of eroded dentine [28]. In particular, that an erosion only group produced the greatest effect on dentine wear, whereas there were no further significant differences in dentine wear with groups involving brushing in addition to erosion [28].

Moreover, in the present study, the micro-hardness of dentine increased significantly from baseline to post erosion-remineralisation cycles for all groups. This included the control group, which itself involved erosion and then artificial saliva remineralisation cycles without brushing or dab on application of dentifrice. This supports the notion, mentioned in the above previous work [28], of the abrasion resistant properties of eroded dentine following remineralisation cycles. Despite this, brushing using the stannous fluoride dentifrice produced the hardest dentine surfaces, which was highly significant compared with both control and dab-on application groups ($p < 0.001$). Taken together with the above deductions on step height, it would appear that brushing with stannous fluoride immediately post erosion would have abrasive effects on eroded dentine, by exposing or uncovering the sound or non-eroded dentine. These findings support a delay in brushing to limit wear in eroded dentine, due to the ability of dentine to re-mineralise [28]. The authors would add that it is sensible to avoid over-zealous brushing and to not brush immediately following acidic consumption to enable remineralisation.

In another previous study, a significant increase in surface roughness was observed following a 400 g (but not lower 100 g) brushing force on eroded dentine using a Novamin dentifrice, compared to the control (eroded only) dentine group [20]. That previous study also showed that a similar effect on surface roughness did not occur following brushing with a sodium fluoride control dentifrice [20]. Therefore, it was demonstrated that both the degree of brushing force and the dentifrice have effects on dentine surface roughness. The present study shows that surface roughness, measured with CP, increases significantly following both brushing (at 200 g) as well as dab-on application of a stannous fluoride dentifrice compared with control group ($p \leq 0.02$). This effect may be due to the presence of stannous fluoride dentifrice constituents at the dentine surface (i.e. due to deposits/uptake of dentifrice at the dentine surface) and the effect of the dentifrice on the dentine (due to higher abrasivity and wear of the dentine itself), as reported previously [20], with dab-on and brushing dentifrice application. Based on the microhardness findings and step height deductions, mentioned above, this effect is again likely caused by exposure of non-eroded dentine when brushing with stannous fluoride. This is also supported by the higher Relative Dentine Abrasivity (RDA) of stannous fluoride compared with sodium fluoride control dentifrice. The RDA of Sensodyne® Rapid Relief with stannous fluoride is 120, which places it higher than other dentifrices used to manage dentine hypersensitivity in one study [30]. Although a reference to the RDA value of Crest® Decay Prevention (0.32 % Sodium fluoride (1450 ppm F) is illusive in the literature, the researchers believe it to be around 90 from a commercial source (R Olley Personal Communication). As an aside, the authors note that the contents of the Sensodyne® Rapid relief dentifrice used in the present study is different to a previously marketed Rapid Relief dentifrice, which is based on Strontium [18] and has a lower RDA of 70 [30].

It may also be inferred that the up to 200 g controlled brushing force applied with the powered toothbrush is too high, as previous work showed that the lower 100 g controlled brushing force produced no increase in surface roughness [20], mentioned above. This might warrant a reduction in the pressure setting on the electric toothbrushes to a lower value of 100 g, in order to limit tooth wear. It should also be noted that manual brushing is likely to produce even wider variations than controlled power brushing, used in the present study.

In regard to the method of dentifrice application specifically, dab on application of dentifrice would reasonably not appear to be as abrasive as toothbrushing with dentifrice. Nonetheless, by its definition, abrasion is caused by physical contact of a foreign body with the surface. Therefore, placement of dentifrice by either a dab on or rubbing activity would presumably contribute abrasion to the dentine surface, due in particular, to the abrasivity of the dentifrice used. In the present study, dab-on using stannous fluoride produced the second highest step height loss after brushing, although this was not significant. In addition, dab-on application of dentifrice produced the least increase in hardness and this increase was significant compared with some brushing groups. Furthermore, dab-on with stannous fluoride produced a significant increase in roughness compared with control. Taken together, these changes may have occurred due to the effect of dentifrice at the surface and inability to remove all eroded dentine with dab on application, despite there also being step height loss. Importantly, the habit of dab on could not be as easily quantified and controlled as a pressure sensitive toothbrush. Therefore, dab on might also produce larger variations, for example, the pressure of dentifrice applied to the dentine surface clinically. Nonetheless, dab-on would not appear, from this study, to offer protective benefits for eroded dentine immediately post acid challenge.

It should be noted that aside from surface changes, this study did not investigate the effects of these dentifrices sub-surface. Such effects have been observed previously in dentine for the purpose of occluding dentine tubules and reducing dentine hypersensitivity [31].

5. Conclusions

CP measured slightly more step height loss than the CLSM methodology for eroded dentine, albeit with statistically significantly good correlation and agreement in dentine step height measures between both methods ($p = 0.01$). There were also no significant differences in step height loss of dentine between control groups and stannous fluoride applied by either electric tooth brushing (with up to 200 g force) or dab-on application measured using both CP and novel CLSM methodology in early aggressive erosion. This study shows that the CLSM and computer algorithm provide a useful non-contacting standalone tool for the three-dimensional measurement of dentine step height loss following tooth wear processes.

Surface roughness increased significantly using stannous fluoride irrespective of a dab-on or electric tooth brushing application, compared with control. This may be attributed to the uptake of dentifrice at the dentine surface as well as greater wear of the dentine itself. Although not significant between groups following wear over three cycles, the greatest step height loss was reported following brushing with stannous fluoride and the smallest increase in step height recorded following brushing with sodium fluoride. Furthermore, brushing application of stannous fluoride increased microhardness significantly compared with control, likely due to more exposure of uneroded dentine. There were no benefits of either a dab-on or brushing application of stannous fluoride dentifrice in reducing tooth wear of eroded dentine following strong acid challenges. The most ideal preventive alternative is avoidance of brushing post erosion in dentine. In addition, reduction in the amount and frequency of erosive challenge and avoidance of over-zealous brushing.

Declarations

Author contribution statement

R. Olley: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

S. Alhaji: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

B. Mohsen: Performed the experiments; Contributed reagents, materials, analysis tools or data.

P. Appleton, G. Chadwick, G. Ball: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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