EFFECT OF FLUORIDE AND ABRASIVES ON ARTIFICIAL ENAMEL CARIES LESIONS

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DEDICATION

To my dear wife “Doaa” for her love, support, and encouragement. To my son “Mohammad” for his smiles and laughs. To my Mom for her prayers and who encouraged me to pursue this degree. Last, I dedicate this degree to my late father “Mohammad Nassar”. I only wish that you were here to witness it. Thank you all.

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ABSTRACT

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Hypothesis: The interaction between the abrasive level and fluoride concentration of dentifrice slurries modulates the surface loss (SL) and remineralization of incipient enamel caries (IEC). Methods: Three types of IEC were created and six experimental slurries with different combinations of fluoride content and abrasive level were tested. In experiment 1, the three IEC were subjected to brushing (with experimental slurries) and remineralization cycles for 5 days. Fluoride concentrations (0 and 275 ppm as NaF) and abrasive levels (Low and High) were tested. SL was determined by optical profilometry at baseline and after 1, 3, and 5 days. In experiment 2, changes in IEC mineral content ($\Delta(\Delta Z)_C$) and depth (ΔL_C) were investigated at baseline and after the 5-day cycling with transverse microradiography. In experiments 3 and 4, SL of MeC and CMC lesions were further studied, respectively; testing not only fluoride concentration (275 and 1250 ppm as NaF) and abrasivity (low and high) of the slurry, but also the brushing frequency (1x, 2x, and 3x/day). Brushing-remineralization cycles were performed for 7 days. Statistical analyses were performed at 5% significance level. Results: Experiment 1: overall, brushing with the high-abrasive slurry caused more SL than with the low-abrasive. For CMC and MeC lesions, 0 ppm F had more SL than 275 ppm F only after day 3. Fluoride had no effect on the SL of HEC lesions. Experiment 2: fluoride and abrasives did not have a significant effect on IEC. HEC had significantly lower $\Delta(\Delta Z)_C$ than CMC and

MeC, with CMC and MeC not differing from each other. Lesion type had no effect on ΔL_C. Experiment 3: brushing CMC lesions 3x/day with 1250 ppm F increased SL compared to 1x/day, after 5 and 7 days. Study 4: brushing MeC lesions with high abrasive slurry containing 1250 ppm F increased SL after 5 and 7 days. Conclusions: The IEC tested showed different SL and remineralization behaviors. The fluoride content and abrasive level of the toothpaste showed to be relevant modulating the SL of enamel caries lesions as well as their remineralization behavior.

Anderson Hara, DDS, MS, PhD, Chair

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INTRODUCTION

Dental caries is the most prevalent infectious disease affecting humans [Al-Malik et al., 2001] and its treatment at advanced stages exhausts large amounts of resources per year [Baelum et al., 2008]. It starts with the metabolism of sucrose by bacteria present in the dental plaque, producing acids as byproducts [Kleinberg, 2002; Marsh, 1995]. As a result, plaque fluid becomes unsaturated with respect to the tooth structure, leading to demineralization and development of incipient caries lesions [Cury and Tenuta, 2009]. Although these lesions have been didactically described as subsurface demineralization with a mineralized surface layer, their mineral distribution profile is largely unknown and we suspect that it may vary according to different clinical factors, including location, stage of development, progression speed, and remineralization level among others. At early stages of the caries process, there is no need for surgical intervention and the treatment usually consists of elimination of causative factors (plaque and fermentable carbohydrates) and increasing the enamel remineralization measures.

An important and widely used approach for managing early caries lesions is the use of fluoride products [Buchalla et al., 2002; Ekstrand and Oliveby, 1999; Ten Cate, 1990]. Fluoride has shown the ability to enhance the remineralization of demineralized enamel [Ogaard and Rolla, 1992; Ten Cate and Mundorff-Shrestha, 1995]. Fluoride-containing products include gels, rinses, varnishes and dentifrices; with the latter standing out due to their wide-spread use and easy access to the population [Ripa, 1991]. Brushing with fluoridated dentifrices has shown to be effective in preventing dental caries [Marinho et al., 2003]. Several studies have demonstrated the ability of fluoridated

dentifrices to enhance mineral gain and surface hardness of the soft demineralized enamel, in laboratorial and clinically relevant conditions [Joyston-Bechal and Kidd, 1986; Wang et al., 1993; White, 1988]. In addition, fluoride has been shown to play a role in the arrestment of incipient caries lesions in vivo [Holmen et al., 1987a]. In pH cycling studies, fluoride-containing dentifrices have been effective in remineralizing enamel lesions with a direct dose-response effect [Ten Cate et al., 2006; Thaveesangpanich et al., 2005]. A similar dose-response effect on the prevention of enamel lesions has been demonstrated clinically [Marinho et al., 2003]. However, a minimal concentration of 500 ppm fluoride was reported to be required to protect enamel from demineralization using dentifrices [Hellwig et al., 2010]. Further, Wefel et al. [1995] found higher mineral gains in white spot lesions exposed to increasing levels of fluoride in situ. These findings are supported by clinical trials that demonstrated the ability of fluoride to prevent and control dental caries [Marinho et al., 2004]. In addition, exposure to fluoridated dentifrices was effective in rehardening softened enamel lesions produced by acid challenges both in vitro [Ganss et al., 2001] and in situ [Ganss et al., 2004; Zero et al., 2006].

Besides fluoride delivery, the toothbrushing procedure disrupts and removes dental plaque, reducing or eliminating the acid production by bacteria [Ainamo, 1971]. This effect results mostly from abrasives present in dentifrice formulations [Joiner et al., 2002; Stookey et al., 1982]. Abrasives are needed not only to achieve cleanness but also to remove extrinsic stains from tooth surfaces [Joiner et al., 2002; Stookey et al., 1982]. The abrasive effects of toothpaste on the dental hard tissues was first reported by Miller in 1907 [Miller, 1907]. Further research has showed that dentin is more prone to

toothbrushing abrasion compared to enamel [Davis and Winter, 1980]. Brushing without toothpaste has negligible effects on the integrity of sound enamel since the hard tissue loss is mainly attributed to the abrasive content of the dentifrice [Absi et al., 1992; Sangnes, 1976]. Negligible effects are also expected when brushing intact enamel with currently marketed dentifrices, as observed in multiple studies [Addy and Hunter, 2003; Hooper et al., 2003; Philpotts et al., 2005]. However, the effect of toothpaste abrasives on the softer, less mineralized early caries lesions has not been fully investigated yet.

A previous study has shown that the wear resistance of erosion-softened enamel is significantly reduced [Attin et al., 1997]. Similarly, incipient lesions tend to have softer surfaces [Arends et al., 1987] and lower mechanical properties [Arends and Christoffersen, 1986] compared to sound enamel. Kielbassa and colleagues [2005] showed that surface loss (SL) values of enamel caries lesions were twice as high as those of sound enamel; indicating that lesion surface layer removal could take place under the effect of toothpaste abrasives. However, the effects of different mineral profiles of the lesion and the interaction between fluoride and abrasives remain unknown. These are important factors for the arrestment of incipient caries [Cury and Tenuta, 2009; Fejerskov et al., 2008], as we hypothesize that the dynamics of remineralization and enamel surface loss during toothbrushing with fluoridated dentifrices can be modified by its abrasive potential.

It has been suggested by some investigators that the reversal of early caries lesions may be related to their mechanical removal by abrasive forces [Artun and Thylstrup, 1989; Cury and Tenuta, 2009; Fejerskov et al., 2008]. Furthermore, the interaction between the remineralization effect of fluoride and the surface abrasion

produced by abrasives is still unknown. Modulating factors, such as brushing frequency, length, and force can be equally important and deserve consideration.

In general, toothbrushing abrasion is time-dependent and is influenced by brushing frequency and duration as well as brushing force [Addy and Hunter, 2003]. In their systematic review, Marinho and collaborators concluded that the anticariogenic effect of fluoridated dentifrices increases with the higher frequency of use [Marinho et al., 2003]. In addition, the force applied during brushing is considered an important factor in the abrasive process [Saxton and Cowell, 1981]. The use of forces of approximately 200 grams is not uncommon during testing tooth wear in vitro. Ganss and colleagues [2009] reported a mean brushing time of 90 seconds. In addition, brushing times between 30 and 180 seconds were associated with a significant increase in surface rehardening of early caries lesions in situ [Zero et al., 2010].

Little information exists on the micro-morphology and mineral composition of incipient lesions. This limits any attempt for their reproduction under in vitro conditions. Therefore, one of the most important factors when studying early caries lesions in vitro is the clinical relevance of the created lesions. There are a number of protocols available [Buskes et al., 1985; Kielbassa et al., 2005; Lynch et al., 2007; Ten Cate et al., 1996; Zhang et al., 2000] with differences in their duration, presence of alternate cycles of demineralization and remineralization, type of acid, type of vehicle for the acid (solution or gel), pH of the solutions, type of buffers, and mineral content. This leads to enamel lesions that differ greatly in their depth, mineral profile, and chemical composition [Arends et al., 1987; Lynch and Ten Cate, 2006]. These characteristics most likely have a direct impact on the remineralization and surface abrasion.

In the present research project, it was hypothesized that the clinical reversal of early caries lesions results from an interaction of factors, involving toothbrushing parameters, dentifrice composition, and structural characteristics of early caries lesion. Hence, our main objective was to investigate the interplay between fluoride and dentifrice abrasives on the SL of in vitro-created enamel lesions, in order to better understand the dynamics involved in the arrestment of incipient carious lesions. In an attempt to capture the possible clinical differences in the mineral distribution of the lesions, three distinct laboratorial protocols were adopted for testing.

We hypothesized that the presence of fluoride would protect against the abrasive effect, regardless of the type of lesion studied. At the same time, we assumed that brushing frequency could modulate this interaction. In addition, we aimed to test the effect of fluoride and abrasive content of dentifrices on the remineralization of early caries lesions. Characterization of depth and mineral content of lesions produced via different protocols was considered as a secondary objective.

This project included four studies (Table 1). The hypotheses were tested with the following specific aims:

Specific aim 1: Investigate the combined effect of fluoride and abrasives on the surface loss of enamel lesions. Hypothesis: Fluoride yields a protective effect on early caries lesions against abrasion; whereas, higher abrasive content increases the surface loss.

Specific aim 2: Determine the effect of fluoride and abrasives on the remineralization behavior of three lesion types with different mineral profiles. Hypothesis: Fluoride enhances the remineralization of caries lesions.

Specific aim 3: Determine the effect of brushing frequency and dentifrice slurry on the surface loss of carboxymethylcellulose lesions. Hypothesis: Increasing the brushing frequency (and fluoride exposure) affects the abrasion behavior of carboxymethylcellulose lesions.

Specific aim 4: Determine the effect of brushing frequency and dentifrice slurry on the surface loss of methylcellulose lesions. Hypothesis: Increasing the brushing frequency (and fluoride exposure) affects the abrasion behavior of methylcellulose lesions.

MATERIALS AND METHODS

Study #1: Dentifrice fluoride and abrasivity interplay on surface loss of artificial caries lesions

Specimen preparation

Enamel slabs (5 x 5 mm) obtained from bovine teeth free from white spots, cracks, and other defects were used in this study (Figure 1). After collection and during the preparation process, the teeth were stored in 0.1% thymol solution. The bottom and top (enamel) sides of the slabs were sequentially ground flat using silicon carbide grinding papers (Struers RotoPol 31/RotoForce 4 polishing unit, USA; Figure 2). A uniform thickness of approximately 2 mm was created. Slabs were then embedded in acrylic resin (Varidur acrylic system, Buehler, USA) utilizing a custom-made silicon mold, leaving the enamel surfaces exposed. The embedded blocks were serially ground and polished up to a 4000-grit grinding paper followed by 1-µm diamond polishing suspension.

Lesions creation

Adhesive UPVC tapes were used to cover the enamel surface of each specimen leaving a 2 x 5 mm central area exposed (Figure 3). One hundred and twenty specimens were randomly assigned to 3 groups, according to the lesion type and submitted to one of the three demineralization protocols (Table 2):

1) Methylcellulose (MeC) acid gel (modification of the method by ten Cate et al. [1996]): 5% methylcellulose covered with an equal volume of 0.1 M lactic acid. KOH was used to adjust the pH at 4.6 and specimens were demineralized for 7 days.

2) Carboxymethylcellulose (CMC) solution (as described by Lippert et al. [2011]): specimens were demineralized for 10 days in a solution containing 0.1 M lactic acid, 4.1 mM Ca (as $CaCl_2 . 2H_2O$), 8 mM PO_4 (as KH_2PO_4) and 1% w/v carboxymethylcellulose (Sigma-Aldrich, USA), pH adjusted to 5.0 using KOH at 37°C.

3) Hydroxyethylcellulose (HEC) gel (modification of the method by Amaechi et al. [1998]): specimens were immersed into a hydroxyethylcellulose gel prepared using a pH 4.5 KOH-adjusted solution of 0.05 M lactic acid at a ratio of 140 g HEC per liter of lactic acid solution. The specimens were demineralized for 7 days at 37°C.

For all demineralization protocols, the demineralizing agent was not stirred or replaced throughout the demineralization period.

Surface loss (SL) measurement

SL was measured using an optical profilometer (Proscan 2000, Scantron, England; Figure 4) after the creation of the lesions as well as after 1, 3, and 5 days. Tapes were removed from the specimens and an area of 3 x 1 mm in the center of the specimen (covering both exposed and tape-covered areas) was scanned. The SL was calculated by subtracting the height of the exposed area from the 2 reference (tape-covered) areas (Figure 5). Dedicated software (Proscan 2000, Scantron) was used. Profilometric analysis readings from each treatment group were used as baseline for subsequent SL analyses

after abrasion process. In addition, baseline measurements were used for balanced randomization of the specimens into the experimental groups for each lesion type.

Daily brushing-remineralizing procedure

Toothbrushing abrasion was conducted using an automated custom-made brushing machine (Oral-B 40 toothbrushes; Figure 6) under 150 g of force [Ganss et al., 2009]. Four slurry variants (Table 3) were used with two levels of abrasives (low, REA=4.01±0.79/RDA=69.24±7.40 and high, REA=7.14±1.96/RDA=208.03±26.57) with 275 ppm fluoride as NaF (representing 1100 ppm F of regular toothpaste at a ratio of 1:3) or without fluoride (0 ppm F). Abrasive slurries were prepared by mixing the ingredients above with an aqueous suspension containing 0.5% (w/w) Blanose 7MF carboxymethylcellulose (CMC) and 10% (w/w) glycerol (Table 1). Sixty grams of the slurry were used in each slot of the brushing machine.

Specimens (n=10) were brushed with their respective assigned slurry for 50 strokes (15 seconds) and then stored in artificial saliva (0.213 g/L of $CaCl_2.H_2O$, 0.738 g/L of KH_2PO_4, 1.114 g/L of KCl, 0.381 g/L of NaCl, 12 g/L of tris buffer, and 2.2 g/L of gastric mucin) for 4 hours with stirring under 150 rpm. After that, the specimens were brushed for an additional 50 strokes after which they were stored in artificial saliva overnight. The brushing protocol was run for 5 days and the specimens were subjected to 500 brushing strokes in total.

Statistical analysis

Analysis of variance (ANOVA) was used to test the effects of lesion type (MeC, CMC, HEC), slurry abrasiveness (low, high), slurry fluoride (0 ppm F, 275 ppm F), and time (1, 3, and 5 days) on SL. An unstructured variance/covariance matrix was used to model the variances and correlations within a specimen over time. Pair-wise comparisons among the treatment combinations were made using Tukey's multiple comparisons procedure to control the overall significance level at 5%. The analyses were performed after a natural logarithm transformation of the data to satisfy the ANOVA assumptions.

Study #2: Effect of fluoride and abrasives on the remineralization of in vitro incipient caries lesions

Microradiographic analysis

Specimens from the first study were mounted on plastic rods and sectioned with a hard tissue microtome (Silverstone-Taylor Hard Tissue Microtome, Series 1000 Deluxe, USA; Figure 7). Two 100 μm sections were obtained from each specimen; one section was acquired through the baseline demineralized area and the other one was obtained from the demineralized area that was subjected to toothbrushing (Figure 8). Sections were mounted on microscope slides along with an aluminum step wedge and subjected to x-ray (Figure 9). X-rayed specimens were analyzed with a dedicated software program (Inspektor TMR 2000, ver.1.25; Figure 10) with sound enamel defined at 87% mineral volume to get two parameters; overall mineral loss (ΔZ) and mean lesion depth (L;

Figure 11). For each specimen, the difference in these terms between the two sections (baseline and after abrasion) was obtained as $\Delta(\Delta Z)$ and ΔL (Figure 12).

Since this project involved structural loss due to toothbrushing abrasion, a modification of the TMR parameters had to be undertaken to compensate for SL (Figure 13). This produced two additional terms: corrected change in mineral loss of the lesion ($\Delta(\Delta Z)_C$) and corrected change in depth of the lesion (ΔL_C) calculated using the following equations:

$$\Delta(\Delta Z)_C = \Delta Z_{base} - (\Delta Z_{post} + SL \times 87)$$

$$\Delta L_C = L_{base} - (L_{post} + SL)$$

Statistical analysis

Analysis of variance (ANOVA) was used to test the effects of lesion type (MeC, CMC, HEC), slurry abrasiveness (low, high), and slurry fluoride (0 ppm F, 275 ppm F) on $\Delta(\Delta Z)_C$ and ΔL_C. Pair-wise comparisons among the treatment combinations were made using Tukey's multiple comparisons procedure. To test changes in the mineral content within each group, a paired t-test was used, comparing ΔZ_{base} and ΔZ_{post}. A 5% significance level was used for all statistical tests.

Study #3: Effect of brushing frequency and dentifrice slurry on the surface loss of carboxymethylcellulose lesions

Experimental design

Overall, a similar design to study 1 was used with some modifications. Bovine enamel slabs measuring 5 × 5 mm were used and lesions were created using CMC with

a sample size of 10 per group. Baseline SL measurements were obtained and used for stratified randomization. Toothbrushing abrasion and remineralization were conducted for 7 days. Four slurry variants (Table 4) were used with two levels of abrasives (low, and high) with 275 ppm fluoride as NaF (representing 1100 ppm F of regular toothpaste at 1:3 ratio) or with 1250 ppm fluoride (representing 5000 ppm F of prescription toothpaste). In addition, a third factor, brushing frequency, was tested. Each fluoride-abrasive combination was allocated into three groups that were brushed either once (1x), twice (2x), or three times (3x) per day with the assigned slurry. SL measurements were obtained at days 1, 3, 5, and 7.

Statistical analysis

Analysis of variance (ANOVA) was used to test the effects of abrasive content (low, high), fluoride level (275 ppm F, 1250 ppm F), brushing frequency (1x, 2x, 3x per day), and brushing time (1, 2, 3, 5 and 7 days) on SL. An unstructured variance/covariance matrix was used to model the variances and correlations within a specimen over time. Pair-wise comparisons among the treatment combinations were made using Tukey's multiple comparisons procedure to control the overall significance level at 5%.

Study #4: Effect of brushing frequency and dentifrice slurry on the surface loss of methylcellulose lesions

Experimental design

The protocol of the third study was used to test the effect of the experimental factors (fluoride content, abrasive level, brushing frequency, and time) on MeC lesions. Lesions were created in 5 × 5 mm bovine enamel slabs by immersion in MeC gel for 10 days. Specimens were randomly allocated to groups (n=8) in a similar design to the third study. Brushing/remineralization cycles were conducted for 7 days and SL measurements were obtained at days 1, 3, 5, and 7.

Scanning electron microscopy

To visualize the surface layer, specimens were imbedded in epoxy resin (EpoThin, Buehler, USA) and then sectioned using a hard tissue microtome. The sectioned surface of each specimen was polished using a series of sand papers up to 4000-grit under de-ionized water irrigation, followed by the application of 37% phosphoric acid for 30 seconds. Sections were washed thoroughly with deionized water under air pressure and placed in a dissector for 2 days. Samples were then sputter coated (Denton Desk II, USA) with a gold target for 75 seconds at 40 micro-amperes to produce a coat of approximately 100 angstroms. They were mounted on aluminum stubs with carbon tape and then had their sides painted with conductive colloidal silver paint. The samples were analyzed under high vacuum with 20 kV of accelerating voltage at a working distance of 10 mm and 58 micro-ampere current (JEOL 5310 LV, Japan).

Statistical analysis

An unstructured variance/covariance matrix was used to model the variances and correlations within a specimen over time for the four experimental factors (fluoride content, abrasive level, brushing frequency, and time).

RESULTS

Study #1

Results of statistical tests are summarized in Tables 5 and 6. For MeC, Day 5 had more SL compared to Day 1 at all comparison levels ($p<0.0001$; Table 5). Day 5 had significantly more SL than Day 3 ($p<0.00001$), with a larger difference for high abrasive level. Day 3 had significantly more SL than Day 1 ($p<0.0001$); with a larger difference for high abrasives and for 0 ppm F.

For CMC, Day 5 had significantly more SL than Day 1 ($p<0.0001$). Day 5 had significantly more SL than Day 3 with high abrasive level ($p=0.0007$), but not at low abrasive levels ($p=0.17$). Day 3 had significantly more SL than Day 1 ($p<0.0001$); with a larger difference for 0 ppm F.

For HEC, Day 5 had significantly more SL than Day 1 for 0 ppm F ($p=0.01$) and for high abrasive ($p=0.004$); but, not for low abrasive level. When comparing Day 3 to both Day 1 and Day 5, no significant differences in SL were found.

Overall, high abrasive slurries had significantly more SL than low abrasivity ($p<0.0001$). Abrasivity had less of an effect on the HEC lesions but was still significant.

For the MeC specimens, 0 ppm F had significantly more SL than 275 ppm F after Day 3 ($p<0.0001$) and Day 5 ($p<0.0001$). However, there was no fluoride difference after Day 1 ($p=0.99$). CMC specimens showed a similar trend with 0 ppm F showing significantly higher SL values compared to 275 ppm F after Day 3 ($p=0.0349$) and Day 5 ($p=0.0490$). Fluoride effect was not significant after 1 day of brushing ($p=0.72$). Fluoride had no effect on the HEC specimens ($p=0.54$).

MeC had significantly more SL than HEC ($p<0.0001$). The difference was larger for high abrasive than for low abrasive and for 0 ppm F than for 275 ppm F. MeC had significantly more SL than CMC for Day 3 ($p<0.0001$) and Day 5 ($p<0.0001$), with a larger difference for high abrasives than for low abrasives and for 0 ppm F than for 275 ppm F, but the difference between MeC and CMC for Day 1 was not statistically significant ($p=0.06$). CMC had significantly more SL then HEC for high abrasive ($p<0.0001$), with a larger difference for 0 ppm F than for 275 ppm F, but the difference between CMC and HEC for low abrasive was not statistically significant ($p=0.91$).

Study #2

Figure 14 shows the difference in appearance of lesions before and after brushing. Overall, brushed areas are less opaque than demineralized area showing clear differences before and after brushing; however, the difference in appearance depended on the lesion type with HEC lesion showing less changes in appearance after brushing.

Baseline comparisons of the lesions created using the three demineralization protocols are shown in Figure 15. Overall, MeC lesions have the least mineral content in the surface layer. CMC lesions were significantly deeper compared to the other two lesion types; while HEC had the smallest ΔZ_{base} values (Table 7).

Microradiographyimages from each group are shown in Figures 16 through 21. All groups showed a distinct surface layer followed by an area of reduced mineral content. Changes in images after brushing were clearer in the MeC and CMC especially in groups with 275 ppm F. In these groups, an increase in the radiopacity of the surface layer was apparent.

Tables 8 and 9 show the results of the statistical analysis for study 2. Both abrasiveness and fluoride did not have a significant effect on $\Delta(\Delta Z)_C$ or ΔL_C. HEC had significantly lower $\Delta(\Delta Z)_C$ than CMC ($p=0.0162$) and MeC ($p<0.0001$); however, CMC and MeC were not significantly different from each other ($p=0.19$). Type of lesion had no effect on ΔL_C.

Results of the paired t-test are shown in Table 10. For MeC groups, there was a significant mineral gain in all groups except the group brushed with high abrasives and 0 ppm F (Figures 22 and 23). Similar results were obtained for CMC lesions (Figures 24 and 25). On the other hand, only the group brushed with low abrasives and 275 F showed a significant mineral gain in the HEC lesions ($p=0.006$; Figures 26 and 27).

Study #3

Results of statistical model are shown in Table 11. High abrasiveness had significantly more surface loss than low abrasiveness when brushing 2x/day ($p=0.0001$) or 3x/day ($p=0.0001$) but not with 1x/day ($p=0.24$; Table 12). The difference between high and low abrasiveness increased as the number of days increased. On the other hand, fluoride had a marginally significant effect ($p=0.06$) between study groups.

Brushing 1x/day had significantly less surface loss than brushing 3x/day after 5 days ($p=0.0102$) and after 7 days ($p=0.0011$; Figures 28 and 29). In addition, when using high abrasive, brushing 1x/day had significantly less surface loss than brushing 2x/day ($p=0.0335$). No other brushing frequency comparisons were statistically significant ($p>0.12$).

Day 1 had significantly more surface loss than Day 3 (p=0.0001), regardless of the other factors (Figures 28 and 29). Day 1 had significantly more surface loss than Day 5 when brushing 1x/day (p=0.0205) but not when brushing 2x/day (p=1.00) or 3x/day (p=0.80). Day 1 also had significantly more surface loss than Day 5 with low abrasive (p=0.0001) but had significantly less loss than Day 5 with high abrasive (p=0.0026). Day 1 had significantly less surface loss than Day 7 when brushing 3x/day (p=0.0043) but not when brushing 1x/day (p=0.07) or 2x/day (p=0.34). Day 1 also had significantly less surface loss than Day 7 with 275 ppm F (p=0.0118) but not with 1250 ppm F (p=0.99). Day 1 had significantly less surface loss than Day 7 with high abrasive (p=0.0001) but had significantly more surface loss than Day 7 with low abrasive (p=0.0001). Day 3 had significantly less surface loss than Day 5 when brushing 2x/day (p=0.0109) and 3x/day (p=0.0001) but not when brushing 1x/day (p=0.99). Day 3 also had less surface loss than Day 5 with high abrasive (p=0.0001) but not with low abrasive (p=0.99). Day 3 had significantly less surface loss than Day 7 when brushing 2x/day (p=0.0001) and 3x/day (p=0.0001) but not when brushing 1x/day (p=0.92). Day 3 also had less surface loss than Day 7 with high abrasive (p=0.0001) but not with low abrasive (p=0.96). Day 5 had significantly less surface loss than Day 7 when brushing 2x/day (p=0.0063) and 3x/day (p=0.0327) but not when brushing 1x/day (p=0.99). Day 5 also had less surface loss than Day 7 with high abrasive (p=0.0001) but not with low abrasive (p=1.00). Day 5 also had less surface loss than Day 7 with 275 ppm F (p=0.0001) but not with 1250 ppm F (p=1.00).

Study #4

Results of the statistical model are shown in Table 13. High abrasiveness had significantly more surface loss under the following conditions: 275 ppm fluoride ($p=0.0001$) but not 1250 ppm fluoride ($p=0.34$); 3x per day ($p=0.0001$) but not 1x per day ($p=0.95$) or 2x per day ($p=0.36$; Table 14). The difference between high and low abrasiveness increased as the number of days increased.

Fluoride at 275 ppm had significantly more surface loss than 1250 ppm fluoride on Day 5 with high abrasive ($p=0.0308$) and on Day 7 with high abrasive ($p=0.0082$) and when used 3x per day ($p=0.0264$). There was no fluoride effect for Day 1 ($p=0.77$) or Day 3 ($p=0.34$), or with low abrasive ($p=0.93$), or with 1x per day ($p=0.99$) or 2x per day ($p=0.68$).

Brushing 1x/day had significantly less surface loss than brushing 3x/day after 5 days with high abrasiveness ($p=0.0249$) and after 7 days with high abrasiveness ($p=0.0160$) and with 1250 ppm fluoride ($p=0.0172$: Figures 30 and 31). No other brushing frequency comparisons were statistically significant ($p>0.14$).

Day 3 and Day 5 had significantly less surface loss than Day 7 ($p<0.0001$; Figures 30 and 31). For high abrasive 3x/day and for high abrasive with 275 ppm fluoride, Day 1 had significantly less surface loss than the other days ($p<0.005$) and Day 3 had significantly less surface loss than Day 5 ($p=0.0001$). For high abrasive 2x/day Day 1 had significantly less surface loss than Day 7 ($p=0.0011$) and Day 3 had significantly less surface loss than Day 5 ($p=0.0019$).

Scanning electron microscopic images are shown in Figure 32. The normal structure of enamel can be seen with the distinct shape of enamel rods (Figure 32a and b).

After demineralization, there was more porosity throughout the lesion (Figure 32c); especially at the surface layer (Figure 32d). After brushing, there was a distinct surface layer that was more compact compared to the remaining lesion (Figure 32e and 32f).

DISCUSSION

The incipient enamel lesion is an important manifestation of the caries process because of its potential for reversal. Attempts to achieve this reversal state have been successfully made using fluoridated dentifrices. However, there is a need for a better understanding of the mechanisms involved in such processes in order to help develop clinical protocols for the arrestment of incipient lesions, especially considering the dynamics involved in the toothbrushing procedure. The main purpose of this project was to investigate the combined effect of fluoride content and abrasive level of dentifrices on incipient caries lesions with different mineral profiles in a setting that simulated the daily oral hygiene practice.

Justifications for experimental parameters

Three lesion types with different mineral profiles and structural compositions were studied, since a complete characterization of the naturally occurring white spot lesions has not been carried out. It has been suggested that the mineral distribution of natural incipient caries lesions is highly variable [Cochrane et al., 2012]. Therefore, artificially-created enamel lesions can be considered as a suitable alternative for the study of enamel incipient caries lesions. The protocols used in this study were chosen since they had previously shown to develop sub-surface enamel lesions with different mineral profiles [Amaechi et al., 1998; Lippert et al., 2011; Ten Cate et al., 1996]. This was done in an attempt to resemble the variation expected in natural lesions. Our assumption was that lesions with different characteristics could behave differently to toothbrushing.

To investigate the study question under clinically relevant conditions, we chose to use a five- to seven-day remineralization protocol, using artificial saliva, and to incorporate the brushing sessions within it. The recommended brushing time to achieve adequate plaque removal is estimated to be 2 minutes [Van der Weijden et al., 1993]; which equates to 20 seconds per sextant to brush the occlusal, facial/buccal, and lingual/palatal surfaces. However, the average brushing time reported in the literature ranges from 30 to 90 seconds [Beals et al., 2000; Ganss et al., 2009; Van der Weijden et al., 1993]. In the present study, we adopted 15 seconds of brushing per surface or approximately 50 brushing strokes in an attempt to simulate highly motivated patients. This gave a total of 500 brushing strokes at the end of the study that would be representative of 5 days of brushing in the first two studies. Since brushing frequency was added as an additional variable in studies 3 and 4, the final number of brushing strokes ranged from 350 to 1050 in these experiments. Meanwhile, other in vitro and in situ studies in the literature used brushing sessions involving 300 – 400 strokes [Ganss et al., 2009; Macgregor and Rugg-Gunn, 1979, 1985] that exceed the clinical situation [Wiegand and Attin, 2011].

The brushing protocol adopted mimics the daily scenario in which the teeth would be exposed to the toothpaste between one and three times per day and remain soaked in saliva for the remainder of the day and during sleeping time. Possibly, this relatively short duration (5 days) may have influenced the remineralization results since most studies in this area last for weeks rather than days [Laheij et al., 2010; Lippert et al., 2011; Ten Cate, 1993]. Still, we could detect some general trends regarding remineralization of the three lesion types.

Dentifrice exposure times between 30 and 180 seconds were associated with a significant increase in surface rehardening of early caries lesions in situ [Zero et al., 2010]. Kielbassa and collaborators used a brushing time of 5 seconds per specimen and kept the specimens exposed to fluoridated dentifrices for 2 minutes to simulate the clinical situation [Kielbassa et al., 2009]. Their protocol was effective in remineralizing bovine enamel softened with a lactic acid solution. In our project, we brushed the specimens for ~15 seconds and left them in the slurry for about 1 minute; which is the average brushing duration reported in the literature [Van der Weijden et al., 1993].

Estimating the brushing force based on the literature yields a lot of discrepancies. The mean reported force of brushing ranges from 2.4 to 4.6 newtons (244.7 to 471 grams) [Fraleigh et al., 1967; Ganss et al., 2009]; however, this could be influenced by factors such as gender and type of brush. In general, males tend to apply higher forces during brushing compared to females and the force delivered while using an electric brush is usually less than that associated with a manual brush [Boyd et al., 1997; Hunter and West, 2000]. Wiegand and Attin [2011] recommended standardizing the brushing force of in vitro studies to be between 1 and 2 newtons (102.0 – 203.0 grams). Therefore, in this project, we used a force of 150 grams for all studies; which is also in line with recommendations by the International Standards Organization (ISO11609) [2010] for testing the abrasivity of toothpastes.

Higher brushing frequency has been reported to increase the anticariogenic effect of fluoridated dentifrices [Marinho et al., 2003]. Brushing twice a day improves the protective effect of fluoride against dental caries [Ashley et al., 1999]. This effect is probably due to better plaque removal as well as more exposure to fluoride [Zero et al.,

2010]. On the other hand, it has been reported that increasing the frequency of toothbrushing is associated with higher degree of abrasion [Bergstrom and Lavstedt, 1979]; especially if brushing is done more than twice a day [Hunter and West, 2000].

The fluoride concentrations used throughout the project are representative of a ratio of 1:3 of regular or prescription toothpastes. These concentrations were combined with different levels of abrasives found in low or highly abrasive toothpastes. This was not done to target any particular product available in the market, but to create a large range of fluoride concentrations that could possibly include most of the products available. This approach helped in controlling for the presence of different ingredients, which would not be possible if commercially available toothpastes were to be used.

The primary measurement throughout the project was surface loss (SL). This parameter has been frequently used to test the resistance of dental hard tissues to toothbrushing abrasion [Ashmore et al., 1972; West et al., 1998]. It was combined with TMR data in the second study in order to link the abrasion resistance behavior of the incipient enamel lesions with their mineral content and remineralization behavior (Figure 13).

Abrasive effect on lesion types

Overall, slurries with high abrasive levels led to more SL compared to low abrasive ones. This was expected since the slurries with higher REA/RDA values have been associated with more abrasive potential on the natural tooth structure [Philpotts et al., 2005]; and a potentially more detrimental effect on the softer demineralized lesions. This abrasive effect was less pronounced on the HEC lesions, possibly due to the higher

mineral content of the surface layer [Lippert et al., 2012]. These results are in agreement with those of Kielbassa and colleagues [2005] who found a direct correlation between SL values of artificial enamel lesions and the abrasivity of toothpaste slurries. In addition, they observed the loss of the surface layer when the lesions were examined using transverse microradiography. However, in their study, excessive brushing simulating 1.5 years of brushing could have led to this result leading to the development of the so-called "pseudo-intact layer". In the present study, a well-defined surface layer was present in all lesion types under TMR imaging even in groups where excessive SL was reported (the highest SL value was recorded for MeC lesions brushed with high abrasives at 0 ppm fluoride). This relates to the less aggressive nature of our model that may resemble active incipient caries lesion.

Fluoride effect on lesion type

The protective effect of fluoride against surface loss has been reported previously and especially in relation to softened erosive lesions [Attin et al., 1998]. A similar effect has been reported for brushing with fluoridated toothpastes that decreased the SL values of enamel and dentin compared to controls [Davis and Winter, 1977]. In the present study, the fluoride effect was dependent on the lesion type and time effect. In general, MeC and CMC showed a similar trend; with the fluoride effect detected after 300 brushing strokes (3 days) and continuously increased until the fifth day. However, in the case of HEC lesions, no fluoride protective effect could be observed even after 5 days (500 brushing strokes). The difference between the fluoride responses across the lesion types could be explained by the inherent differences in the structure of the surface layer

between those lesions. HEC lesions have a surface layer with high mineral content (~70 vol.%) [Lippert et al., 2012], and possibly less porous structure, that could potentially inhibit the reprecipitation of minerals on the surface. The remineralization potential of fluoride could be seen more clearly in the other lesion types possibly due to the less mineralized surface layer, in the range of 35 to 55 vol.%, that could invite more minerals leading to more resistance to abrasion [Lippert et al., 2012; Lippert et al., 2011]. In our TMR analysis (study 2), we recorded similar values for the surface layer with HEC, CMC, and MeC lesions showing average values of 70, 60, and 45 vol.% mineral content at the surface, respectively (Figure 15).

Effect of lesion type, fluoride, and abrasivity on surface loss

The association between mineral content and hardness has been shown in the past [Featherstone et al., 1983; Kielbassa et al., 1999]. Higher hardness numbers can lead to a more resistant surface layer and consequently more abrasion resistance. So, it is not surprising to record the highest SL values for MeCand the lowest for HEC lesions. Overall, the fluoride-abrasivity interaction was not significant ($p=0.14$) in the statistical model (Table 5); however, when a “lesion type” term was added, a significant interaction was found ($p=0.038$). This indicates that the protective effect of fluoride against a specific level of abrasivity is dependent on the inherent characteristics of a particular lesion type. That is why we could observe a protective effect of fluoride when MeC and CMC lesions were brushed while no such effect was seen with HEC lesions. We can attribute this to differences in the mineral composition of the surface layer of each lesion

type; as the characteristics of the deeper parts of the lesion could probably have a smaller effect on the surface properties.

Effect of lesion type, fluoride, and abrasivity on mineral content

MeC lesions tend to have the least mineralized surface layer followed by CMC and lastly HEC had the highest concentrations of mineral at the surface. For HEC, this could translate into a more compact surface that could hinder the penetration of minerals into the body of the lesion. Most of the mineral gain was observed at the surface layer (Figures 22 through 25) with minor mineral changes in the body of the lesion. Although statistical comparisons for the studied parameters (difference in mineral change and lesion depth change) were not significant using the ANOVA model, there is an obvious trend of more mineral deposition in the surface layer when fluoride slurries were used to brush MeC, and to lesser extent CMC, lesions regardless of the level of abrasives. However, this effect was not observed for HEC lesions that had the most mineralized surface layer. The more compact layer at the surface could potentially lead to a less porous structure that will hinder the penetration of minerals to the deeper parts of the lesion hindering further remineralization [Ten Cate, 1990]. Further discussion of this point will be made when interpreting SEM images from study 4.

It has been reported that higher values of ΔZ_{base} are associated with higher tendency of lesions to remineralize [Schafer et al., 1992; Strang et al., 1987]. Although all lesions showed some remineralization in the present study, such relationship between remineralization and ΔZ_{base} could not be observed in the $\Delta(\Delta Z)_C$ analysis. HEC lesions had significantly lower ΔZ_{base} values compared to MeC and CMC lesions, yet, no

differences in mineral gain ($\Delta(\Delta Z)_C$) could be observed. One explanation could be the differences in the remineralization protocols of the cited studies, which was done in in situ conditions and involved periods of acid challenge. Acid challenges could dissolve minerals at the surface forming a more porous layer that could allow minerals to penetrate deeper and enhance remineralization. Another difference is the duration of remineralization. In the present study, remineralization was performed for 5 days, which is shorter than the duration of most in situ studies (2 to 5 weeks).

To test the changes in the mineral content before and after brushing within each group, a paired t-test was utilized (Table 10). Overall, all groups within MeC and CMC lesions showed significant mineral gain except for groups brushed with high abrasives in the absence of fluoride (Figures 22 through 25). In the latter groups, we suspect that the effect of structural loss due to the high abrasivity of the slurry had affected the mineral gain by removing the surface layer. A similar finding has been reported previously by Kielbassa and colleagues [2005]. Although high abrasive slurries were expected to improve remineralization of the body of the lesion by preventing the formation of a highly mineralized surface layer mineralization, this effect was not observed. This may have happened possibly due to the more prominent effect of high abrasives compared to the result of remineralization. Use of slurries with intermediate abrasive levels may allow us to find this effect, deserving further investigations. Within groups showing significant mineral gain, those brushed with fluoride-containing slurries showed more mineral gain in the surface layer as seen by a hump in the mineral profile (a compact surface layer can be seen in MeC lesions examined with SEM in study 4; Figure 32). Contrary to this, HEC lesions showed significant mineral gain only in the group brushed with low abrasives in

the presence of fluoride (Table 10 and Figures 26 and 27). This could be explained by the fact that higher driving force for mineral deposition of the fluoride was needed to allow some mineral gain past the highly mineralized surface layer of the HEC lesion.

Brushing effect on surface loss and mineral content

It has been hypothesized that toothbrushing abrasion leads to structural loss that might play a role in the arrestment of white spot lesions [Artun and Thylstrup, 1989; Fejerskov et al., 2008; Holmen et al., 1987b; Kielbassa et al., 2005; Stookey and Muhler, 1968]. More abrasive slurries would remove the surface layer at a higher rate leading to wear of the surface. Although we assumed that this phenomenon could lead to more porosities in the surface layer and opening the lesion to remineralization, this effect was not seen in HEC lesions brushed with high abrasives. It is possible that the combination of the precipitation action of fluoride and the high abrasivity caused a smear layer that inhibited the penetration of minerals (this is based on SEM images done in study 3 and are not shown in this thesis). Still, this assumption could be valid since MeC and CMC lesions brushed with high abrasives in the presence of fluoride showed significant mineral gain.

However, Arends and colleagues [1987] have reported that initial caries lesions obtained from patients after orthodontic therapy had softer surfaces than sound enamel. To our knowledge, only one in vitro study [Kielbassa et al., 2005] has investigated the effect of toothpaste abrasives on the abrasion resistance of enamel caries lesions produced by a lactic acid demineralization protocol [Kielbassa et al., 2001]. Resultant lesions had TMR parameters different from lesions created in the present investigation

(for comparison, refer to Table 7 and Figure 15) with L= 88.7 ± 26.2 µm, mineral content in the surface layer between 62.3 and 69.9 vol.%, and ΔZ_{base} between 4181.6 and 5362.4 vol.% [Kielbassa et al., 2005; Kielbassa et al., 2001]. In that study [Kielbassa et al., 2005], 50% less abrasion was found in sound enamel compared to initial caries lesions. Further, greater surface loss values and loss of the surface layer were reported for the higher abrasive pastes. The authors recommended the use of oral hygiene products with low abrasivity for patients with white spot lesions. Studies investigating the effect of toothpaste on eroded enamel have reported supporting evidence. Attin and colleagues [1997] found more toothbrushing abrasion associated with eroded enamel in vitro. In addition, Turssi et al. [2004] reported greater wear of softened compared to sound enamel in an in situ study.

Brushing frequency

In the last two studies, brushing frequency was investigated since it has been previously shown to influence the brushing abrasive wear [Bergstrom and Lavstedt, 1979; Sangnes, 1976]. In studies 1 and 2, specimens were brushed twice daily representing the minimum recommended frequency for oral hygiene practice [ADA, 2012]. However, different frequencies such as 1x and 3x/day are not uncommon and could have an impact on the reversal of early caries lesions. Sheiham [1977] has reported little benefit to periodontal health if teeth were brushed more than twice per day, with higher frequencies causing excessive dental abrasion. Therefore, in studies 3 and 4, 1x and 3x/day brushing frequencies were added to further investigate their modulating effects on fluoride and abrasives. Although higher brushing frequency can be linked to

more structural loss, the effect of repeated exposure to varying levels of fluoride could have a protective effect against SL.

In study 3, the effect of fluoride on SL of CMC lesions was marginally significant ($p=0.06$). It can be speculated that a significant effect could have been shown if cycling was extended for additional days. This should be considered in subsequent investigations because a cumulative effect of fluoride would probably be present in clinical scenarios.

Similar to the first study, high abrasive slurries were associated with higher SL values. However, this finding was modulated by the brushing frequency and abrasive level. Only when high abrasive slurries were used twice or three times per day ($p=0.0001$) a significant effect was recorded. If a lower frequency was used, the use of either low or high abrasives did not affect the SL. Moreover, the difference between low and high abrasive groups increased as the brushing/cycling continued. This is in line with previous research showing abrasion as a time-dependent process [Addy and Hunter, 2003].

Another significant variable in study 3 was brushing frequency. In general, higher SL values were associated with brushing 3 times per day regardless of the abrasive level. This could have an important implication for clinical situations; since white spot lesions could be progressively abraded with the increase in the number of the brushing sessions by the patient. Furthermore, the use of highly abrasive toothpastes in cases with incipient lesions should be thought carefully since significantly higher SL values were recorded even when brushing twice per day.

Considering the time effect in the third study, there were some inconsistences regarding SL trends. Although a progressive abrasion effect can be seen in some groups, others showed some mineral precipitation on the surface at the early stages of cycling. In addition, data from Day 3 do not fit with the overall trend seen in the experiment. These could be explained by technical difficulties with the optical profilometer between days 1 and 3 that could have affected the data. Statistical analyses were performed with and without data for "Day 3" and, since the results were the same in both cases, the complete set of data was kept.

In the last study, all four factors –fluoride, abrasives, brushing frequency, and time– were significant indicating a very complex model. High abrasive level was associated with higher SL values on MeC lesions compared to low abrasives at 275 ppm fluoride but not at the 1250 ppm level. This indicates that the dose increase in fluoride was effective in protecting the weak surface layer of the MeC lesions. A similar fluoride effect was previously reported by Dunipace et al. [1997] and Wefel et al. [1995] when dentifrices with variable levels of fluoride in their in situ models were used. This is contrary to study 3, were fluoride had a non-significant effect on SL of CMC lesions further illustrating the variation of responses for lesions with different mineral profiles. Additional data show that SL values were higher at 275 ppm fluoride compared to 1250 after brushing 3x/day, for 5 days. Although a protective fluoride effect was present in relation to high abrasives in study 1, the effect of higher fluoride concentration (1250 ppm) in study 4 was dependent on frequency. Fluoride was able to decrease the SL values when brushing took place once or twice per day. However, when brushed three times

daily with high abrasives, the fluoride effect faded since it could not counter act the abrasive effect.

The increase in the brushing frequency increased the exposure of incipient lesions to both fluoride and abrasives. Higher abrasives increased the surface abrasion while higher frequency of fluoride exposure protected the surface from abrasion. This makes the model used in this study unique since both processes play important roles clinically. Based on results from study 4, the use of low abrasive toothpastes will not cause a great amount of abrasion of incipient lesions in the presence of fluoride in the concentrations found in over-the-counter dentifrices. On the other hand, highly abrasive toothpastes such as whitening formulations might cause more damage to the surface of white spot lesions if the patient brushed at higher frequencies. In these cases, increasing the amount of fluoride to the range found in prescription toothpastes could be considered.

Limitations

As with other laboratory reports, this study had some limitations. First, the artificial lesions might not represent white spot lesions completely. However, this is a common problem with almost all in vitro caries studies; and until the characterization of white lesions is done (which is extremely difficult to accomplish) the use of artificial enamel lesions is justified. Second, the remineralization protocol involves the use of artificial saliva that does not completely mimic natural saliva. Third, we did not include episodes of acidic challenges similar to what would happen clinically, assuming non-effective plaque removal by brushing and continuing exposure to fermentable carbohydrates. In such conditions, the acid generated by bacteria could demineralize the

compact surface layer of the lesions making it more porous, and supposedly, more prone to remineralization. However, in the present study we simulated a situation where patients develop with incipient active lesions would start brushing with toothpaste, following dentist's recommendations. Lastly, the use of a laboratory setting eliminated variability related to differences in brushing behavior observed in the population. The last two points could be further explored by conducting an in situ study.

Clinical extrapolations

Toothbrushing with fluoridated toothpaste can be considered as one of the most common recommendations from dental professionals to patients with white spot lesions. Based on results from this investigation, the abrasive level and fluoride content of the toothpaste used influences the reversal of the incipient caries lesion. Results from this investigation suggest that lesions developing under severe acidic challenges and having a weak surface layer can be a more easily abraded by highly abrasive dentifrices, if used more than twice a day. This seems to encourage the use of low abrasive toothpastes avoiding more abrasive formulations, such as most whitening toothpastes, for patients with active caries. Alternatively, patients could also be advised to decrease the number of toothbrushing sessions or to use higher fluoride concentration formulations. The use of formulations with either level of abrasivity tested does not greatly influence the remineralization effect produced by the toothpaste; however, the structural integrity of the white spot lesion could be affected.

CONCLUSIONS

The disappearance of the clinical signs of incipient caries has been reported in the literature. The remineralization effect enhanced by the use of fluoridated oral care products is well documented. However, the interaction between fluoride and abrasives found in dentifrices in incipient caries lesions is largely unknown. Based on the results from this investigation, we can conclude that:

1. The effect of higher abrasive content of toothpastes can be seen in all three lesion types tested in this study. Whereas, the effect of fluoride is dependent on the type of lesion tested. MeC lesions showed the least abrasion resistance and HEC was the most resistant to abrasion.
2. The combined effect of fluoride and abrasives within the toothpaste formulation showed little influence on remineralization. However, lesions with a less mineralized surface were more prone to surface loss compared to lesions with a higher mineral content at the surface layer.
3. The protective effect of higher fluoride concentration (1250 ppm) was shown with MeC but not CMC lesions indicating an effect dependent on the structural composition of the lesions.
4. Brushing frequency is an important modulating factor in the relation between fluoride and abrasives within toothpaste slurries either by exposing the lesion to more fluoride or by abrading the surface more due to more abrasive exposure.

TABLES

Table 1 – Summary of the four studies conducted in the project.

Study	Demineral-ization protocol	Factors	Factorial levels	Sample size	Number of groups	Method of analysis
1	MeC CMC HEC	Lesion type Fluoride content Abrasive level Time	MeC CMC HEC 0 ppm 275 ppm Low High Day 1 Day 3 Day 5	10	12	Surface profilometry
2	MeC CMC HEC	Lesion type Fluoride content Abrasive level	MeC CMC HEC 0 ppm 275 ppm Low High	10	12	Transverse micro-radiography
3	CMC	Fluoride content Abrasive level	275 1250 Low High	10	12	Surface profilometry
4	MeC	Brushing frequency Time	1x/day 2x/day 3x/day Day 1 Day 3 Day 5 Day 7	8	12	Surface profilometry

MeC: Methylcellulose gel
CMC: Carboxymethylcellulose solution
HEC: Hydroethylcellulose gel

Table 2 – Summary of the demineralization protocols used throughout the project with the corresponding reference.

Protocol	pH	Ca and PO_4	Duration (days)	Reference
Methylcellulose gel (MeC)	4.6	No	7	Ten Cate et al., 1996
Carboxymethylcellulose solution (CMC)	5.0	Yes	10	Lippert et al., 2011
Hydroethylcellulose gel (HEC)	4.5	No	7	Amaechi et al., 1998

Table 3 – The contents of the four slurries used in studies 1 and 2. Slurries 1 and 2 are considered low abrasive; while slurries 3 and 4 are considered high abrasive. The sum of the slurry ingredients adds up to 60 grams which were used to brush each specimen individually.

Slurry	Carboxymethyl -cellulose	Glycerol	NaF	Abrasives	Deionized water
1	2.5g	5g	0g	3g Zeodent® 113[*]	49.5g
2	2.5g	5g	0.036g[#]	3g Zeodent® 113	49.5g
3	2.5g	5g	0g	9g Zeodent® 103[§]	43.5g
4	2.5g	5g	0.036g	9g Zeodent® 103	43.5g

[*]This abrasive level has REA and RDA values of 4.01 and 69.24, respectively.
[#]This level represents 275ppm fluoride.
[§]This abrasive level has REA and RDA values of 7.14 and 208.03, respectively.

Table 4 – The contents of the four slurries used in studies 3 and 4. Slurries 1 and 2 are considered low abrasive; while slurries 3 and 4 are considered high abrasive. The sum of the slurry ingredients adds up to 60 grams which were used to brush each specimen individually.

Slurry	Carboxymethyl -cellulose	Glycerol	NaF	Abrasives	Deionized water
1	2.5g	5g	0.036g[#]	3g Zeodent® 113[*]	49.5g
2	2.5g	5g	0.144g[Ø]	3g Zeodent® 113	49.5g
3	2.5g	5g	0.036g	9g Zeodent® 103[§]	43.5g
4	2.5g	5g	0.144g	9g Zeodent® 103	43.5g

[*]This abrasive level has REA and RDA values of 4.01 and 69.24, respectively.
[#]This level represents 275 ppm fluoride.
[Ø]This level represents 1250 ppm fluoride.
[§]This abrasive level has REA and RDA values of 7.14 and 208.03, respectively.

Table 5 – Results of statistical analysis for surface loss from study 1 showing the p-values for each term of the model.

Effect	p-value
Brush position	0.87
Type of lesion	<.0001
Fluoride	<.0001
Type of lesion * Fluoride	0.0101
Abrasiveness	<.0001
Type of lesion * Abrasiveness	<.0001
Fluoride * Abrasiveness	0.14
Type of lesion * Fluoride * Abrasiveness	0.0389
Time	<.0001
Type of lesion * Time	<.0001
Fluoride * Time	<.0001
Type of lesion * Fluoride * Time	0.0013
Abrasiveness * Time	<.0001
Type of lesion * Abrasiveness * Time	<.0001
Fluoride * Abrasiveness * Time	0.65
Type of lesion * Fluoride * Abrasiveness * Time	0.36

Table 6 – Mean and SD of surface loss (SL) over time in the three lesion types from study 1, methylcellulose (MeC), carboxymethylcellulose (CMC), and hydroxyethylcellulose (HEC) divided by fluoride content and abrasive level.

Protocol	Abrasive Level	F (ppm)	Surface loss (µm)		
			Day 1	Day 3	Day 5
MeC	Low	0	0.28 (0.06) [a]	0.84 (0.13) [b]	1.13 (0.14) [c]
		275	0.35 (0.10) [a]	0.55 (0.15) [b]	0.78 (0.23) [c]
	High	0	1.70 (0.28) [a]	6.32 (1.22) [b]	10.97 (1.43) [c]
		275	1.07 (0.10) [a]	2.14 (0.35) [b]	3.90 (0.56) [c]
CMC	Low	0	0.23 (0.07) [a]	0.55 (0.14) [b]	0.65 (0.16) [b]
		275	0.07 (0.05) [a]	0.13 (0.05) [b]	0.21 (0.06) [b]
	High	0	1.21 (0.17) [a]	2.11 (0.29) [b]	2.73 (0.43) [c]
		275	0.97 (0.13) [a]	1.36 (0.22) [b]	1.72 (0.32) [c]
HEC	Low	0	0.14 (0.06) [a]	0.22 (0.06) [a]	0.26 (0.05) [a]
		275	0.14 (0.04) [a]	0.16 (0.05) [a]	0.19 (0.05) [a]
	High	0	0.33 (0.03) [a]	0.5 (0.07) [a,b]	0.66 (0.08) [b]
		275	0.3 (0.06) [a]	0.41 (0.06) [a,b]	0.5 (0.08) [b]

Same letters indicate non-significant effect of time (p>0.05) within each group (same row). Groups conjoined with brackets are not significantly different (p>0.05) indicating a fluoride effect. Groups subjected to high abrasive level were always associated with more surface loss (p<0.05) compared to groups brushed with low abrasives at the same fluoride level.

Table 7 – Mean, standard deviation (in parenthesis), and coefficient of variation of TMR parameters of the three lesion types at baseline. Different superscript letters indicate a significant difference ($p<0.05$) within a particular parameter.

Protocol	n	ΔZ_{base} (vol.%)	CV (%)	L_{base} (µm)	CV (%)
MeC	40	2576.7 (504.7)[a]	19.6	80.9 (11.2)[a]	13.8
CMC	40	2505.7 (476.9)[a]	19.0	108.1 (13.8)[b]	12.7
HEC	40	1313.3 (272.2)[b]	20.7	79.4 (12.8)[a]	16.1

Table 8 – Results of statistical analysis for TMR parameters from study 2 showing the p-values for each term of the model.

Effect	ΔZ_{base}	L_{base}	$\Delta(\Delta Z)_C$	ΔL_C
Brush position	0.32	0.54	0.37	0.34
Type of lesion	<.0001	<.0001	0.0001	0.18
Fluoride	0.21	0.58	0.59	0.51
Type of lesion * Fluoride	0.15	0.11	0.14	0.73
Abrasiveness	0.08	0.08	0.31	0.36
Type of lesion * Abrasiveness	0.62	0.30	0.83	0.68
Fluoride * Abrasiveness	0.49	0.41	0.40	0.55
Type of lesion * Fluoride * Abrasiveness	0.28	0.99	0.30	0.83

Table 9 – Means and SD of TMR parameters from study 2. Within each lesion type, fluoride and abrasives did not have a significant effect (p>0.05).

Lesion type	Abrasive level	Fluoride (ppm)	$\Delta(\Delta Z)_C$ (vol.%)	ΔL_C (µm)
MeC	low	0	617.0 (696.2)	-9.4 (12.0)
		275	630.0 (706.7)	-8.7 (11.3)
	high	0	649.0 (719.9)	-8.2 (11.0)
		275	629.0 (713.1)	-5.9 (11.6)
CMC	low	0	746.3 (523.9)	-13.5 (14.7)
		275	436.0 (522.8)	-7.8 (10.6)
	high	0	326.0 (557.0)	-7.5 (10.7)
		275	276.5 (562.8)	-7.8 (10.4)
HEC	low	0	204.4 (276.5)	-4.4 (11.9)
		275	372.0 (324.9)	-9.4 (16.2)
	high	0	316.0 (363.6)	-5.1 (17.2)
		275	297.0 (361.9)	-4.8 (17.1)

Table 10 – Means and SD of mineral content before and after brushing from study 2. Bold face type of the p-value indicates significant difference (p<0.05) of the paired t-test before and after treatment.

Lesion type	Abrasive level	Fluoride (ppm)	ΔZ_{base} (vol.%)	ΔZ_{post} (vol.%)	p-value*
MeC	low	0	2665.0 (478.3)	2048.0 (480.5)	**0.021**
		275	2398.0 (384.9)	1483.0 (347.2)	**<0.001**
	high	0	2185.3 (508.9)	1665.6 (447.1)	0.062
		275	2165.7 (517.5)	1220.0 (198.3)	**<0.001**
CMC	low	0	2472.5 (508.0)	1726.3 (341.2)	**0.005**
		275	2235.0 (509.0)	1799.0 (302.3)	**0.027**
	high	0	2377.9 (321.6)	2117.8 (390.5)	0.152
		275	2805.4 (345.8)	2232.0 (648.1)	**0.03**
HEC	low	0	1241.1 (303.3)	1036.7 (123.6)	0.057
		275	1332.0 (286.7)	960.0 (137.0)	**0.006**
	high	0	1338.9 (202.2)	1147.8 (271.5)	0.082
		275	1342.5 (322.4)	1295.0 (625.6)	0.827

* P-values are for the paired t-test.

Table 11 – Results of statistical analysis for surface loss from study 3 showing the p-values for each term of the model.

Effect	All Data	Exclude Day 3
	p-value	p-value
Abrasive Load	0.0000	0.0000
Fluoride Level	0.06	0.06
Abrasive Load * Fluoride Level	0.36	0.24
Brushing Frequency	0.0065	0.0019
Abrasive Load * Brushing Frequency	0.08	0.0464
Fluoride Level * Brushing Frequency	0.84	0.75
Abrasive Load * Fluoride Level * Brushing Frequency	0.69	0.62
Day	0.0000	0.0000
Abrasive Load * Day	0.0000	0.0000
Fluoride Level * Day	0.0000	0.0000
Abrasive Load * Fluoride Level * Day	0.20	0.35
Brushing Frequency * Day	0.0000	0.0000
Abrasive Load * Brushing Frequency * Day	0.13	0.10
Fluoride Level * Brushing Frequency * Day	0.80	0.96
Abrasive Load * Fluoride Level * Brushing Frequency * Day	0.62	0.73

Table 12 – Means and standard deviations of surface loss from study 3 involving CMC lesions showing comparisons for fluoride and abrasives. Fluoride did not have a significant effect (p=0.06). Groups brushed with high abrasive slurries had a significantly more surface loss (p<0.0001) compared to low abrasive groups when brushed 2x and 3x.

Brushing Frequency	Abrasive level	Day 1		Day 3		Day 5		Day 7	
		275 F	1250 F	275 F	1250 F	275 F	1250 F	275 F	1250 F
1x	Low	-0.18 (0.10)	-0.21 (0.09)	-0.44 (0.14)	-0.61 (0.15)	-0.55 (0.14)	-0.52 (0.14)	-0.49 (0.13)	-0.70 (0.13)
	High	-0.09 (0.12)	-0.12 (0.10)	-0.19 (0.14)	-0.42 (0.22)	-0.15 (0.13)	-0.22 (0.16)	0.04 (0.16)	-0.19 (0.23)
2x	Low	-0.27 (0.13)	-0.28 (0.05)	-0.54 (0.14)	-0.65 (0.10)	-0.62 (0.12)	-0.55 (0.10)	-0.48 (0.13)	-0.64 (0.10)
	High	0.19 (0.11)	-0.14 (0.15)	0.02 (0.26)	-0.23 (0.11)	0.55 (0.25)	0.10 (0.27)	0.89 (0.29)	0.31 (0.18)
3x	Low	-0.14 (0.11)	-0.25 (0.08)	-0.36 (0.17)	-0.54 (0.13)	-0.37 (0.16)	-0.31 (0.13)	-0.22 (0.17)	-0.36 (0.14)
	High	0.16 (0.12)	0.14 (0.11)	0.03 (0.10)	0.01 (0.15)	0.57 (0.17)	0.44 (0.17)	0.93 (0.16)	0.48 (0.22)

Table 13 – Results of statistical analysis for surface loss from study 4 showing the p-values for each term of the model.

Effect	p-value
Abrasive Load	0.0000
Fluoride Level	0.0045
Abrasive Load * Fluoride Level	0.0446
Brushing Frequency	0.0271
Abrasive Load * Brushing Frequency	0.0226
Fluoride Level * Brushing Frequency	0.16
Abrasive Load * Fluoride Level * Brushing Frequency	0.46
Day	0.0000
Abrasive Load * Day	0.0001
Fluoride Level * Day	0.0018
Abrasive Load * Fluoride Level * Day	0.0323
Brushing Frequency * Day	0.07
Abrasive Load * Brushing Frequency * Day	0.0419
Fluoride Level * Brushing Frequency * Day	0.34
Abrasive Load * Fluoride Level * Brushing Frequency * Day	0.49

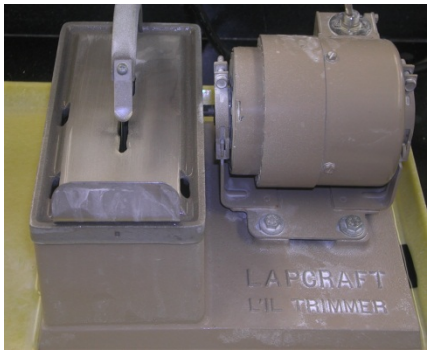
Table 14 – Means and standard deviations of surface loss from study 4 involving MeC lesions showing comparisons for fluoride and abrasives. For abrasive effect, at 275 ppm F, groups brushed with high abrasive slurry were statistically different ($p<0.05$) from groups brushed with low abrasives for all days; however, for 1250 ppm F, no abrasive effect was seen at any day. Different superscript letters indicate significantly different ($p<0.05$) surface loss values for fluoride levels at the same day.

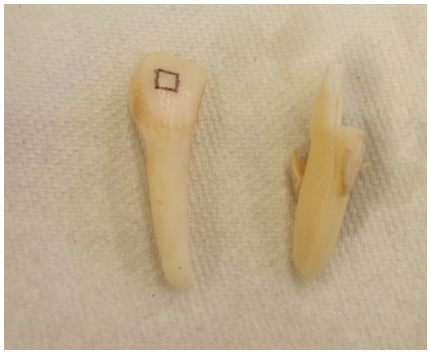
Brushing Frequency	Abrasive level	Day 1		Day 3		Day 5		Day 7	
		275 F	1250 F	275 F	1250 F	275 F	1250 F	275 F	1250 F
1x	Low	0.55 (0.18)^{a}	0.38 (0.10)^{a}	0.36 (0.17)^{a}	0.32 (0.08)^{a}	0.32 (0.17)^{a}	0.30 (0.09)^{a}	-0.19 (0.16)^{a}	-0.12 (0.08)^{a}
	High	-0.02 (0.23)^{a}	0.26 (0.17)^{a}	-0.29 (0.30)^{a}	0.09 (0.18)^{a}	-0.58 (0.33)^{a}	0.04 (0.16)^{b}	-1.24 (0.41)^{a}	-0.41 (0.14)^{b}
2x	Low	0.22 (0.23)^{a}	0.31 (0.16)^{a}	0.07 (0.22)^{a}	0.31 (0.15)^{a}	-0.16 (0.30)^{a}	0.31 (0.17)^{a}	-0.68 (0.34)^{a}	-0.04 (0.21)^{a}
	High	-0.35 (0.20)^{a}	0.08 (0.17)^{a}	-0.99 (0.39)^{a}	-0.11 (0.23)^{a}	-2.13 (0.75)^{a}	-0.45 (0.35)^{b}	-3.50 (1.12)^{a}	-1.22 (0.53)^{b}
3x	Low	0.32 (0.22)^{a}	0.56 (0.13)^{a}	0.09 (0.20)^{a}	0.66 (0.14)^{a}	0.00 (0.23)^{a}	0.63 (0.15)^{a}	-0.57 (0.23)^{a}	0.17 (0.13)^{a}
	High	-0.89 (0.49)^{a}	-0.41 (0.43)^{a}	-3.22 (1.78)^{a}	-0.71 (0.66)^{a}	-5.12 (2.10)^{a}	-1.04 (0.69)^{b}	-6.70 (2.30)^{a}	-1.64 (0.79)^{b}

FIGURES

Figure 1 – Photographs of the procedure for obtaining enamel slabs. Low speed cutting machine (a), bovine incisors before and after cutting (b), crown sections were mounted on plastic plates using sticky wax (c), slow-speed cutting machine (d), two parallel saw blades were used to cut specimens (e), 5×5 mm enamel slabs were obtained (f).

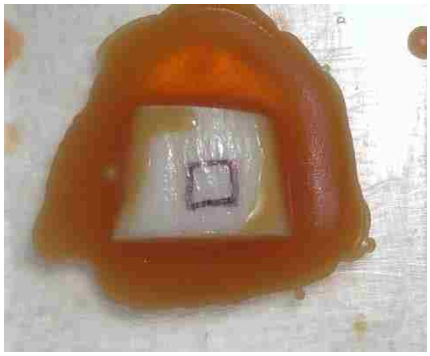
(a)





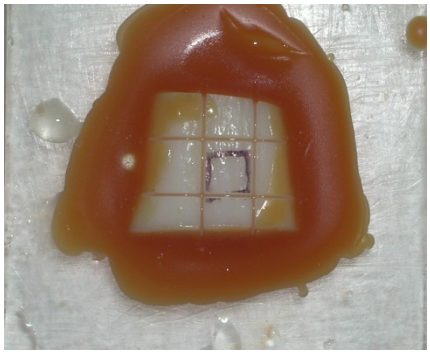
(b)

(c)



(d)

(e)



(f)

Figure 2 – Photographs of the steps of polishing the enamel slabs. The polishing machine (top), enamel slabs mounted on acrylic blocks using sticky wax (middle), and then ground and polished on the rotary part of the machine (bottom).

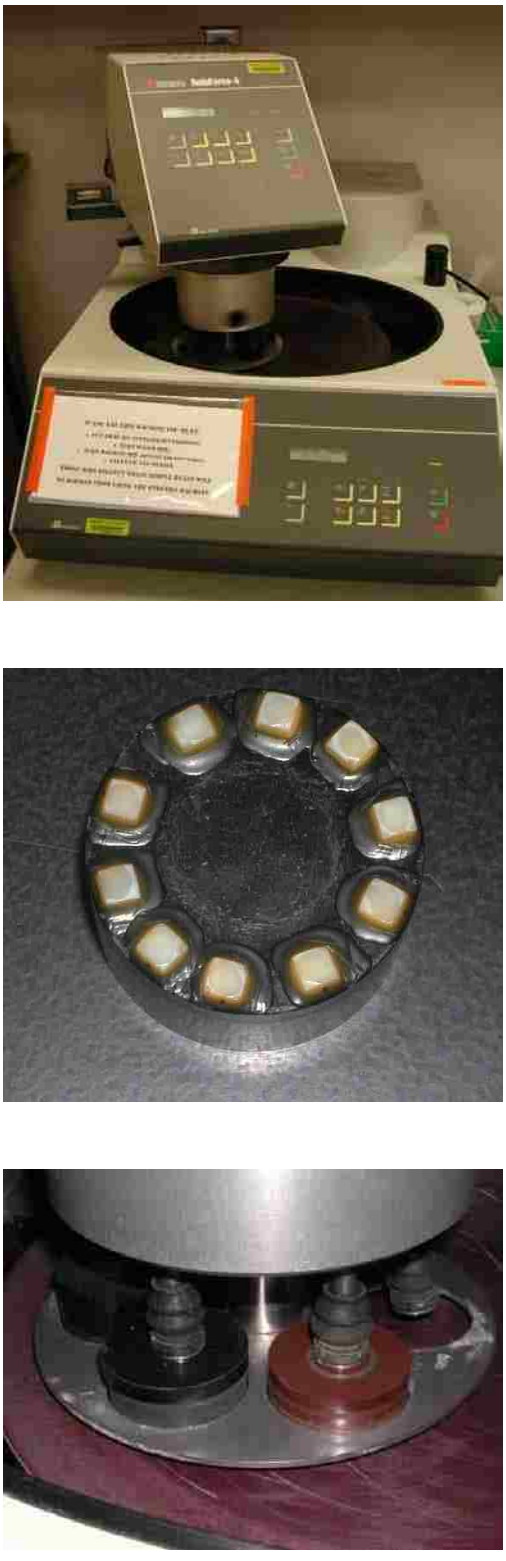
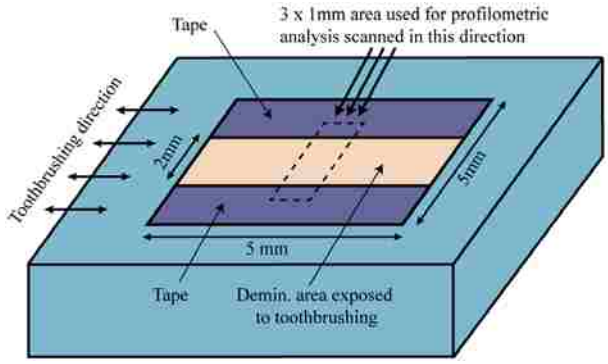


Figure 3 – A diagram illustrating the specimen setup used in study 1 (top). A photograph of the enamel slab after embedding in acrylic resin (middle). A photograph of the specimen after placing the adhesive tapes to protect the reference areas (bottom).



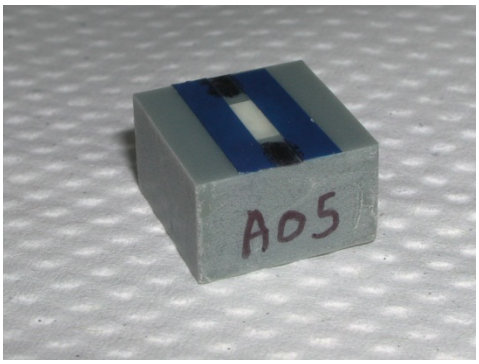


Figure 4 – A photograph of the optical profilometer used in the project (top) and a close-up photograph of the optical sensor with a specimen ready for scanning (bottom).

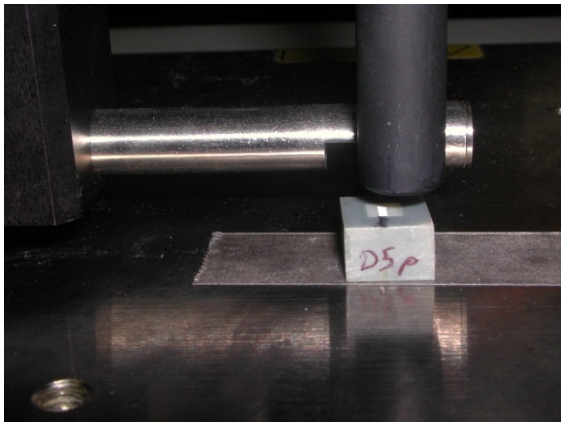


Figure 5 – An output screen from the optical profilometer analysis software.

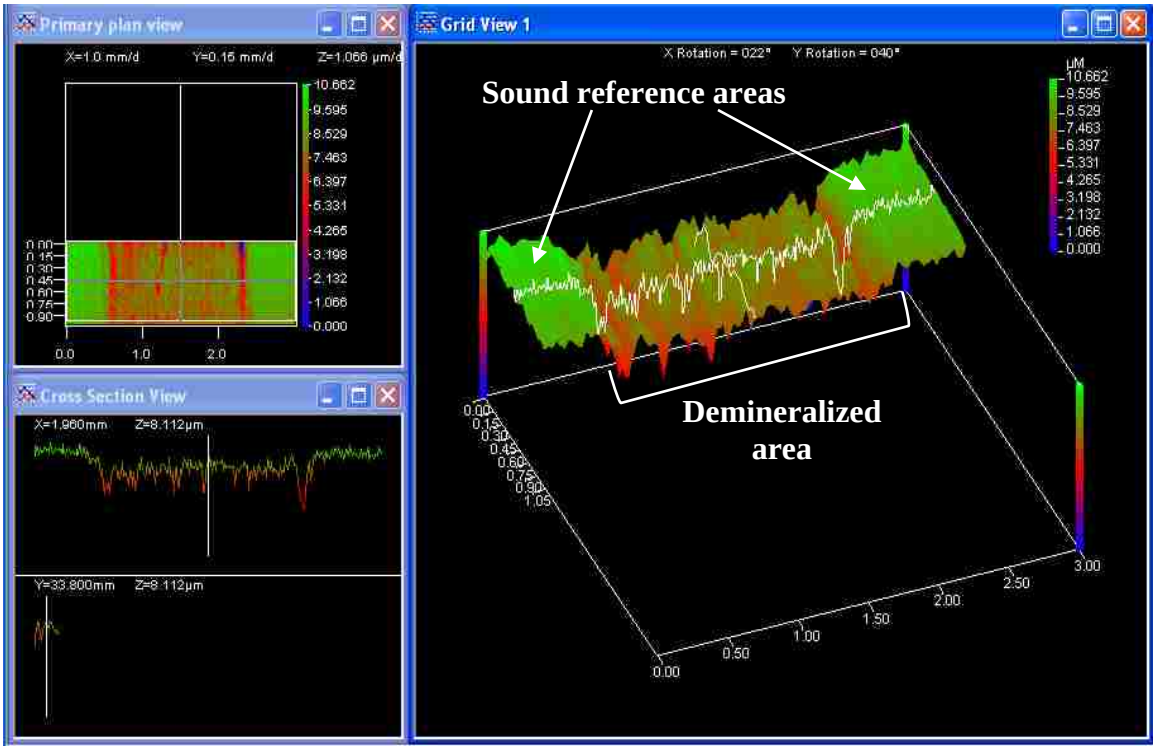


Figure 6 – Photographs of the automated brushing machine used in the project.

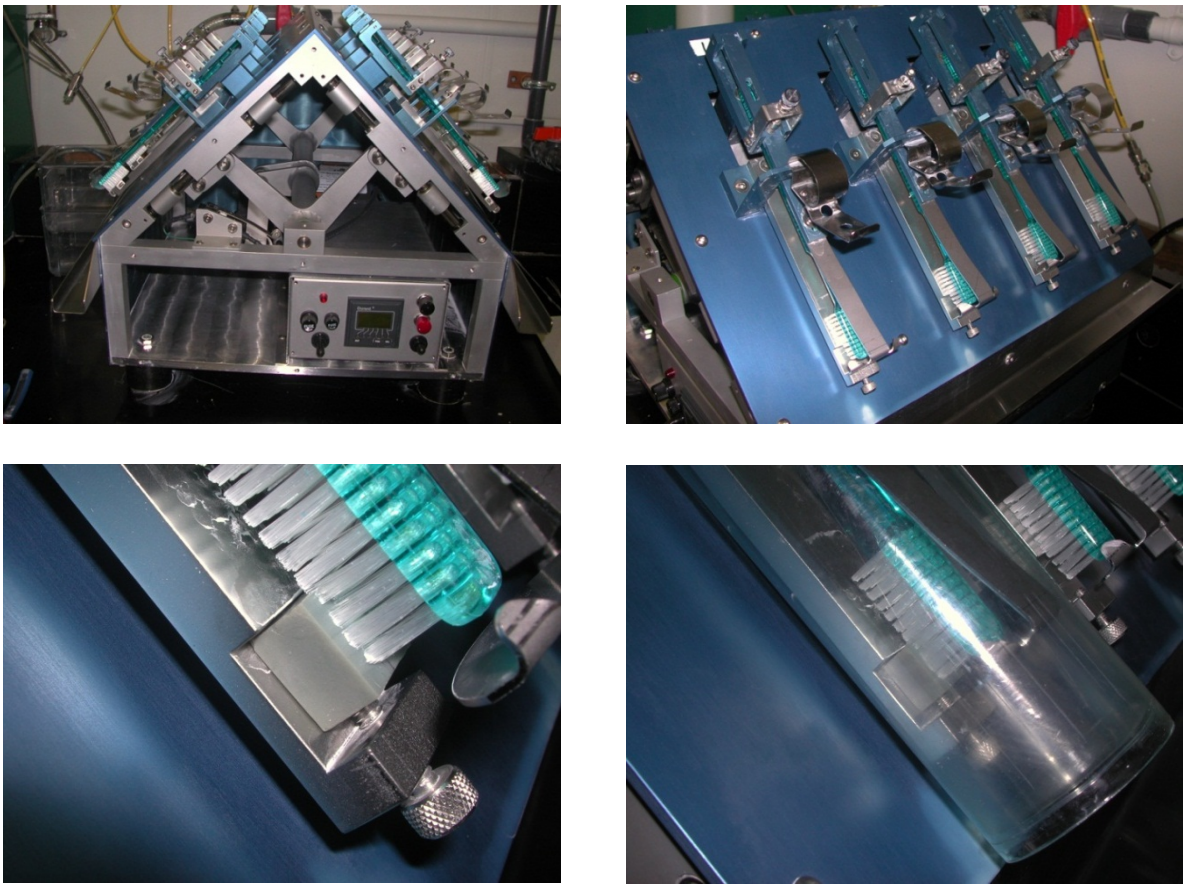
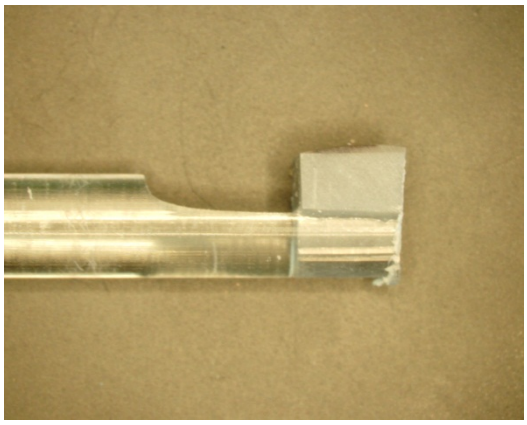


Figure 7 – Photograph of the hard tissue microtome for obtaining sections for TMR (top). A specimen glued on plastic rods before sectioning (b). A section examined using a stereomicroscope (c).



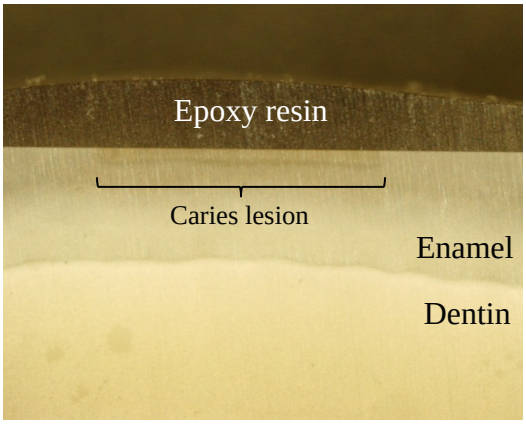
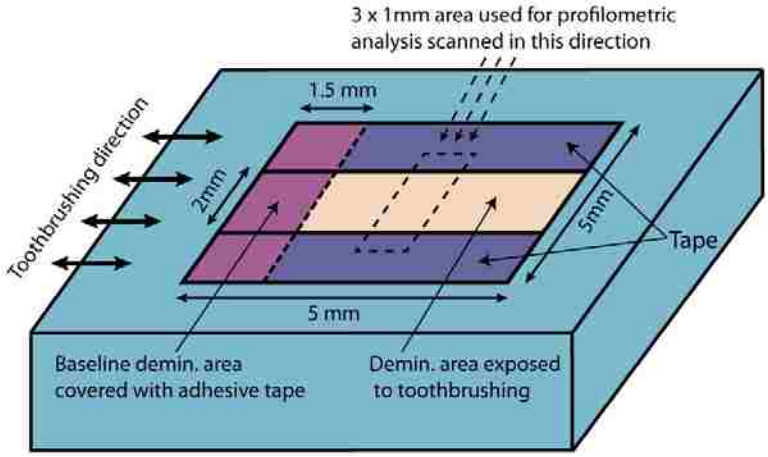


Figure 8 – A diagram illustrating the specimen setup used in study 2 (top). A photograph of the specimen after placing the adhesive tapes to protect the reference areas and the baseline demineralized area (bottom).



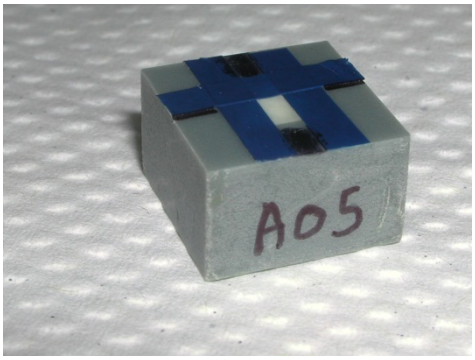


Figure 9 – The process of obtaining microradiography images. The X-ray machine (a), specimens sections arranged on a microscopic slide and wrapped in thin plastic (b), a microradiography plate after exposure and development in the dark room (c), a microscope attached to a digital camera used to examine the microradiography plates and to transfer images to a computer for analysis (d).

(a)

(b)

(c)

(d)

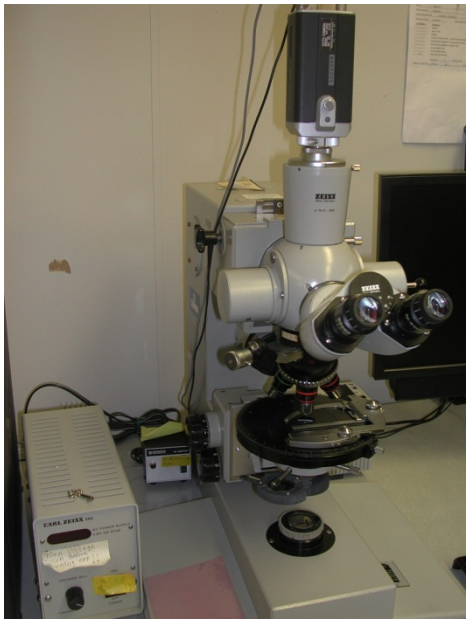


Figure 10 – An output screen from the TMR analysis software showing the mineral distribution curve of the specimen (a), calibration curve for the step-wedge (b), and the microradiography image of the specimen (c).

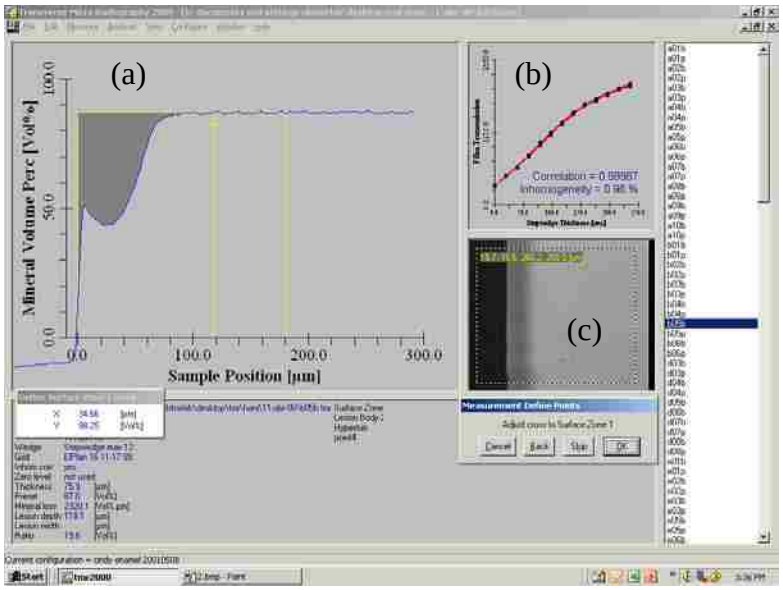


Figure 11 – A graph showing the parameters used in the TMR analysis.

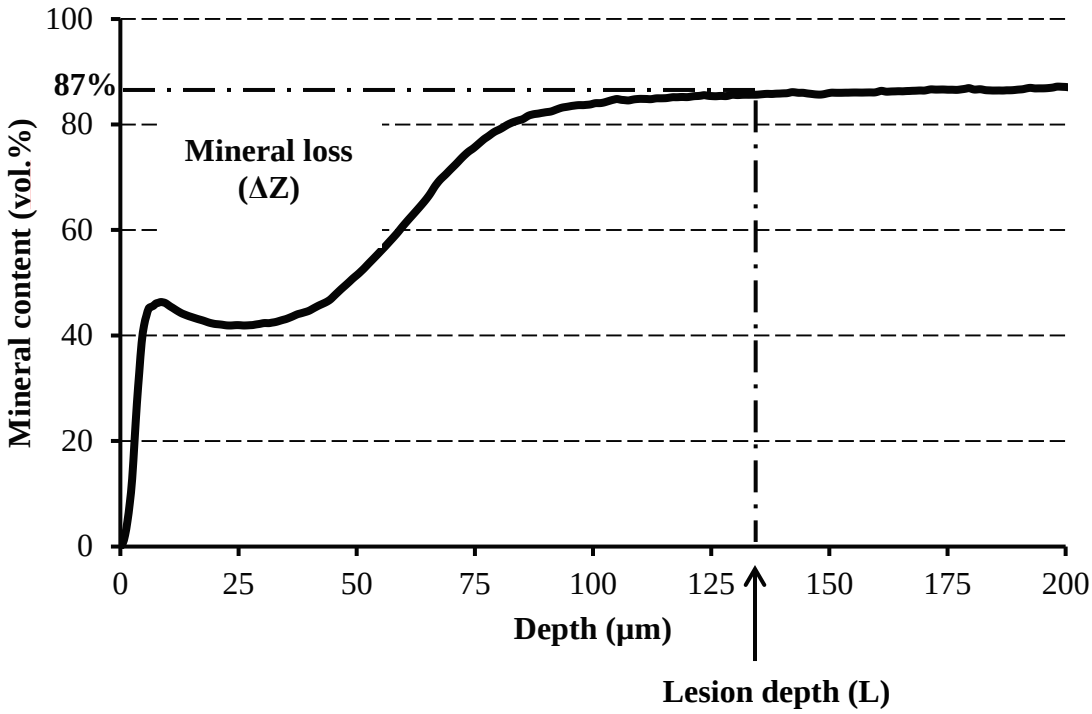


Figure 12 – Graphs showing a step-by-step procedure for deriving $\Delta(\Delta Z)$ and ΔL. Baseline parameters (ΔZ_{base} and L_{base}) are determined (top). Post-brushing parameters (ΔZ_{post} and L_{post}) are determined (middle). Final parameters are calculated (bottom) using formulas: $\Delta(\Delta Z) = \Delta Z_{base} - \Delta Z_{post}$ and $\Delta L = L_{base} - L_{post}$.

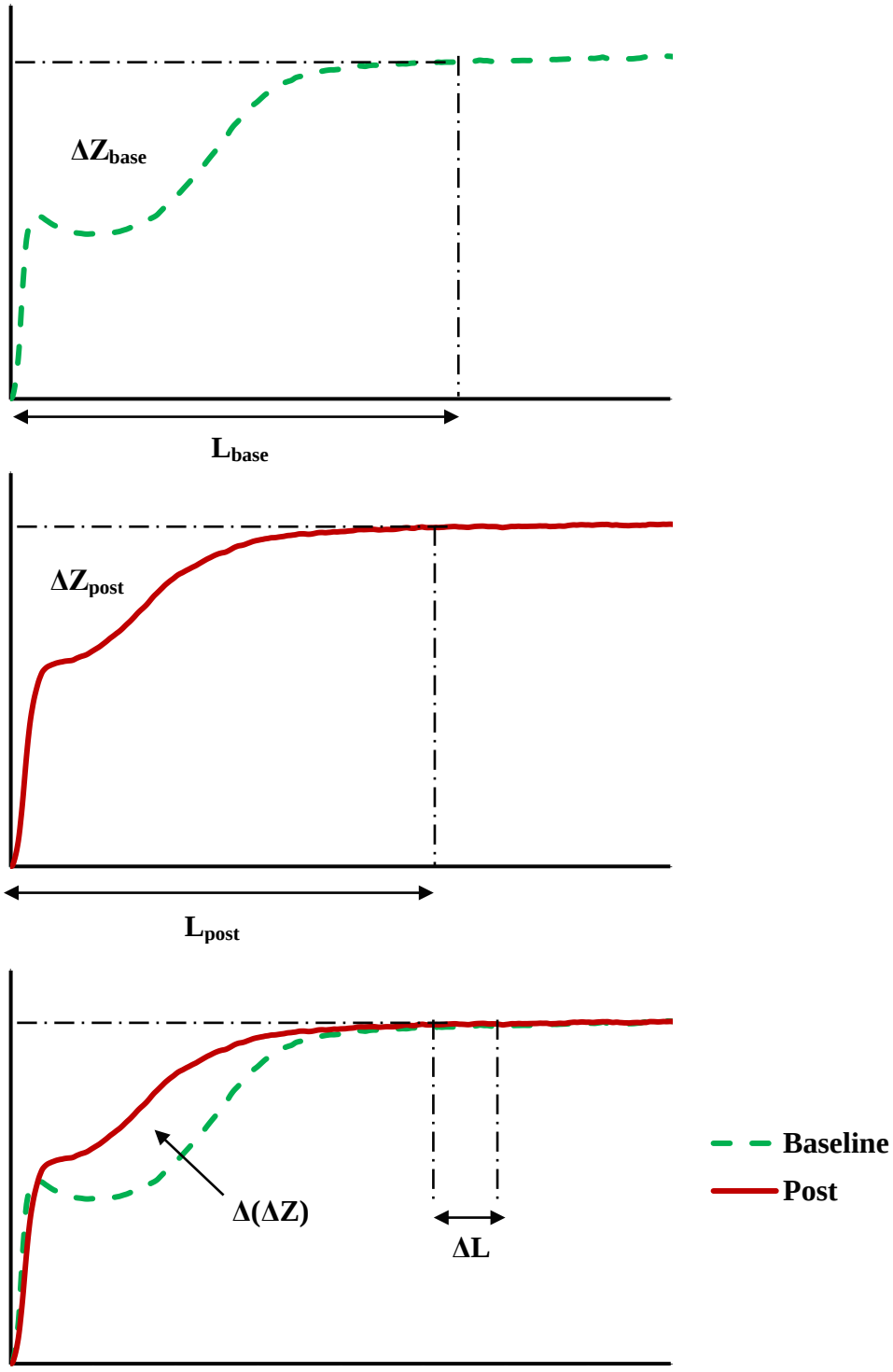


Figure 13 – Graphs showing a step-by-step procedure for deriving $\Delta(\Delta Z)_C$ and ΔL_C. Baseline parameters (ΔZ_{base} and L_{base}) are determined (top). Post-brushing parameters (ΔZ_{post} and L_{post}) are determined (middle). Final parameters are calculated (bottom) using formulas: $\Delta(\Delta Z)_C = \Delta Z_{base} - (\Delta Z_{post} + SL \times 87)$ and $\Delta L_C = L_{base} - (L_{post} + SL)$.

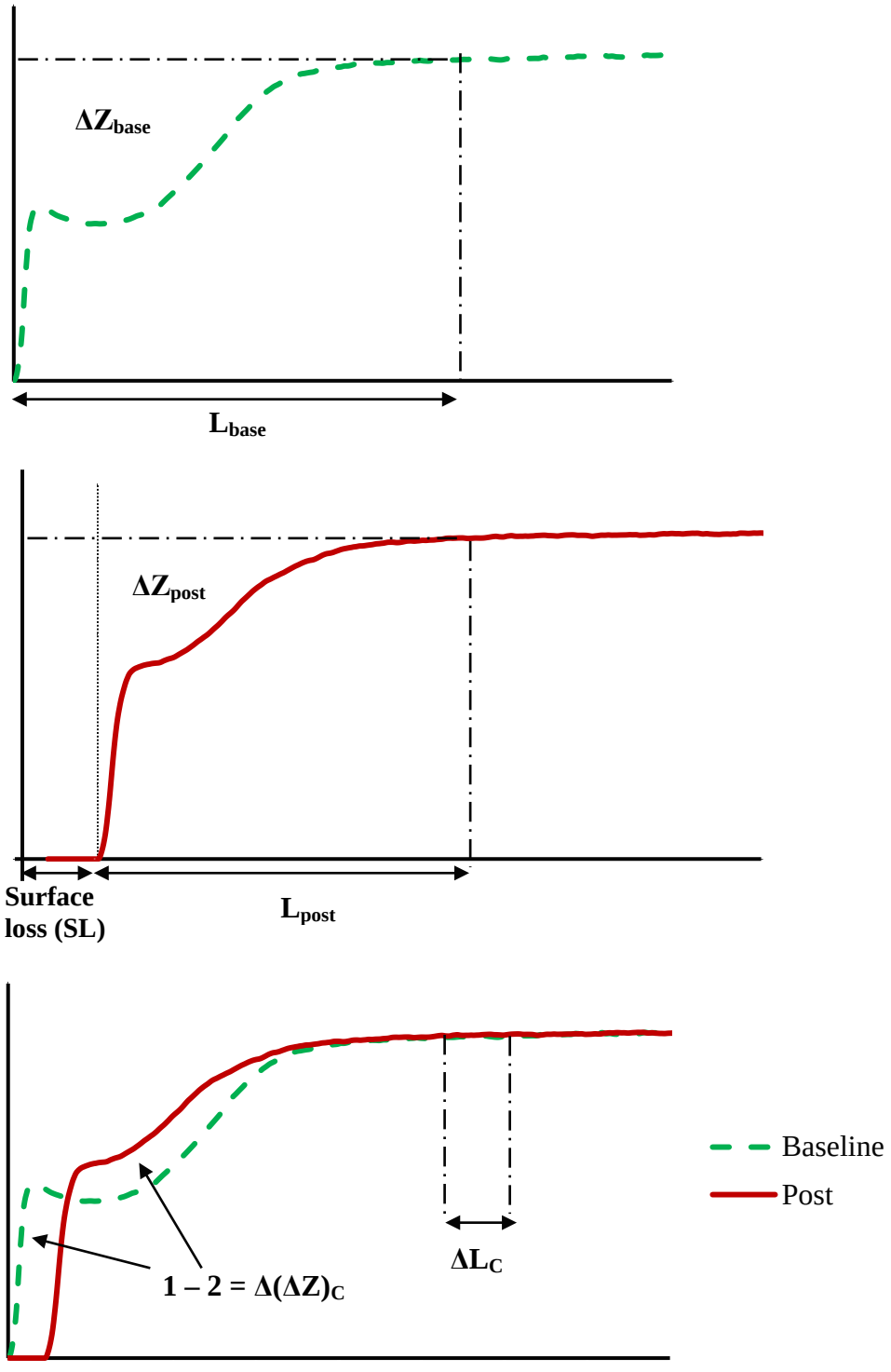
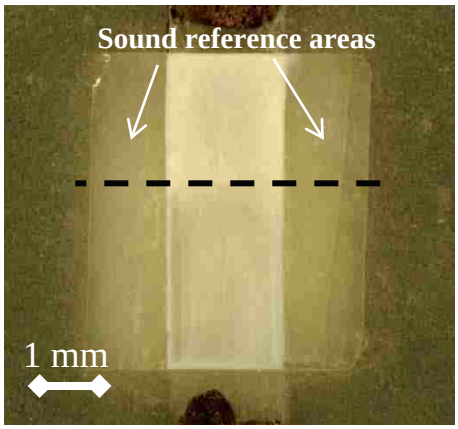
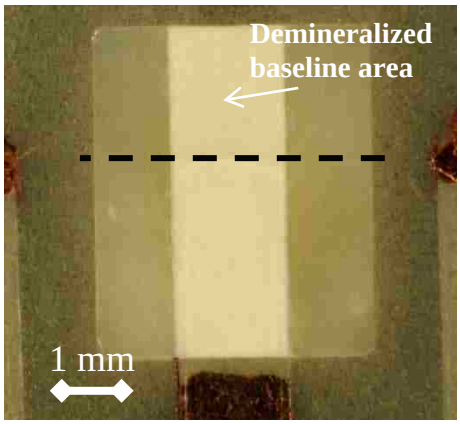


Figure 14 – Photographs of specimens' surface showing the sound reference areas, baseline demineralized area, and demineralized area subjected to toothbrushing. An MeC specimen (a), a CMC specimen (b), and an HEC specimen (c).

(a)



(b)



(c)

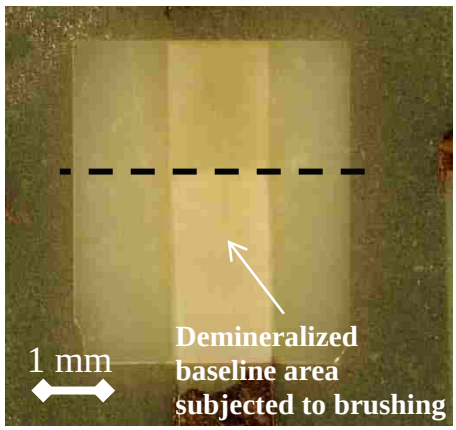


Figure 15 – A graph showing the average mineral distribution for MeC, CMC, and HEC lesions at baseline.

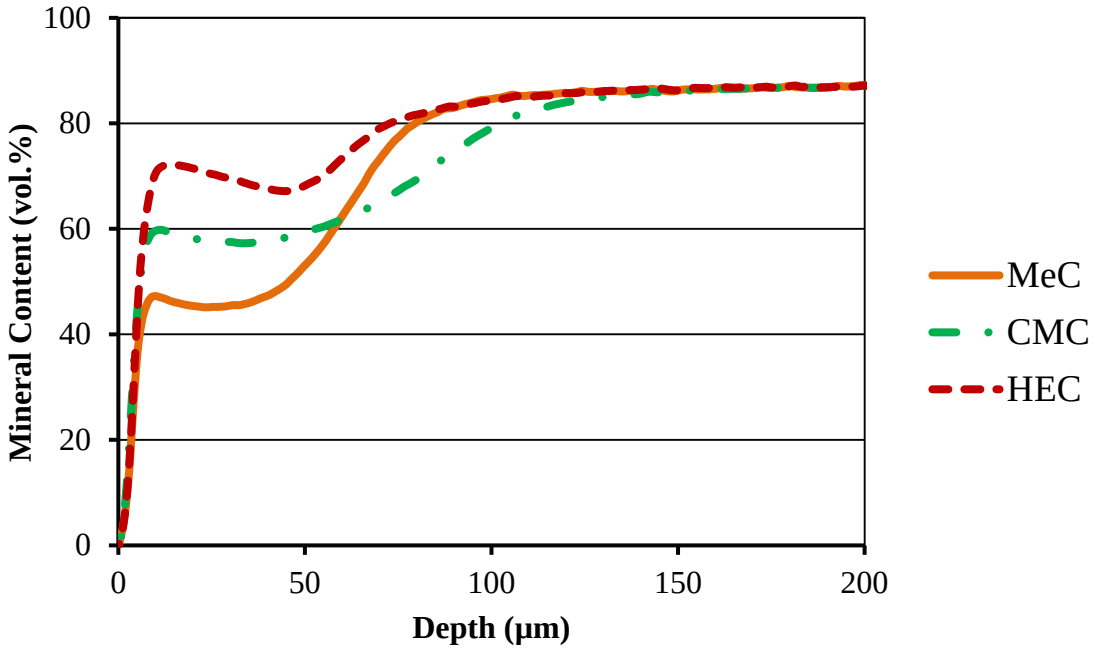
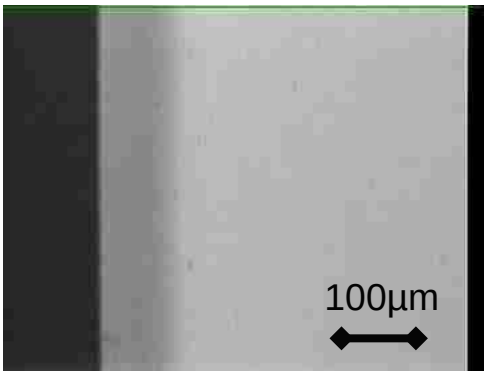
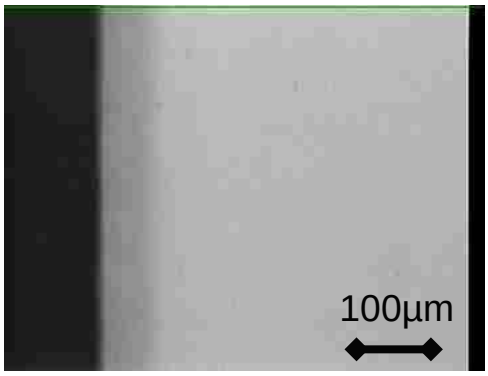


Figure 16 – Microradiography images of specimens from MeC groups brushed with low abrasive slurries. Baseline demineralized area in the 0 ppm fluoride group (a), post-brushing in the 0 ppm fluoride group (b), baseline demineralized area in the 275 ppm fluoride group (c), post-brushing in the 275 ppm fluoride group (d).

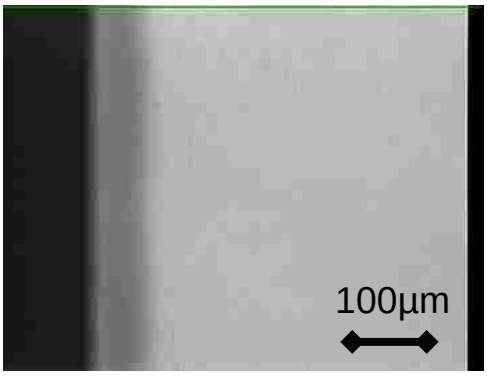
(a)



(b)



(c)



(d)

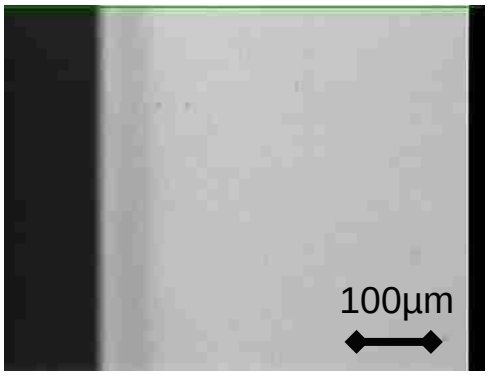
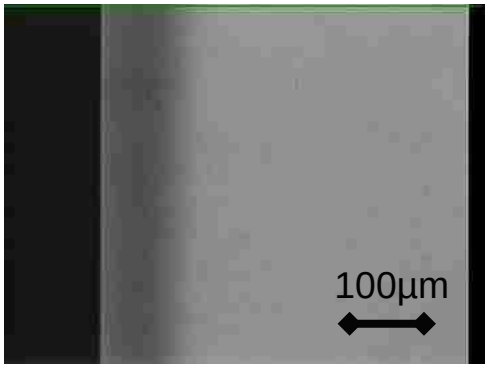
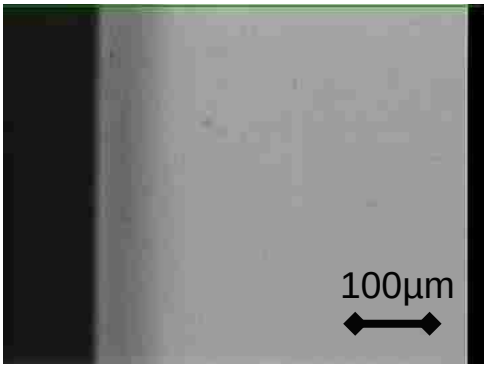


Figure 17 – Microradiography images for selected specimens from MeC groups brushed with high abrasive slurries. Baseline demineralized area in the 0 ppm fluoride group (a), post-brushing in the 0 ppm fluoride group (b), baseline demineralized area in the 275 ppm fluoride group (c), post-brushing in the 275 ppm fluoride group (d).

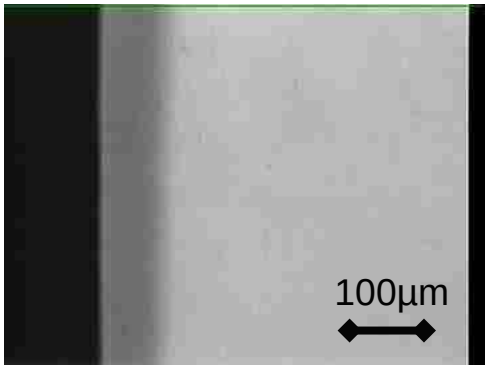
(a)

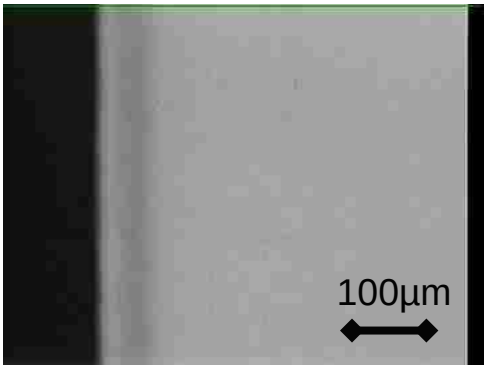




(b)

(c)





(d)

Figure 18 – Microradiography images of specimens from CMC groups brushed with low abrasive slurries. Baseline demineralized area in the 0 ppm fluoride group (a), post-brushing in the 0 ppm fluoride group (b), baseline demineralized area in the 275 ppm fluoride group (c), post-brushing in the 275 ppm fluoride group (d).

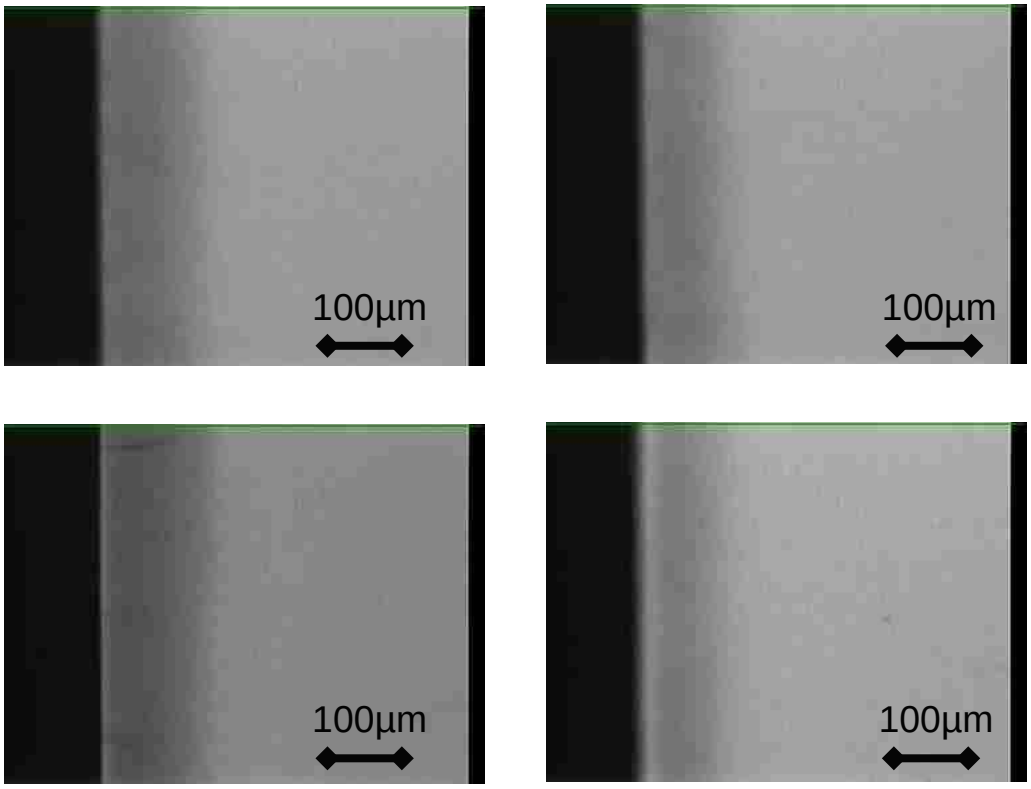


Figure 19 – Microradiography images for selected specimens from CMC groups brushed with high abrasive slurries. Baseline demineralized area in the 0 ppm fluoride group (a), post-brushing in the 0 ppm fluoride group (b), baseline demineralized area in the 275 ppm fluoride group (c), post-brushing in the 275 ppm fluoride group (d).

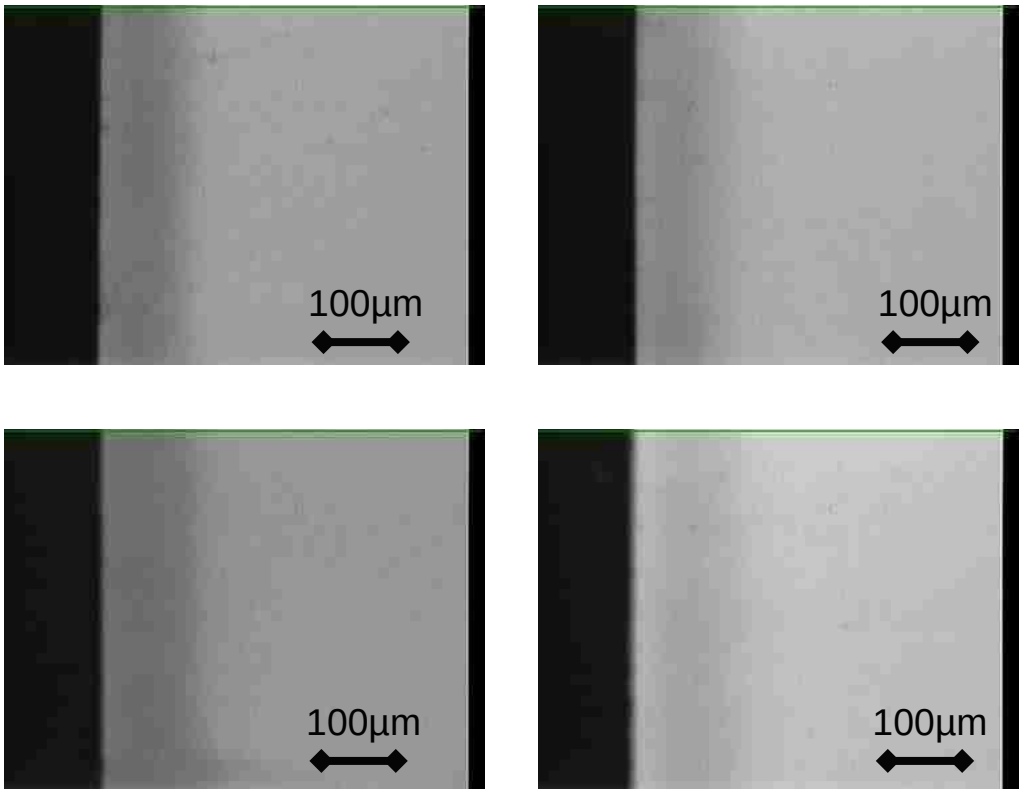


Figure 20 – Microradiography images of specimens from HEC groups brushed with low abrasive slurries. Baseline demineralized area in the 0 ppm fluoride group (a), post-brushing in the 0 ppm fluoride group (b), baseline demineralized area in the 275 ppm fluoride group (c), post-brushing in the 275 ppm fluoride group (d).

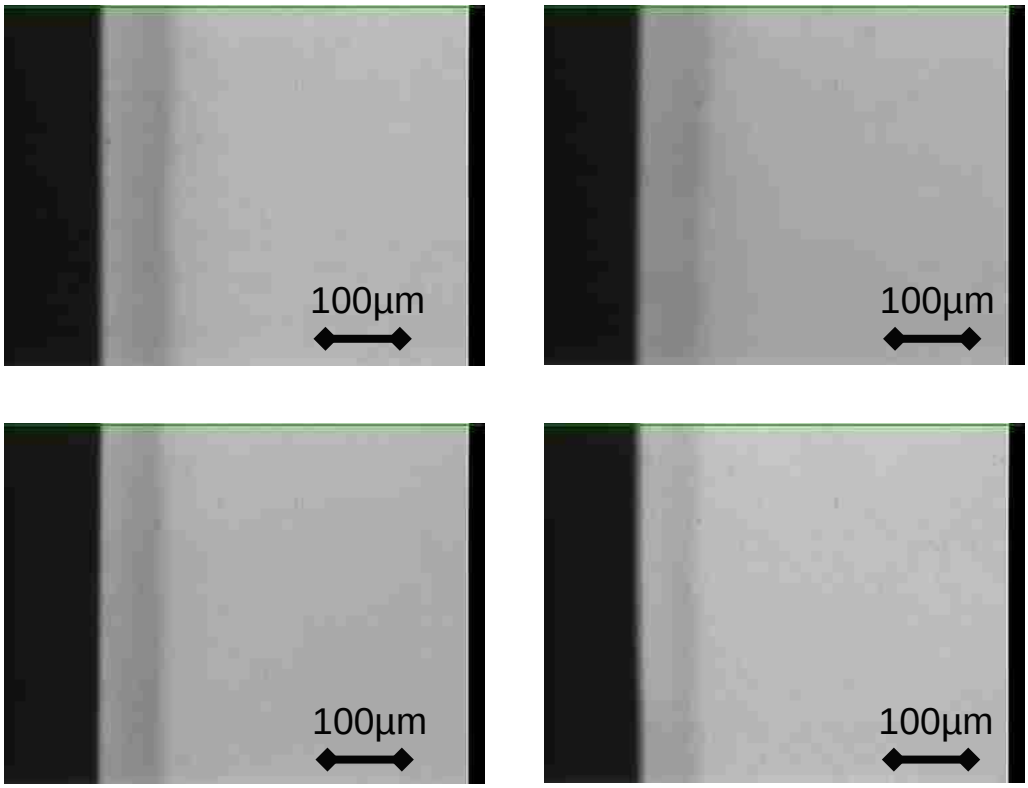


Figure 21 – Microradiography images for selected specimens from HEC groups brushed with high abrasive slurries. Baseline demineralized area in the 0 ppm fluoride group (a), post-brushing in the 0 ppm fluoride group (b), baseline demineralized area in the 275 ppm fluoride group (c), post-brushing in the 275 ppm fluoride group (d).

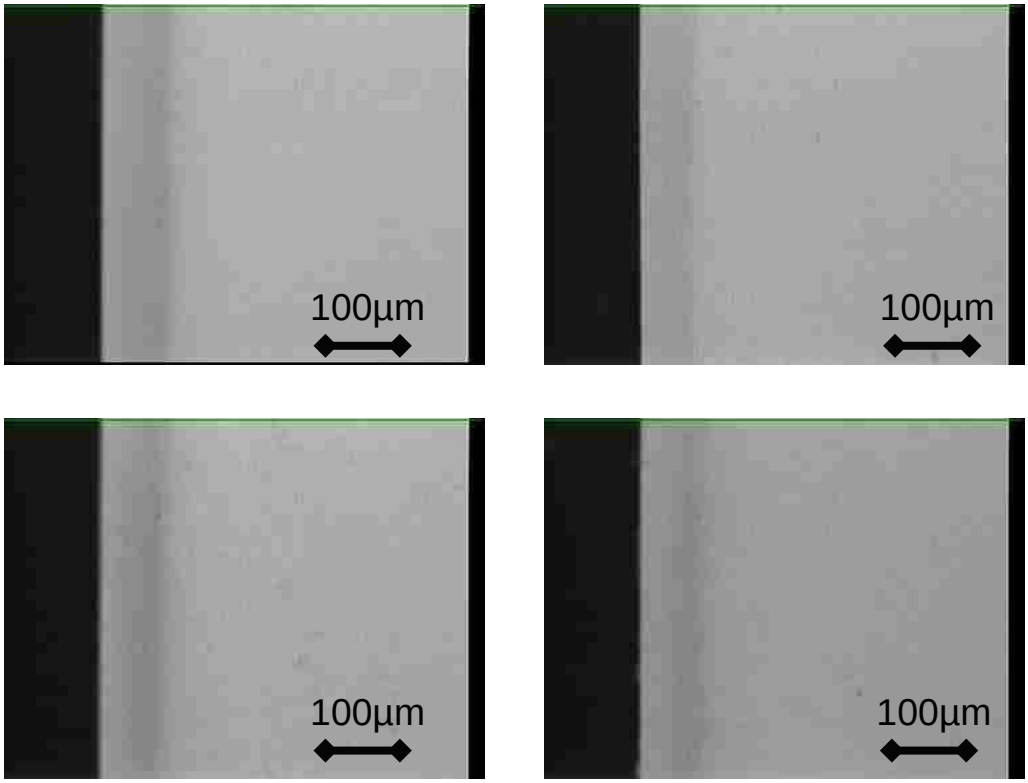


Figure 22 – Graphs showing the average mineral distribution at baseline and post-brushing for MeC lesions in groups subjected to low abrasive slurries with 0 ppm fluoride (top) and 275 ppm fluoride (bottom).

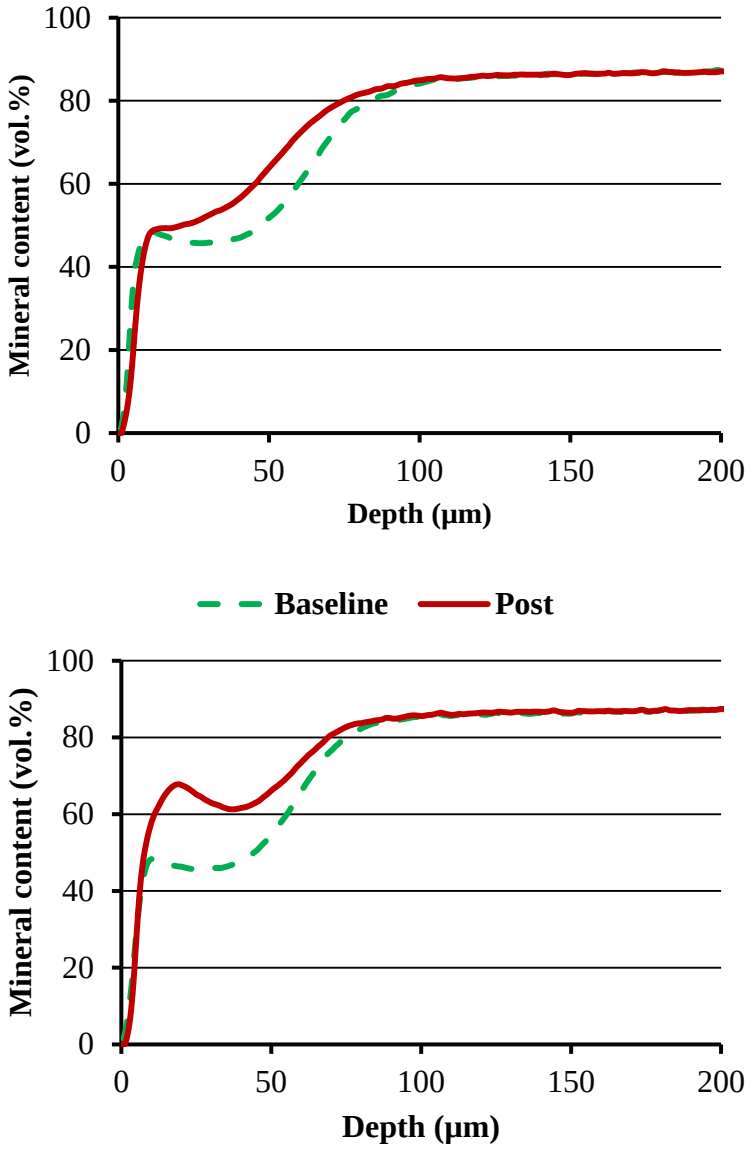


Figure 23 – Graphs showing the average mineral distribution at baseline and post-brushing for MeC lesions in groups subjected to high abrasive slurries with 0 ppm fluoride (top) and 275 ppm fluoride (bottom).

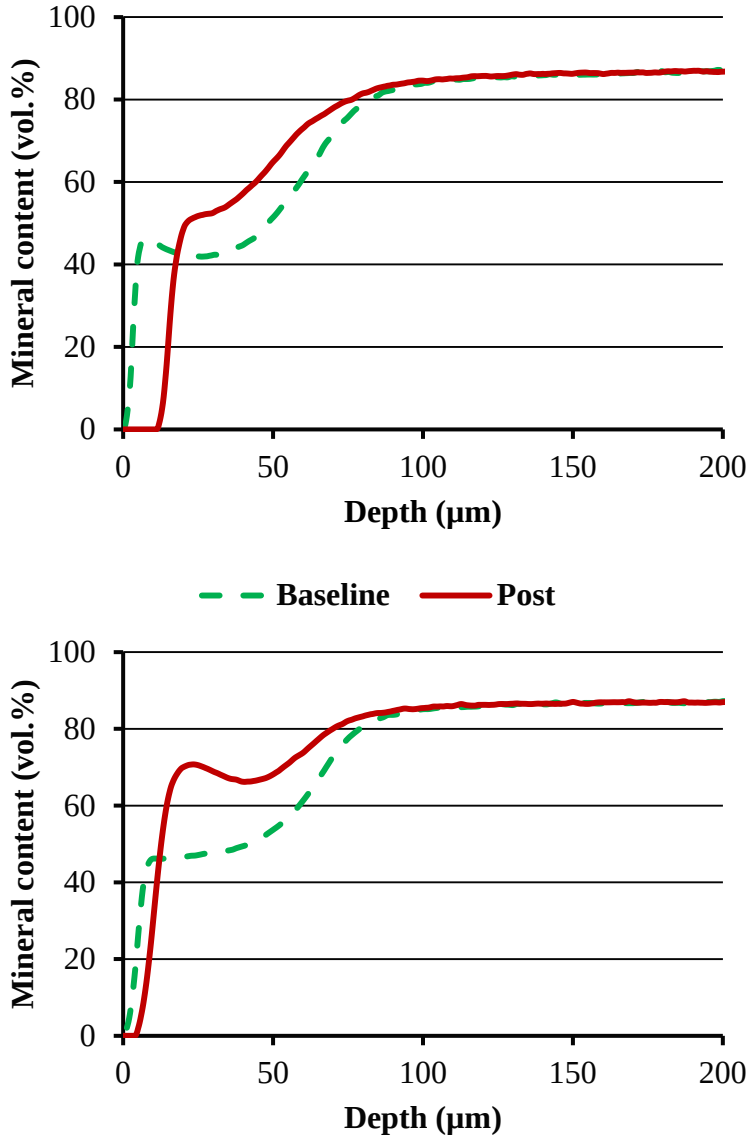


Figure 24 – Graphs showing the average mineral distribution at baseline and post-brushing for CMC lesions in groups subjected to low abrasive slurries with 0 ppm fluoride (top) and 275 ppm fluoride (bottom).

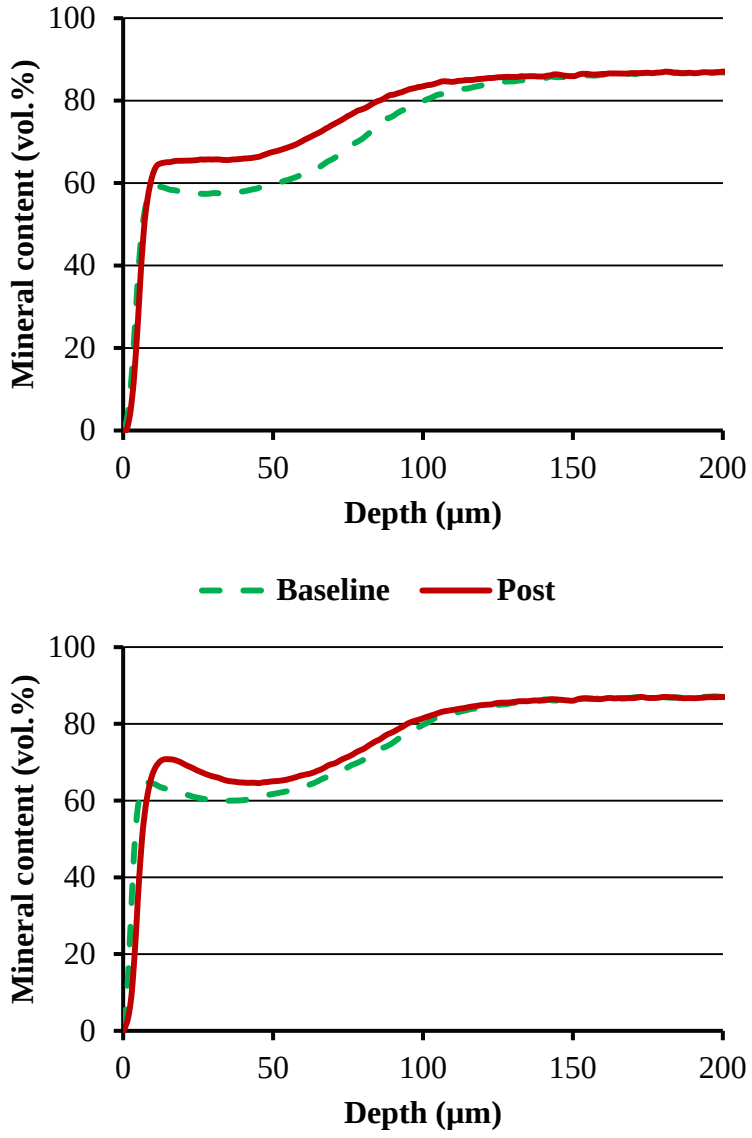


Figure 25 – Graphs showing the average mineral distribution at baseline and post-brushing for CMC lesions in groups subjected to high abrasive slurries with 0 ppm fluoride (top) and 275 ppm fluoride (bottom).

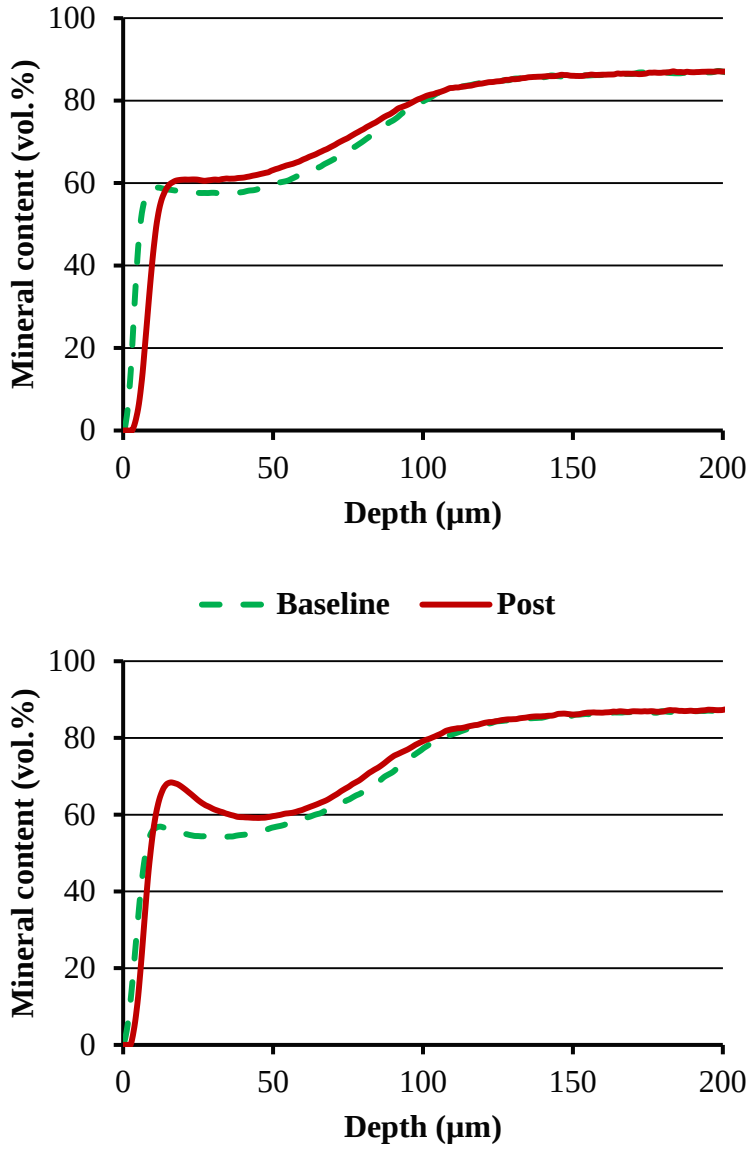


Figure 26 – Graphs showing the average mineral distribution at baseline and post-brushing for HEC lesions in groups subjected to low abrasive slurries with 0 ppm fluoride (top) and 275 ppm fluoride (bottom).

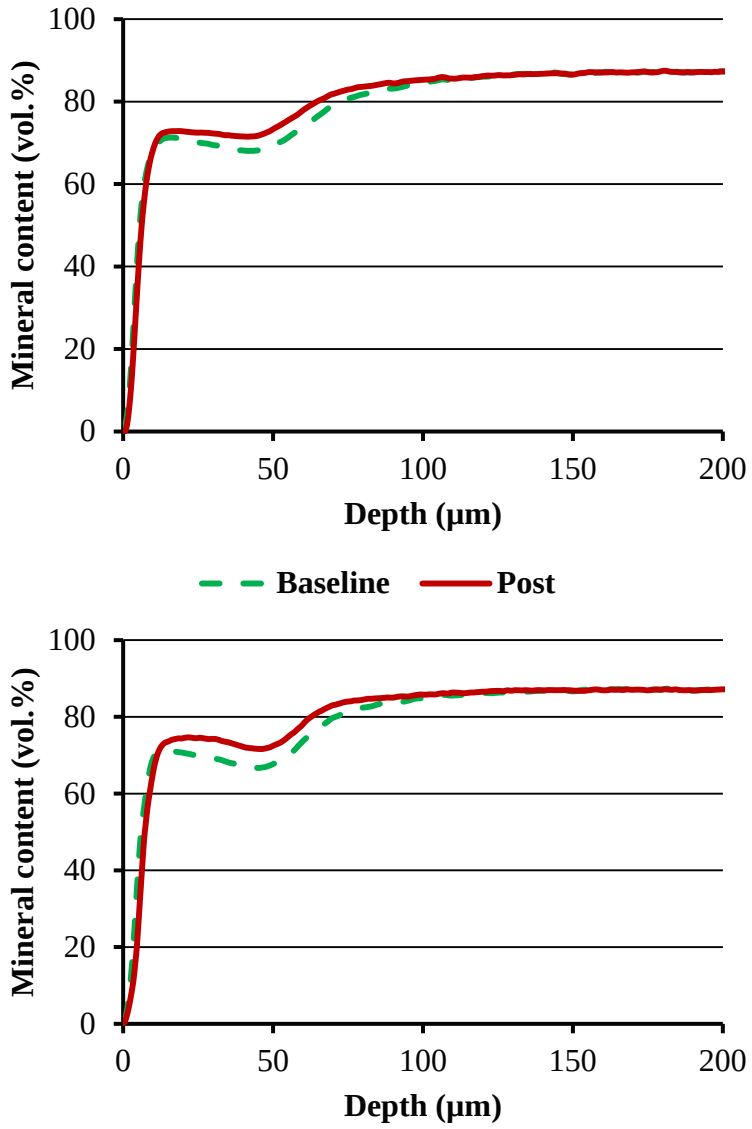


Figure 27 – Graphs showing the average mineral distribution at baseline and post-brushing for HEC lesions in groups subjected to high abrasive slurries with 0 ppm fluoride (top) and 275 ppm fluoride (bottom).

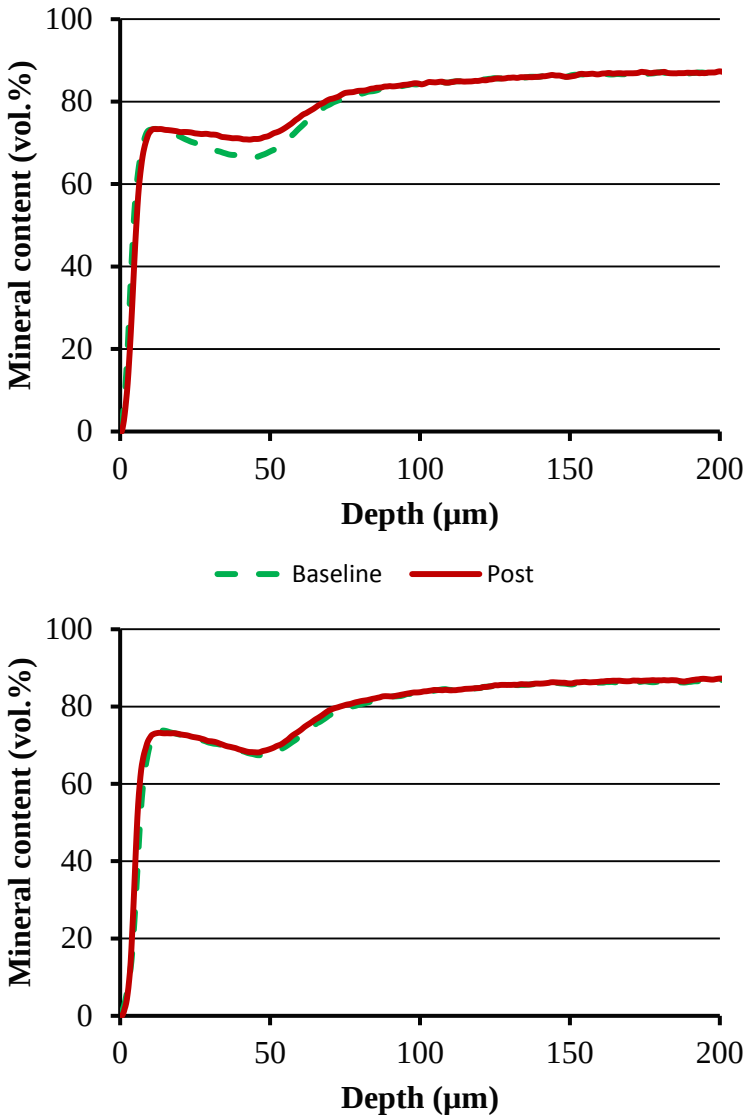


Figure 28 – Line graphs showing means of surface loss in CMC lesions in study 3 in groups brushed with low abrasive slurries containing 275 (top) and 1250 ppm fluoride (bottom). Different letters indicate significantly different (p<0.05) surface loss value (time effect) at each frequency level. For brushing frequency, 1x/day had significantly less surface loss than 3x/day at Day 5 (p=0.0122) and 7 (p=0.0011).

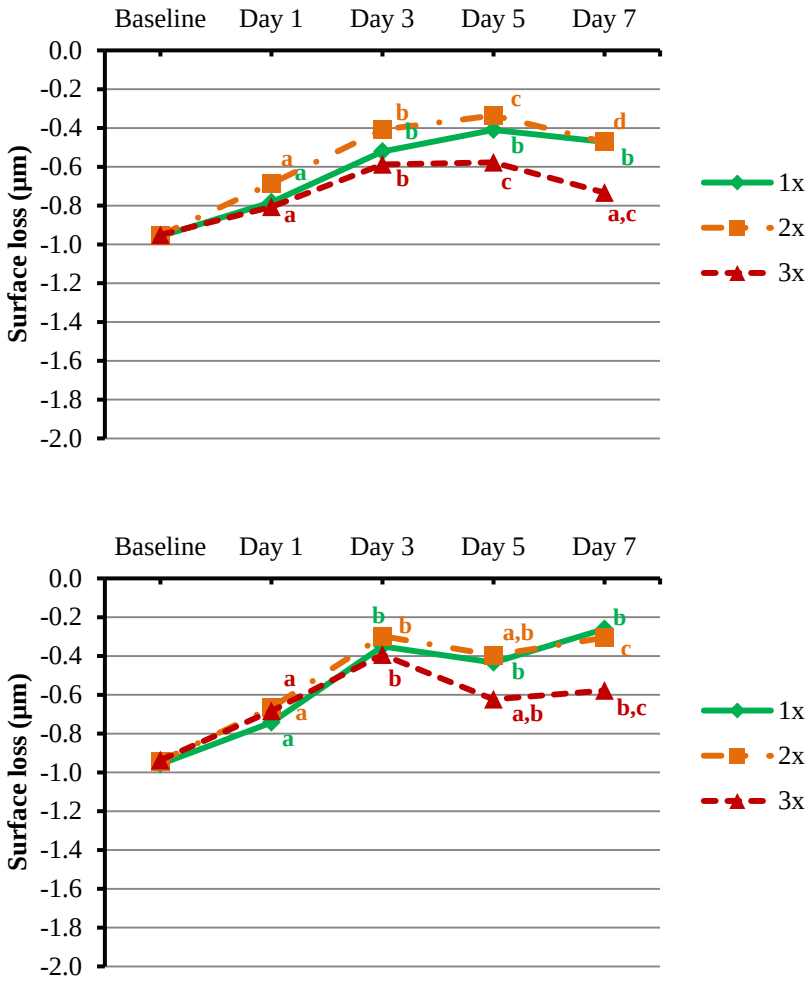


Figure 29 – Line graphs showing means of surface loss in CMC lesions in study 3 in groups brushed with high abrasive slurries containing 275 (top) and 1250 ppm fluoride (bottom). Different letters indicate significantly different (p<0.05) surface loss value (time effect) at each frequency level. For brushing frequency, 1x/day had significantly less surface loss than 3x/day at Day 5 (p=0.0122) and 7 (p=0.0011).

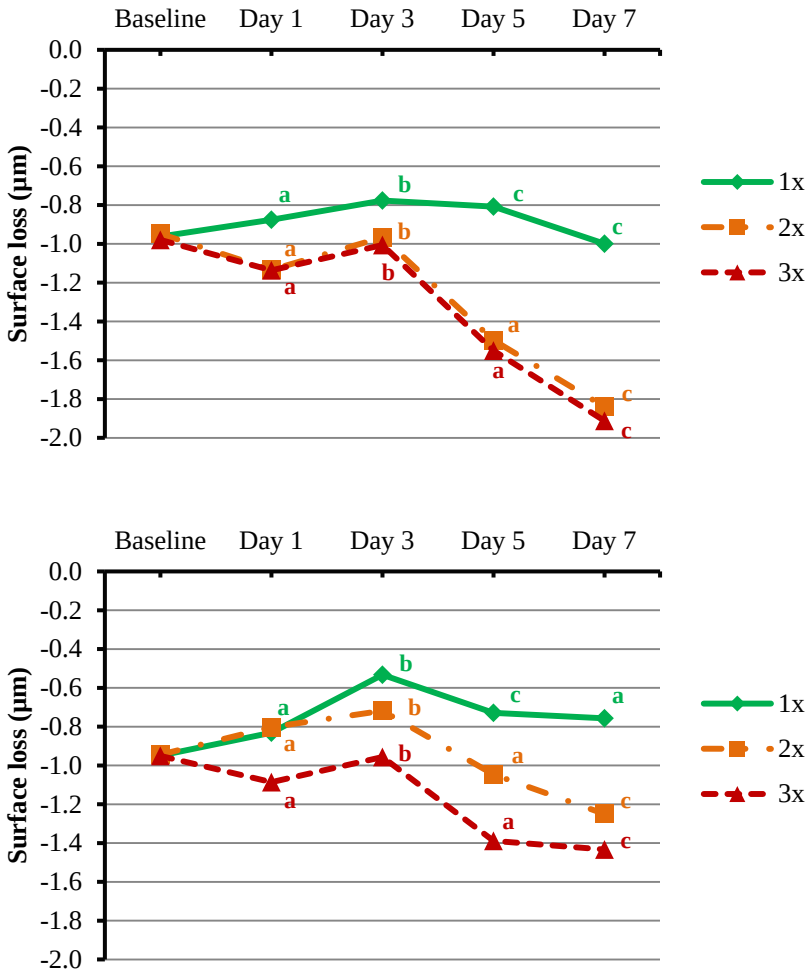


Figure 30 – Line graphs showing means of surface loss in MeC lesions in study 4 in groups brushed with low abrasive slurries containing 275 (top) and 1250 ppm fluoride (bottom). Different letters indicate significantly different (p<0.05) surface loss value (time effect) at each frequency level. For brushing frequency, 1x/day had significantly less surface loss than 3x/day at Day 5 (p=0.0122) and 7 (p=0.0011).

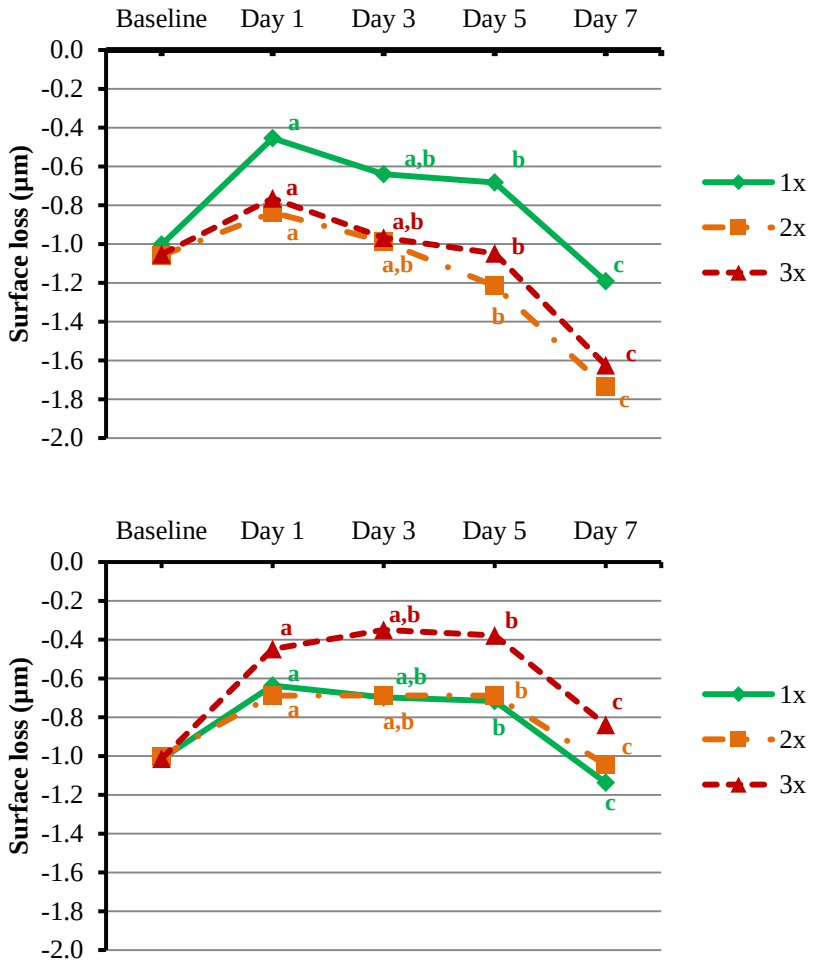


Figure 31 – Line graphs showing means of surface loss in MeC lesions in study 4 in groups brushed with high abrasive slurries containing 275 (top) and 1250 ppm fluoride (bottom). Different letters indicate significantly different (p<0.05) surface loss value (time effect) at each frequency level. For brushing frequency, 1x/day had significantly less surface loss than 3x/day at Day 5 (p=0.0122) and 7 (p=0.0011). [Scale of each graph is different].

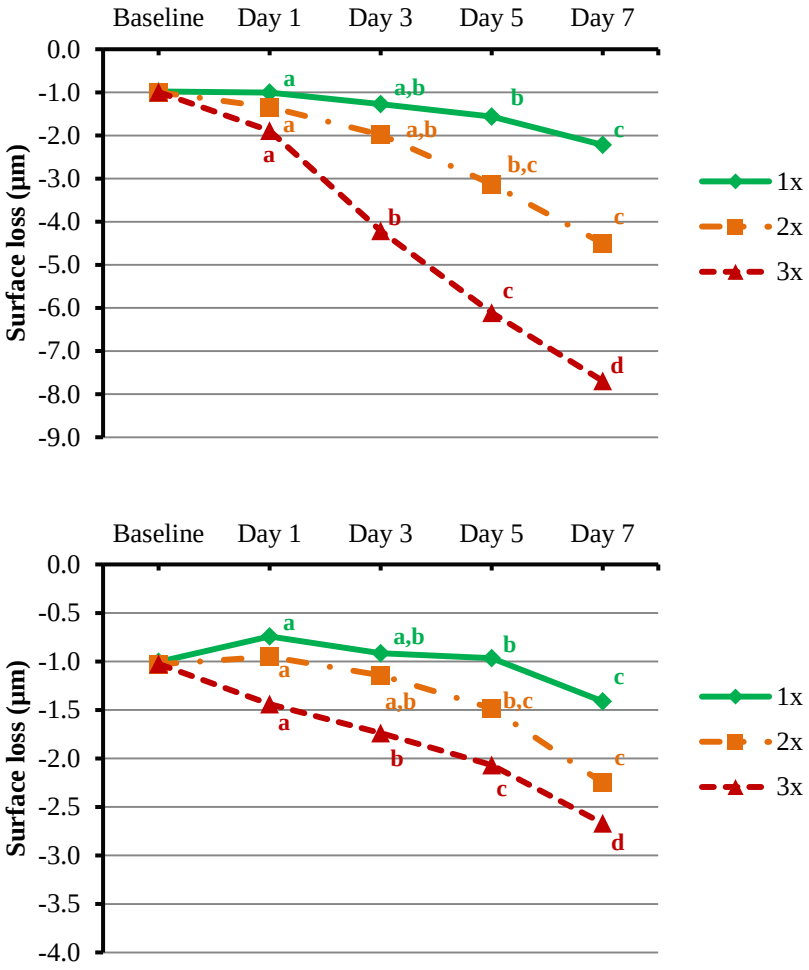
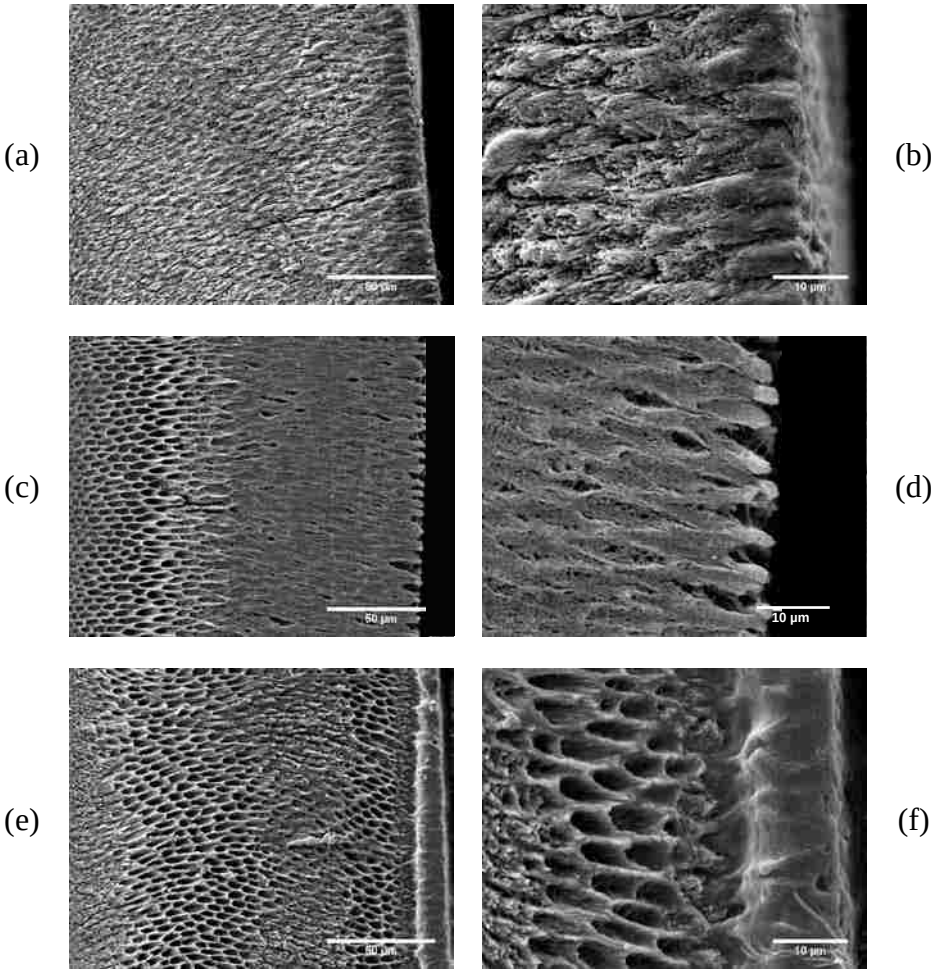


Figure 32 – Scanning electron microscopic images from sections of MeC lesion from study 4. Sound enamel at 1,000x (a) and 3,500x (b). Demineralized area at 1,000x (c) and 3,500x (d). Lesion after brushing at 1,000x (e) and 3,500x (f).



APPENDICES

Appendix A – Surface loss raw data in study 1.

Lesion Type	Fluoride (ppm)	Abrasive level	Specimen	Pre-brush	Surface loss (μm)		
					Day 1	Day 3	Day 5
MeC	0	low	A01	-0.70	-0.79	-1.25	-1.51
			A02	-0.92	-1.17	-1.89	-2.12
			A03	-0.52	-0.73	-0.79	-0.91
			A04	-0.78	-1.19	-2.07	-2.29
			A05	-0.38	-1.05	-1.84	-2.01
			A06	-0.20	-0.36	-0.73	-1.01
			A07	-0.36	-0.47	-1.02	-1.75
			A08	-0.71	-1.11	-1.87	-2.15
			A09	-0.25	-0.72	-1.30	-1.76
			A10	-0.32	-0.37	-0.80	-0.90
MeC	275	low	B01	-0.65	-0.85	-0.78	-0.87
			B02	-0.42	-0.86	-1.17	-1.32
			B03	-0.41	-0.88	-1.22	-1.63
			B04	-0.41	-0.86	-1.22	-2.27
			B05	-0.39	-1.44	-1.87	-2.35
			B06	-0.62	-1.14	-1.44	-1.68
			B07	-0.36	-0.34	-0.49	-0.37
			B08	-0.21	-0.28	-0.50	-0.42
			B09	-0.34	-0.44	-0.41	-0.43
			B10	-0.11	-0.29	-0.28	-0.43
MeC	0	high	C01	-0.51	-2.08	-6.70	-14.45
			C02	-0.89	-2.61	-6.73	-10.02
			C03	-0.79	-2.54	-4.26	-5.96
			C04	-0.72	-2.04	-8.17	-13.53
			C05	-0.38	-3.86	-15.60	-18.58
			C06	-0.38	-1.74	-4.92	-10.44
			C07	-0.26	-1.57	-5.41	-11.48
			C08	-0.15	-2.55	-6.67	-13.64
			C09	-0.14	-4.75	-14.64	-27.12
			C10	-0.33	-0.73	-2.86	-5.04
MeC	275	high	D01	-0.44	-1.30	-1.89	-2.77
			D02	-0.58	-1.68	-2.16	-3.95
			D03	-0.18	-1.07	-1.05	-1.98
			D04	-0.41	-1.67	-1.79	-2.96
			D05	-0.31	-1.17	-1.98	-2.72
			D06	-0.36	-1.92	-4.37	-6.98

			D07	-1.02	-2.41	-5.07	-7.84
			D08	-0.35	-0.86	-1.86	-4.29
			D09	-0.31	-1.41	-2.52	-4.56
			D10	-0.08	-1.23	-2.73	-4.94
CMC	0	low	E01	-0.99	-1.28	-1.58	-1.40
			E02	-0.64	-0.71	-1.08	-1.02
			E03	-0.27	-0.40	-0.43	-0.48
			E04	-0.31	-0.47	-0.74	-0.87
			E05	-0.50	-0.60	-0.97	-1.04
			E06	-0.23	-0.61	-0.59	-0.69
			E07	-0.64	-1.31	-2.15	-2.49
			E08	-0.31	-0.32	-0.55	-0.91
			E09	-0.48	-0.98	-1.65	-1.63
			E10	-0.16	-0.16	-0.27	-0.46
CMC	275	low	F01	-0.29	-0.47	-0.41	-0.38
			F02	-0.28	-0.48	-0.31	-0.28
			F03	-0.25	0.04	-0.12	-0.25
			F04	-0.69	-0.71	-0.85	-0.72
			F05	-0.40	-0.50	-0.50	-0.49
			F06	-0.64	-0.60	-0.80	-1.18
			F07	-0.54	-0.85	-0.86	-0.91
			F08	0.00	-0.04	-0.02	-0.17
			F09	-0.28	-0.28	-0.35	-0.62
			F10	-0.25	-0.44	-0.65	-0.69
CMC	0	high	G01	-0.13	-0.95	-1.36	-1.62
			G02	-0.61	-2.63	-2.65	-2.91
			G03	-0.81	-1.66	-1.67	-1.91
			G04	-0.58	-1.50	-1.73	-2.01
			G05	-0.57	-2.38	-3.05	-3.02
			G06	-1.34	-2.24	-5.15	-6.57
			G07	-0.18	-0.93	-2.91	-4.25
			G08	-0.45	-2.20	-3.74	-4.48
			G09	-0.19	-1.34	-3.20	-4.88
			G10	-0.27	-1.12	-2.43	-3.30
CMC	275	high	H01	-0.80	-2.03	-1.70	-1.71
			H02	-0.42	-1.41	-1.82	-2.11
			H03	-0.44	-1.18	-1.21	-1.30
			H04	-0.72	-1.59	-1.75	-1.92
			H05	-1.26	-2.49	-3.11	-2.20
			H06	-0.26	-0.69	-1.35	-1.61
			H07	-0.23	-1.01	-1.28	-1.87
			H08	-0.10	-1.42	-2.85	-3.82

			H09	-0.54	-1.21	-1.62	-1.81
			H10	-0.46	-2.18	-2.65	-3.28
HEC	0	low	I01	-0.22	-0.38	-0.44	-0.36
			I02	-0.64	-0.55	-0.64	-0.69
			I03	-0.54	-0.73	-0.66	-0.79
			I04	-0.42	-0.96	-0.99	-1.04
			I05	-0.35	-0.32	-0.40	-0.53
			I06	-0.23	-0.26	-0.46	-0.46
			I07	-0.11	-0.29	-0.33	-0.40
			I08	-0.01	-0.09	-0.10	-0.16
			I09	-0.06	-0.18	-0.43	-0.36
			I10	-0.18	-0.45	-0.55	-0.54
HEC	275	low	J01	-0.14	-0.41	-0.36	-0.25
			J02	-0.13	-0.24	-0.11	-0.30
			J03	-0.34	-0.77	-0.80	-0.88
			J04	-0.02	-0.21	-0.05	-0.09
			J05	-0.66	-0.81	-0.87	-0.87
			J06	-0.05	-0.09	-0.13	-0.16
			J07	-0.03	-0.05	-0.28	-0.18
			J08	-0.10	-0.14	-0.28	-0.38
			J09	-0.21	-0.22	-0.21	-0.19
			J10	-0.20	-0.36	-0.41	-0.43
HEC	0	high	K01	-0.06	-0.49	-0.55	-0.86
			K02	-0.05	-0.34	-0.53	-0.75
			K03	-0.20	-0.56	-0.81	-0.83
			K04	-0.05	-0.26	-0.35	-0.52
			K05	-0.15	-0.55	-0.66	-0.63
			K06	-0.02	-0.24	-0.45	-0.78
			K07	-0.20	-0.35	-0.26	-0.38
			K08	-0.33	-0.68	-1.05	-1.36
			K09	-0.06	-0.55	-0.99	-1.05
			K10	-0.11	-0.46	-0.57	-0.71
HEC	275	high	L01	-0.26	-0.63	-0.78	-0.77
			L02	-0.29	-0.40	-0.44	-0.47
			L03	-0.17	-0.57	-0.66	-0.59
			L04	-0.02	-0.52	-0.62	-0.75
			L05	-0.18	-0.28	-0.31	-0.46
			L06	-0.27	-0.44	-0.56	-0.58
			L07	-0.04	-0.65	-0.72	-1.03
			L08	-0.08	-0.32	-0.60	-0.90
			L09	-0.13	-0.47	-0.45	-0.52
			L10	-0.14	-0.27	-0.53	-0.53

Appendix B – Transverse microradiogrphy raw data in study 2.

Lesion type	Fluoride (ppm)	Abrasive level	Specimen	L baseline (μm)	ΔZ baseline (vol.%)	Δ(ΔZ) (vol.%)	$\Delta(\Delta Z)_C$ (vol.%)	ΔL (μm)	ΔL_C (μm)
MeC	0	low	A01	87.2	2650	999.7	1070	-23.9	-23.09
			A02	81.9	2650	886.0	990	-17.2	-16.01
			A03	83.3	2430	926.3	960	-16.4	-16.01
			A04	90.2	2790	498.9	630	-7.7	-6.19
			A05	84.6	3220	577.8	720	8.2	9.83
			A06	79.3	2320	139.7	210	-4.9	-4.09
			A07	100.5	3710	1739.6	1860	-30.7	-29.32
			A08	82.7	2130	-494.8	-370	0.4	1.83
			A09	90.7	2460	-611.5	-480	-1.7	-0.19
			A10	74	2290	529.5	580	-11.7	-11.12
MeC	275	low	B01	79.2	2490	1180.8	1200	-16	-15.78
			B02	74	2560	1101.8	1180	-11.5	-10.60
			B03	73.3	2410	653.9	760	5.8	7.02
			B04	82.4	2740	1257.8	1420	-10.1	-8.24
			B05	67.6	2140	-409.8	-240	3.7	5.65
			B06	63.7	1680	408.0	500	1.9	2.96
			B07	69.9	2330	1109.1	1110	-3.6	-3.59
			B08	69.1	2040	571.5	590	-4.2	-3.99
			B09	85.4	2520	1121.7	1130	-16.9	-16.81
			B10	88.6	3070	1472.2	1500	-9.1	-8.78
MeC	0	high	C01	64.5	2060	-363.3	291.3	-9.4	4.55
			C02	73.4	2750	95.8	415.6	-5.2	3.93
			C03	97.1	3010	780.3	707.9	-22.6	-17.43
			C04	78.8	2570	-194.7	355.3	-16.8	-3.99
			C05	92.2	3310	776.6	1085.8	-50.2	-32.00
			C06	94.2	3190	494.8	851.8	-24.1	-14.04
			C07	90.3	3320	943.8	1300.6	-32.2	-20.98
			C08	74	1850	-1833.8	-1176.8	13.4	26.89
			C10	94.2	3030	810.9	845.9	-23.4	-18.70
MeC	275	high	D01	45.8	1090	-102.3	-24.8	16.7	19.03
			D02	82.7	2820	1546.5	1583.5	-18.1	-14.73
			D03	72.6	1970	603.2	760	-7.9	-6.10
			D04	77.2	2370	658.2	770.8	-12.7	-10.15
			D05	91.1	3020	1450.6	1337.3	-10.5	-8.09
			D06	75.3	2350	494.4	416.3	-0.7	5.92
			D07	89.9	2660	816.3	1049.4	-23	-16.18
			D08	76.9	2840	976.6	988.4	-6.3	-2.35

			D09	100.3	3020	1540.7	1409.5	-42.2	-37.96
			D10	75.8	2630	1196.8	1166.1	-18	-13.14
CMC	0	low	E01	121.1	2740	734.2	770	-10.4	-9.99
			E02	82	1700	36.6	70	-0.2	0.18
			E03	89	2330	1041.6	1060	-8.8	-8.59
			E05	125.6	2800	753.3	800	-8.6	-8.06
			E06	88.4	1950	619.2	660	-15.5	-15.03
			E07	101.8	2500	389.0	550	-12	-10.15
			E08	137.7	3330	1738.5	1790	-49	-48.41
			E09	109.2	2430	170.5	270	-9.3	-8.16
CMC	275	low	F01	120.6	3160	772.2	780	-9.1	-9.01
			F02	109.1	2320	490.3	490	0.5	0.50
			F03	120.9	2640	749.7	750	-11.6	-11.60
			F04	101.7	1680	-253.4	-250	-5	-4.96
			F05	99.4	1560	-497.7	-490	5.3	5.39
			F06	106.8	2730	1042.5	1090	-19.7	-19.15
			F07	95.8	2000	297.2	330	-1.3	-0.92
			F08	108.5	2110	855.0	870	-24.7	-24.53
			F09	110.6	2370	740.3	770	-19.2	-18.86
			F10	91.4	1780	-18.4	20	5	5.44
CMC	0	high	G01	120.7	2360	-450.1	-320	-7.4	-5.91
			G02	105.5	2690	329.8	-5.1	-5	-2.70
			G03	93.8	2020	-305.6	-210	7.6	8.70
			G04	119.4	3010	675.4	800	-18.3	-16.87
			G05	109.6	2830	366.9	449.4	-14.1	-11.65
			G07	134.1	3140	1216.4	943.1	-42.2	-38.14
			G08	84.7	1840	-230.1	641	7.5	11.52
			G09	95.9	2040	-38.6	382.9	-3.6	1.10
			G10	102.6	2750	-84.2	-340.6	8.3	11.34
CMC	275	high	H01	108.9	2700	-209.5	-130	3.7	4.61
			H02	110.7	2170	353.1	500	-7.7	-6.01
			H03	120	3040	1185.0	1260	-25.5	-24.64
			H04	115.4	2690	905.3	1010	-11.2	-10.00
			H05	135.3	3120	18.2	100	2.3	3.24
			H06	103.2	2690	-847.5	-730	15.8	17.15
			H07	115.1	3060	1267.4	1410	-26.2	-24.56
			H08	99.3	2730	576.3	699.5	-23.7	-19.98
			H09	97	2680	169.8	280	-4.4	-3.13
			H10	109.4	3020	734.8	1334.9	-9.9	-7.08
HEC	0	low	I01	67.9	1140	127.9	140	5.7	5.84
				53.6	850	-24.3	-20	4.2	4.25
				96	1610	468.9	490	-24.1	-23.86
				72.4	1070	153.9	170	0	0.19

				71.2	1380	300.0	320	-10.9	-10.67
				78.7	1460	434.9	460	-12.2	-11.91
				84.8	1650	566.6	580	-17.8	-17.65
				84.7	1180	-135.3	-110	3.8	4.09
				54.8	830	-221.2	-190	9.5	9.86
HEC	275	low	J01	82.9	1410	400.6	410	-25	-24.89
			J02	66.2	1310	415.2	430	-9.2	-9.03
			J03	69.5	1410	552.6	600	-7.5	-6.96
			J04	84.7	1660	773.5	780	-23.6	-23.53
			J05	86.9	1680	741.5	760	-23	-22.79
			J06	65	990	-189.7	-180	28.4	28.51
			J07	81.4	880	-22.7	-10	-19.9	-19.75
			J08	96.4	1670	495.0	520	-14.5	-14.21
			J09	76.3	1150	51.3	50	0.2	0.19
			J10	63	1160	340.1	360	-1.5	-1.27
HEC	0	high	K02	63.7	950	-211.0	-150	16.7	17.40
			K03	84	1580	184.8	240	-6.1	-5.46
			K04	80.8	1520	-71.2	-30	35.8	36.27
			K05	84.1	1370	-91.3	-50	9.7	10.18
			K06	82.1	1400	653.7	720	-28.6	-27.84
			K07	78.3	1180	-75.5	-60	6	6.18
			K08	102.6	1520	370.1	460	-33	-31.97
			K09	79.5	1340	193.7	280	-1	-0.01
			K10	77.5	1190	257.9	310	-13.9	-13.30
HEC	275	high	L01	77.5	1330	545.4	590	-17	-16.49
			L02	90.2	1590	103.8	120	15.4	15.59
			L03	83.4	1430	423.9	460	-8.7	-8.29
			L06	101.8	1550	-1217.6	-1190	21	21.32
			L07	97.5	1690	554.5	640	-20.7	-19.72
			L08	58.6	710	-301.1	-230	6.9	7.72
			L09	79.1	1040	-144.4	-110	-12.1	-11.71
			L10	102	1400	66.2	100	12.3	12.69

Appendix C – Surface loss raw data in study 3.

Abrasive level	Fluoride (ppm)	Brushing frequency	specimen	Surface loss (μm)				
				Baseline	1 day	3 days	5 days	7 days
low	275	1x	A1	-0.43	-0.52	-0.51	-0.47	-0.53
			A2	-0.58	-0.41	-0.33	-0.48	-0.37
			A3	-0.66	-0.89	-0.64	-0.48	-0.47
			A4	-0.82	-0.88	-0.69	-0.53	-0.58
			A5	-0.92	-1.12	-0.74	-0.62	-0.70
			A6	-0.96	-0.74	-0.22	-0.23	-0.20
			A7	-1.11	-0.73	-0.22	-0.31	-0.26
			A8	-1.19	-0.89	-1.03	-0.33	-0.80
			A9	-1.45	-0.76	-0.43	-0.47	-0.52
			A10	-1.45	-0.88	-0.40	-0.19	-0.30
low	275	2x	B1	-0.31	-0.50	-0.59	-0.41	-0.66
			B2	-0.63	-0.40	-0.16	-0.27	-0.13
			B3	-0.71	-0.63	-0.39	-0.33	-0.36
			B4	-0.79	-0.83	-0.56	-0.45	-0.51
			B5	-0.88	-0.65	-0.11	-0.17	-0.27
			B6	-0.98	-0.71	-0.31	-0.22	-0.30
			B7	-1.13	-0.89	-0.27	-0.42	-0.43
			B8	-1.20	-1.26	-0.96	-0.10	-1.15
			B9	-1.37	-0.66	-0.41	-0.38	-0.39
			B10	-1.53	-0.33	-0.33	-0.60	-0.54
low	275	3x	C1	-0.33	-0.55	-0.54	-0.19	-0.29
			C2	-0.54	-0.81	-0.54	-0.79	-0.93
			C3	-0.76	-1.17	-0.73	-0.76	-0.88
			C4	-0.77	-0.50	-1.14	-0.92	-1.13
			C5	-0.92	-0.77	-0.37	-0.37	-0.41
			C6	-0.96	-0.92	-0.81	-0.89	-0.99
			C7	-1.09	-0.60	-0.41	-0.38	-0.38
			C8	-1.23	-0.63	-0.34	-0.14	-0.27
			C9	-1.35	-1.10	-0.72	-1.05	-1.49
			C10	-1.57	-1.02	-0.28	-0.29	-0.57
high	275	1x	D1	-0.39	-0.41	-0.32	-0.45	-0.56
			D2	-0.58	-0.47	-0.92	-0.57	-0.94
			D3	-0.74	-0.62	-0.54	-0.45	-0.50
			D4	-0.77	-0.81	-1.14	-0.94	-1.18
			D5	-0.89	-1.59	-1.14	-1.36	-1.80
			D6	-1.04	-1.11	-0.93	-1.00	-1.16
			D7	-1.06	-1.05	-0.78	-1.13	-1.29
			D8	-1.15	-0.87	-0.14	-0.80	-0.90
			D9	-1.42	-1.04	-0.70	-0.62	-0.75

			D10	-1.59	-0.78	-1.17	-0.76	-0.91
high	275	2x	E1	-0.23	-0.97	-1.69	-2.41	-2.94
			E2	-0.58	-0.80	-0.40	-0.82	-1.02
			E3	-0.64	-1.22	-1.28	-1.36	-1.78
			E4	-0.76	-1.31	-1.18	-1.50	-2.05
			E5	-0.91	-0.74	-0.50	-0.65	-0.90
			E6	-0.98	-1.02	-0.65	-0.59	-0.86
			E7	-1.12	-1.20	-1.54	-2.38	-2.84
			E8	-1.15	-0.92	-0.68	-0.95	-1.12
			E9	-1.42	-1.65	-1.61	-2.37	-2.69
			E10	-1.69	-1.51	-0.14	-1.94	-2.22
high	275	3x	F1	-0.37	-0.29	-0.56	-0.74	-1.11
			F2	-0.55	-1.40	-0.17	-2.48	-2.58
			F3	-0.73	-0.83	-1.00	-1.17	-1.40
			F4	-0.79	-0.65	-0.94	-1.11	-1.33
			F5	-0.90	-1.68	-1.58	-1.84	-2.43
			F6	-1.00	-0.63	-0.72	-0.88	-1.25
			F7	-1.14	-1.32	-1.04	-1.54	-1.93
			F8	-1.18	-1.43	-1.19	-1.79	-2.39
			F9	-1.44	-1.43	-1.48	-2.01	-2.35
			F10	-1.69	-1.72	-1.40	-1.94	-2.37
low	1250	1x	G1	-0.25	-0.62	-0.40	-0.52	-0.31
			G2	-0.62	-0.49	-0.44	-0.47	-0.18
			G3	-0.69	-0.35	-0.42	-0.34	-0.14
			G4	-0.86	-0.59	-0.16	-0.14	-0.11
			G5	-0.96	-0.86	-0.57	-0.70	-0.54
			G6	-0.99	-0.66	-0.11	-0.29	-0.12
			G7	-1.13	-0.76	-0.21	-0.22	-0.26
			G8	-1.17	-1.19	-0.70	-0.88	-0.61
			G9	-1.28	-0.89	-0.30	-0.47	-0.23
			G10	-1.64	-1.01	-0.20	-0.32	-0.12
low	1250	2x	H1	-0.40	0.04	-0.11	-0.06	-0.14
			H2	-0.51	-0.31	-0.11	-0.14	-0.13
			H3	-0.73	-0.80	-0.50	-0.59	-0.64
			H4	-0.83	-0.62	-0.22	-0.48	-0.22
			H5	-0.87	-0.75	-0.50	-0.72	-0.37
			H6	-0.97	-0.59	-0.06	-0.02	-0.10
			H7	-1.10	-0.84	-0.34	-0.55	-0.35
			H8	-1.23	-0.82	-0.45	-0.41	-0.27
			H9	-1.32	-0.98	-0.21	-0.38	-0.35
			H10	-1.47	-0.99	-0.49	-0.62	-0.49
low	1250	3x	I1	-0.39	-0.70	-0.53	-0.67	-0.52
			I2	-0.49	-0.41	-0.11	-0.16	-0.26

			I3	-0.63	-0.55	-0.51	-0.39	-0.44
			I4	-0.85	-0.57	-0.30	-0.40	-0.56
			I5	-0.94	-0.70	-0.79	-1.27	-1.33
			I6	-0.98	-0.73	-0.13	-0.65	-0.47
			I7	-1.14	-0.75	-0.48	-0.83	-0.77
			I8	-1.18	-0.83	-0.33	-0.60	-0.64
			I9	-1.28	-0.76	-0.56	-0.87	-0.61
			I10	-1.48	-0.82	-0.20	-0.39	-0.19
high	1250	1x	J1	-0.41	-0.14	-0.18	-0.09	0.06
			J2	-0.53	-1.18	-1.87	-1.56	-2.25
			J3	-0.76	-0.84	-0.78	-0.81	-0.86
			J4	-0.79	-0.55	-0.09	-0.22	-0.17
			J5	-0.94	-0.87	-0.52	-0.95	-0.83
			J6	-0.99	-0.80	-0.22	-0.60	-0.31
			J7	-1.05	-0.72	-0.17	-0.36	-0.32
			J8	-1.19	-0.61	-0.16	-0.40	-0.45
			J9	-1.38	-1.41	-0.72	-1.02	-1.12
			J10	-1.46	-1.18	-0.64	-1.29	-1.31
high	1250	2x	K1	-0.45	-0.85	-0.44	-0.96	-1.07
			K2	-0.50	-0.60	-0.83	-1.90	-1.98
			K3	-0.75	-0.66	-0.53	-0.91	-0.99
			K4	-0.79	-0.49	-0.31	-0.73	-0.98
			K5	-0.87	-0.78	-0.55	-0.87	-0.64
			K6	-0.98	-1.43	-1.34	-1.90	-2.06
			K7	-1.05	-0.65	-0.49	0.83	-0.77
			K8	-1.22	-1.37	-1.01	-1.25	-1.38
			K9	-1.32	-0.13	-0.84	-1.30	-1.27
			K10	-1.50	-1.06	-0.84	-1.49	-1.36
high	1250	3x	L1	-0.30	-0.93	-0.84	-1.25	-1.22
			L2	-0.47	-0.54	-0.41	-0.44	-0.38
			L3	-0.72	-0.55	-0.51	-0.93	-0.72
			L4	-0.85	-1.14	-1.01	-1.43	-1.65
			L5	-0.86	-0.83	-0.44	-0.80	-0.60
			L6	-1.04	-0.95	-0.62	-1.02	-1.20
			L7	-1.11	-1.99	-2.13	-2.58	-3.18
			L8	-1.16	-1.00	-0.82	-1.04	-1.18
			L9	-1.29	-1.29	-1.40	-1.94	-2.00
			L10	-1.70	-1.64	-1.38	-2.46	-2.20

Appendix D – Surface loss raw data in study 4.

Abrasive level	Fluoride (ppm)	Brushing frequency	specimen	Surface loss (µm)				
				Baseline	1 day	3 days	5 days	7 days
low	275	1x	A1	-0.46	-0.35	-0.33	-0.43	-0.65
			A2	-0.90	-0.29	-0.51	-0.55	-1.40
			A3	-1.04	-0.30	-0.50	-0.52	-1.54
			A4	-1.13	-1.07	-1.43	-1.36	-1.82
			A5	-0.71	-0.59	-0.76	-0.86	-1.23
			A6	-0.81	-0.53	-0.61	-0.66	-0.84
			A7	-1.67	-0.15	-0.47	-0.49	-1.26
			A8	-1.30	-0.35	-0.50	-0.59	-0.79
low	275	2x	B1	-0.71	-0.32	-0.34	-0.47	-0.73
			B2	-1.07	-1.23	-1.34	-2.16	-2.34
			B3	-0.53	-0.48	-0.55	-0.69	-0.94
			B4	-1.15	-1.41	-1.53	-1.35	-1.22
			B5	-0.99	-0.52	-0.66	-0.82	-1.67
			B6	-0.81	-0.06	-0.35	-0.38	-1.28
			B7	-1.32	-2.10	-2.31	-2.95	-4.10
			B8	-1.90	-0.58	-0.82	-0.90	-1.63
low	275	3x	C1	-0.82	?	-0.64	-0.60	-0.90
			C2	-0.96	-1.52	-1.80	-2.19	-2.66
			C3	-1.38	-0.51	-0.76	-0.90	-1.53
			C4	-0.60	-0.72	-0.94	-1.03	-1.47
			C5	-1.02	-0.75	-1.20	-1.30	-2.22
			C6	-1.82	-0.71	-0.90	-1.01	-1.92
			C7	-0.65	-0.51	-0.71	-0.69	-1.27
			C8	-1.18	-0.64	-0.79	-0.66	-1.03
high	275	1x	D1	-1.27	-1.15	-1.47	-1.89	-3.38
			D2	-1.04	-2.24	-2.92	-3.53	-4.43
			D3	-0.65	-0.77	-1.20	-1.30	-1.87
			D4	-1.13	-0.78	-0.46	-1.01	-1.07
			D5	-1.49	-0.70	-1.11	-1.21	-2.06
			D6	-0.53	-0.38	-0.50	-0.64	-0.81
			D7	-0.89	-0.41	-0.58	-0.73	-1.45
			D8	-0.83	-1.61	-1.95	-2.17	-2.69
high	275	2x	E1	-0.71	-0.53	-0.52	-0.67	-0.63
			E2	-1.17	-1.63	-2.86	-5.45	-7.37
			E3	-1.40	-2.00	-3.86	-5.60	-7.41
			E4	-1.05	-0.87	-1.27	-1.50	-2.55
			E5	-0.98	-1.85	-2.24	-4.16	-6.74
			E6	-1.25	-2.60	-3.59	-5.89	-8.85
			E7	-0.54	-0.61	-0.73	-0.83	-1.32

			E8	-0.87	-0.65	-0.78	-0.89	-1.11
high	275	3x	F1	-1.31	-5.50	-16.57	-19.17	-21.40
			F2	-1.08	-1.47	-2.42	-3.45	-5.17
			F3	-1.54	-1.79	-2.62	-4.73	-6.00
			F4	-0.74	-1.23	-1.82	-2.96	-4.51
			F5	-0.91	-0.65	-1.02	-1.17	-2.14
			F6	-0.45	-0.61	-0.93	-1.02	-1.45
			F7	-0.73	-1.89	-2.94	-5.57	-7.21
			F8	-1.21	-1.95	-5.40	-10.84	-13.68
low	1250	1x	G1	-1.34	-0.45	-0.66	-0.67	-1.47
			G2	-0.99	-0.71	-0.88	-0.87	-0.98
			G3	-0.56	-0.37	-0.41	-0.45	-0.99
			G4	-1.08	-0.94	-1.00	-0.95	-1.22
			G5	-1.58	-1.55	-1.46	-1.56	-1.66
			G6	-1.10	-0.63	-0.74	-0.73	-1.56
			G7	-0.71	-0.25	-0.29	-0.30	-0.60
			G8	-0.76	-0.19	-0.15	-0.19	-0.63
low	1250	2x	H1	-1.52	-0.86	-0.87	-0.81	-1.16
			H2	-1.16	-1.16	-0.94	-1.01	-1.35
			H3	-1.01	-0.71	-1.09	-1.27	-2.08
			H4	-0.77	-0.57	-0.48	-0.40	-0.65
			H5	-0.57	-0.73	-0.68	-0.75	-0.98
			H6	-0.95	-0.79	-0.76	-0.73	-1.25
			H7	-0.67	-0.55	-0.52	-0.44	-0.41
			H8	-1.36	-0.13	-0.16	-0.11	-0.48
low	1250	3x	I1	-1.07	-0.32	-0.39	-0.44	-1.09
			I2	-1.17	-0.29	-0.13	-0.11	-0.93
			I3	-1.28	-0.93	-0.81	-0.84	-1.35
			I4	-0.97	-0.37	-0.14	-0.22	-0.52
			I5	-0.57	-0.25	-0.17	-0.19	-0.52
			I6	-0.73	-0.73	-0.67	-0.68	-0.87
			I7	-0.78	-0.32	-0.30	-0.35	-0.87
			I8	-1.54	-0.39	-0.19	-0.19	-0.57
high	1250	1x	J1	-0.72	-0.69	-1.04	-1.10	-1.31
			J2	-1.53	-0.71	-1.00	-1.04	-2.05
			J3	-0.75	-0.60	-1.13	-1.09	-1.55
			J4	-1.26	-0.96	-0.99	-1.01	-1.87
			J5	-0.61	-1.23	-1.20	-1.22	-1.42
			J6	-1.02	-0.97	-1.10	-1.16	-1.30
			J7	-1.23	-0.55	-0.52	-0.63	-1.14
			J8	-0.91	-0.23	-0.35	-0.49	-0.65
high	1250	2x	K1	-0.76	-0.59	-0.70	-0.75	-1.37
			K2	-1.74	-1.91	-2.86	-4.14	-5.89

			K3	-0.57	-0.94	-1.28	-1.35	-1.72
			K4	-0.63	-0.33	-0.41	-0.48	-0.70
			K5	-0.88	-1.51	-1.38	-1.86	-2.58
			K6	-1.05	-1.03	-0.83	-0.85	-1.07
			K7	-1.22	-0.25	-0.31	-0.37	-0.87
			K8	-1.37	-1.06	-1.36	-2.06	-3.82
high	1250	3x	L1	-0.96	-3.68	-5.70	-6.02	-7.30
			L2	-1.07	-1.23	-1.76	-2.53	-3.67
			L3	-1.10	-2.53	-2.75	-3.45	-3.75
			L4	-0.71	-1.05	-1.15	-1.51	-1.92
			L5	-0.42	-0.62	-0.62	-0.55	-0.83
			L6	-0.80	-0.97	-0.76	-0.80	-1.33
			L7	-1.37	-0.90	-0.69	-1.05	-1.47
			L8	-1.79	-0.53	-0.48	-0.64	-1.08

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