INTERACTION BETWEEN TOOTHPASTE ABRASIVITY AND TOOTHBRUSH FILAMENT STIFFNESS ON THE DEVELOPMENT OF EROSIVE-ABRASIVE LESIONS

by

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DEDICATION

To my Father and Mother for their prayers and encouragement. To my husband, Mahmud, for love, support, and encouragement. To my princess,Malak, for her smiles and laughs. To my cousin Anecie for his support and who encouraged me to pursue this degree.
Thank you all.

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INTRODUCTION

Tooth brushing is considered the most common method used to maintain good oral hygiene.[1] However, several studies have indicated that tooth brushing with toothpaste is a major contributor to dental abrasion. Moreover, it should be clarified that toothbrush abrasion is of minor importance to maintaining sound dental hard tissue,[2-5] especially for enamel, because most abrasives in toothpaste are softer than enamel, except for the rarely used hydrated alumina.[6] It has been reported, based on several *in-situ* studies, that the mean of enamel loss after 10 years is around 20 µm, which is considered clinically irrelevant.[1] On the other hand, various researchers have indicated that dentin is less resistant to abrasion, and the mean of dentin loss after 10 years of tooth brushing was estimated to be 1 mm.[1] However, several *in-vitro* and *in-situ* studies have shown that toothbrush abrasion is considered a significant risk factor for tooth wear and especially when associated with acid softening of enamel and dentin.[2,5,7] It has been suggested that after a short exposure to an erosion challenge (1 min to 3 min) the tooth surface will be softened by up to several hundred nanometers. This softened layer can be completely or partially removed depending on several factors related to tooth brushing.[1]

A number of factors have a potential impact on the abrasion process of dental hard tissue. These factors include the abrasivity and concentration of the toothpaste, brushing frequency, brushing duration, force of brushing, and toothbrush bristle stiffness.[5] However, the abrasivity of the toothpaste is the most important parameter that affects the abrasion process of dental hard tissue.

Toothpaste abrasivity is determined by the radioactive dentine abrasivity (RDA) value and radioactive enamel abrasivity (REA) value. RDA and REA values are numerical scales that determine the abrasivity of toothpaste, and they are used for comparison between toothpastes.[6,8] However, because sound dentin is more susceptible than enamel to abrasion, the RDA value is the main factor determining the abrasivity of toothpaste.[1] The REA and RDA impact on dental hard tissue has been investigated in several studies. These studies investigated whether toothpastes of high abrasivity might cause more harmful effects to the tooth surface than low-abrasion toothpastes. For sound and eroded dentin, the RDA of toothpaste was shown to be associated significantly with the amount of dentin wear in many studies.[9-11] However, for enamel, an *in-situ* study suggested that the RDA of toothpaste was of minor relevance to enamel wear, [2] whereas an *in-vitro* study reported a high association between toothpaste abrasivity and the abrasion of acid-softened enamel.[12]

Overall, it has been indicated that interpreting toothpaste abrasivity studies is very difficult, especially because the RDA and REA of brands obtained from different sources may differ depended on the country of origin.[13,14]

Toothbrushes also play a role in the abrasive process; they are considered to be the delivery vehicles that modulate the action of toothpaste abrasivity.[4,12] Several toothbrush variables may have an influence on dental hard tissue, such as filament stiffness, the brand of tooth brush (as hardness among brands is not consistent), time and frequency of brushing, and brushing force.[11,12,15] Several studies indicated that toothbrush filament stiffness is an important parameter that may affect tooth wear and its extent, depending on toothpaste abrasivity.[9,16]

Patients suffering from erosion are typically instructed to brush their teeth with a low-RDA toothpaste and a toothbrush with soft filaments to reduce abrasion of the eroded tooth surface.[11] This is done even though the impacts of toothbrush filament stiffness and toothpastes of different abrasivities are not fully understood.

A need exists to accurately assess the interaction between toothbrushes and different abrasive levels on dental tissue. Little research has taken place using a clinically relevant erosion-abrasion cycling model. Previous studies were limited in their approach; many studies used non-fluoridated toothpaste, several hundred to several thousand brush strokes, and erosion-abrasion treatments without remineralization in between. Our present *in-vitro* investigation aimed to resolve the interaction between toothbrushes and the abrasives used in toothpastes using a clinically relevant erosion/abrasion cycling model.

OVERALL AIM

The objective of this *in-vitro* study was to investigate the interaction between different abrasive levels of toothpaste and toothbrush filament stiffness on the development of erosion/abrasive lesions. Specific focus: The surface loss of eroded enamel and dentin surfaces subjected to brushing abrasion with soft, medium and hard toothbrush associated to high and low abrasive slurries.

Null Hypotheses

1. The interplay between the abrasive levels of toothpaste and different types of toothbrushes cannot modulate tooth-brushing abrasive wear on eroded enamel and dentin.

2. The amount of enamel and dentin loss is not dependent on the abrasivity of the toothpaste.

3. The amount of enamel and dentin loss is not affected by the filament stiffness of the toothbrush.

4. The amount of enamel and dentin loss is not affected by the cycling time.

Alternative Hypothesis

1. The interplay between the abrasive levels of toothpaste and different types of toothbrushes can modulate tooth-brushing abrasive wear on eroded enamel and dentin.

2. The amount of enamel and dentin loss is dependent on the abrasivity of toothpaste.

3. The amount of enamel and dentin loss is dependent on the filament stiffness of the toothbrush.

4. The amount of enamel and dentin loss is affected by cycling time.

REVIEW OF LITERATURE

Tooth wear can be described as a loss of dental hard tissue by processes other than dental caries. The term tooth wear is also used to describe a variety of mechanisms that cause tooth surface loss, e.g. attrition, abrasion, erosion and abfraction. These terms have been used when the specific etiological factor are known.[17] It has been shown in a number of clinical and experimental observations that the interaction between different types of tooth wear is very common, especially between abrasion and erosion. In addition, this interaction is considered the major factor in tooth wear,[18,19] and leads to significant increased surface loss of dental hard tissue.[3,20]

THE ROLE OF EROSION IN THE ETIOLOGY OF TOOTH WEAR

Dental erosion is a multifactorial condition that plays a major role in tooth wear. It is defined as a chemical process characterized by chronic, localized loss of dental hard tissue as a result of acid dissolution and/or chelation absent any bacterial involvement.[21,22] During the erosion process, acid attack results in bulk loss of dental tissue and the remaining partially demineralized surface layer. This demineralized layer is highly susceptible to mechanical wear.[16] Currently, there is increased attention to dental erosion by clinicians and researchers. This is probably due to an increased prevalence of dental erosion and an increase in knowledge about its etiology and diagnosis.[23,24]

The erosive acids that cause dental erosion can be of intrinsic or extrinsic origin. Intrinsic sources of acid are related to gastric abnormalities such as bulimia, anorexia nervosa, and regurgitation.[25] Extrinsic sources of acid may be from dietary components including citrus fruits, carbonated soft drinks, fruit juices, and from medication and/or environmental acid sources.[26,27] Several studies found correlations between erosion and dietary habits. For example, it has been shown that the risk of erosion increased three-fold for children and young adults who drink Coca-Cola several times a week. This is of great concern, especially because young adults and children are the primary consumers of soft drinks.[28] Dietary acids that have naturally low pH values, such as citric and malic acids (organic hydroxyl acids found in the fruits), phosphoric acid (weak mineral acid), and ascorbic acid (contained in sport drinks) are the most consumed erosive acids and play an important role in the erosion process.[29] Citric acid is considered to have more erosive potential than phosphoric acid due to its chelating properties. It has been shown that the citric acid in orange juice depletes up to 32 percent of calcium in saliva, leading to decreased saturation of saliva and increased dissolution of tooth minerals.[30]

Factors Involved in Dental Erosion

Dental erosion is a result of the interaction between different chemical, biological, and behavioral factors. *In situ* studies showed different susceptibilities in individuals exposed to the same acidic challenge.[29] This may be explained by the modifying role of chemical, behavioral, and biological factors in the erosive process.[25,31]

Chemical Factors

There are a number of chemical characteristics that influence the erosive potential of extrinsic acids. These include pH value, pKa, titratable acidity or buffering capacity, concentration, temperature, and calcium-chelation ability.[29] It has been shown that the pH value of acids is probably one of the most important parameters affecting the erosive potential of acidic products. However, other parameters, such as titratable acidity and concentration, should also be considered. Hughes and collaborators demonstrated in their *in-vitro* study that by decreasing pH value and increasing the concentration of acid, the rate of enamel dissolution increased.[29] Barbour et al. investigated enamel dissolution in citric acid with a pH range over $2.30 < pH < 6.30$. They found that enamel reached the lowest hardness when the pH value was less than 2.90.[28]

Behavioral Factors

Time of and frequencies of acid exposure contribute to the development of dental erosion, and are significantly important, especially for the protective measurement of dental erosion. Exposure to acid before sleeping (from drinking acidic beverages) may increase the risk of dental erosion due to decreased salivary flow during nighttime. Furthermore, any behavioral factors that increase the contact time of acid with the tooth surface probably lead to an increased risk of erosion.

Biological Factors

Saliva, dental pellicle, and tooth structure play important roles in the erosion process. Saliva is considered the most relevant factor for the prevention of dental erosion. It acts against dental erosion in different ways, including decreasing demineralization by forming the acquired dental pellicle, and increasing remineralization by providing phosphate and calcium to the demineralized tissue.[32,33] It has been established *in vitro*, *in situ* and *in vivo* that acid-softened dental tissue can be rehardened after exposure to saliva.[34] However, remineralization of early eroded dental tissue can be achieved not only from natural saliva but from artificial saliva too, and the potential remineralization effect of artificial saliva has been well established.[34]

THE ROLE OF ABRASION IN THE ETIOLOGY OF TOOTH WEAR

Abrasion is defined as the physical wear of dental hard tissue produced by the interaction between teeth and other materials, such as toothpaste and toothbrushes.[35,36] Tooth brushing with toothpaste is considered the major contributor to dental abrasion.[2-4] In 1907 Miller was the first to state the effects of toothpaste abrasivity on dental hard tissue.[9,37] Since that time, it has become obvious that dentin is probably affected by the different toothpastes available on the international market.[38] However, toothpaste abrasivity seems to have a negligible effect on sound enamel.

It is well known that enamel is the hardest tissue in the human body.[39] The hardness of enamel ranges between 272 KHN to 440 KHN (Knoop Hardness Number), while that of dentin is between 50 KHN to 70 KHN.[40] The hardness of the commonly used toothpaste abrasives in developed countries ranges between 50 KHN to 150 KHN.[41] Furthermore, some studies have indicated a strong correlation between the microhardness of enamel and its resistance to the abrasives.[38,42] Consequently, the abrasive effect of toothpastes on sound enamel is generally very low.[38,39]

Several authors have studied the effects of different toothpastes on enamel. In 2002 Joiner et al. stated in a study comparing whitening toothpaste with a conventional one that it is very difficult to wear away any significant amount of enamel by brushing with toothpaste. In addition, several *in-vitro* and *in-situ* studies have indicated that less than 1 μm of enamel wear by toothpaste may not be clinically significant.[26,43,44]

This may just apply to areas covered with enamel; but in patients with exposed dentin, toothpaste abrasivity could be considered a problem because dentin has shown more susceptibility to abrasion than has sound enamel.[45,46]

Factors Involved in Dental Abrasion

It is well documented that tooth brushing abrasion is significantly related to the abrasiveness of toothpastes.[45] However, toothpaste abrasivity is dependent on several parameters including the type of abrasive (chemical composition), concentration, particle size, size distribution, surface structure of the abrasive particle, diluents, and the dilution rate of toothpaste.[47,48] There is a linear relation between abrasive wear and size and the concentration of abrasive particles. As particle size and concentration increase, abrasive wear increases as well.[46,49,50] Furthermore, an increased dilution rate of toothpaste may lead to a decrease in wear, as the concentration of abrasive particles decreases as a result of slurry dilution.[51]

Different studies have investigated the abrasivity of toothpaste, which can be described by REA and RDA.[52] RDA and REA values are determined by the radiotracer method (ISO 11609),[7,9,11] which is the most commonly used method to determine RDA and REA as compared with the standard abrasive, calcium pyrophosphate.[2,11] However, there are other methods that have been developed to assess abrasivity of toothpastes to enamel and dentin, and each method has its advantages and disadvantages.[13] These methods include surface profilometry, microscopic methods, and the weight-loss technique. [13]

Several studies have investigated whether toothpastes of high abrasivity (RDA) cause more damaging effects to tooth surface than do low-abrasion toothpastes. For eroded and sound dentin, many studies have shown that abrasive wear of dentine is associated significantly with RDA.[9,11] For instance, Hooper et al. in their *in-situ* study found a positive correlation between RDA value and dentin wear.[2] However, for the eroded enamel, Hooper and colleagues reported in the same study that there is no difference in enamel wear when brushed with toothpastes of different RDA values.[2] Philpotts et al. also tested toothpastes with a range of REA and RDA values *in vitro* and demonstrated similar results as Hooper et al. [13] In contrast, a separate *in-vitro* investigation suggested that the abrasion of eroded enamel is mainly affected by toothpaste abrasivity.[12]

There are other factors that could affect toothpaste abrasivity and consequently affect abrasive wear of teeth, including tooth brush characteristics, brushing time, force of brushing, temperature during brushing, and slurry viscosity. The toothbrush is considered the delivery vehicle which modulates the action of toothpaste abrasivity.[4,12] Several toothbrush variables could have an influence on dental hard tissue, such as filament stiffness, brand of tooth brush (as hardness among brands is not consistent), time and frequency of brushing, and brushing force.[11,12,15] Toothbrush stiffness is also dependent on several factors such as bristle diameter and modulus of elasticity, number of tufts, number of bristles packed into tuft holes, and tuft diameter. These characteristics of the toothbrush identify their efficiency as delivery aids for the various toothpastes.[53]

A number of *in-situ* and *in-vitro* studies have indicated that filament diameter or stiffness may affect the abrasion process. In 2000 Dyer et al. tested different types of toothbrushes on acrylic plates that have hardness similar to that of dentin. They stated that a soft toothbrush may abrade dental tissue more than a hard one.[15] In 2009 Wiegand et al. investigated the impact of soft, medium, and hard toothbrushes on eroded dentin. Their findings were similar to those of Dyer et al.[11] Furthermore, in 2011 Teche et al. also tested the abrasion capacity of four different brands of soft toothbrushes. They found a positive association between softer bristles and abrasion (the softer the bristles the greater the abrasion capacity).[53] Dyer et al. have given an explanation for these results.[15] They assumed that during brushing, the bristles of the toothbrush harbor abrasive particles of toothpaste across the tooth surface. Since a soft toothbrush (approximate bristle diameter of 0.15 mm) contains a high number of bristles, it probably retains a greater amount of the abrasive.[10] In addition, a soft toothbrush is more flexible and could create greater contact area with the tooth surfaces, thereby increasing the amount of abrasive moving across the tooth surface.[4,15]

In contrast, very few studies support the opposite theory, that a hard toothbrush might cause more abrasion to the tooth surface than a soft toothbrush.[4] This result might be due to using extra-hard natural bristle toothbrushes in some studies.[37] For instance, Manly and Hart found that a hard toothbrush caused more abrasion on sound enamel than a soft one.[9] Furthermore, a recent clinical evaluation also strengthened the common idea held by many dentists, that hard toothbrushes cause greater abrasion.[54]

On the other hand, one *in-vitro* study has indicated that filament stiffness is considered a secondary factor affecting toothbrush abrasion,[15,55] and enamel loss is more correlated to toothpaste abrasivity than to filament stiffness.[12] Voronets et al. found no significant difference between hard and soft toothbrushes. In an *in-vitro* assessment, it has been reported that soft, medium, and hard toothbrushes are not capable of significantly abrading the sound enamel surface.[56]

Tellefsen et al. showed that toothbrush abrasion was negligible when the brushing was performed with water, and that only small differences were found between different toothbrush types. However, when brushing was performed with toothpaste, the wear increased up to 10 times depending on the toothpaste and the toothbrush (shape cut of bristle and roughness).[4]

Even though toothbrush abrasion is significantly related to the abrasivity of toothpaste, it can be concluded from the previous information that it is difficult to distinguish the effect of the toothpaste from the effect of the toothbrush on the abrasion process. Furthermore, abrasivity probably depends on the interaction between both toothpaste and toothbrush.

COMBINED ROLE OF EROSION AND ABRASION

It has been shown in a number of clinical and experimental observations that the interaction between different types of tooth wear is very common, especially between abrasion and erosion. In addition, this interaction is considered the major factor in tooth wear.[18,19,57] In 1980 Davis and Winter were the first ones who introduced the concept that toothbrush abrasion is accelerated by acid softening of enamel and dentin. The acid-softened surface becomes highly susceptible to mechanical abrasion due to mineral loss that forms a softened layer with reduced surface hardness. This layer can be partially or completely removed by toothbrushing.[1] Since that time, considerable evidence drawn largely from *in-vitro* and *in-situ* studies has supported their concept.[3,58,59] For instance, one study indicated that tooth wear will increase 50 percent with the combined effect of erosion and abrasion over erosion or abrasion alone.[57] Furthermore, Ganss et al. have shown that abrasion can increase surface loss in association with 1-min and 15-min erosion challenges with an erosive beverage four-fold and thirteen-fold, respectively.[58] In contrast, De Menezes and collaborators stated that there is no statistical difference in surface loss between uneroded and eroded dentin subjected to brushing abrasion. However, these results may be related to the study design, in which specimens were exposed to remineralization solution for 1 minute after 5 minutes exposure to the erosive challenge (Diet Sprite, Coca-Cola Co., USA).[45]

Several studies have assessed the interplay between erosion and abrasion *in vitro*, *in situ* or under combined *in-vitro* and *in-situ* conditions. In addition, the impact of different parameters on the development of erosion/abrasion lesions have been evaluated, such as fluoride, different abrasive levels of toothpaste (low, medium, and high), and different toothbrush characteristics.[19] These studies have used erosion/abrasion cyclic models to simulate everyday life situations, where teeth are subjected to erosion (from acidic food or beverages) and abrasion (tooth brushing or hard food) several times a day. However, there were some differences in these erosive/abrasion models, including a number of erosion/abrasion cycles, acid type and exposure time, and number of strokes during brushing.

The *in-vitro* model has been preferred in many studies due to its advantage over *in-situ* and *in-vivo* models. For instance, the *in-vitro* model provides good control of the specific parameters involved in a study. [60] The experiment also can be conducted within a short-time period, with a small budget and the use of fewer staff members. However, interpretation of *in-vitro* studies should be done very carefully to avoid over- or underestimating the actual tooth wear. Furthermore, since the behavioral and biological aspects of the erosion/abrasion process cannot be fully applied, the clinical relevance of clinical aspects is limited. Nevertheless, valid and reasonable data can be obtained from *in-vitro* erosion/abrasion studies.[60]

Bovine teeth have been widely used as a substitute for human dental hard tissue in many erosion/abrasion experiments.[61] For a variety of reasons, about 50 percent of *in-vitro* studies have used bovine teeth.[19] It is difficult to obtain a sufficient number of human teeth, and to manipulate and standardize their use, especially in regard to the dentin substrate.[45] On the other hand, a sufficient number of bovine teeth can be collected easily. Furthermore, bovine incisor teeth have a wide surface area, so more than one specimen can be obtained from one tooth. This increases homogeneity among the separate groups, as different specimens from one tooth can be distributed among the different groups.[62] However, the difference between human and bovine samples must be taken into consideration, especially as bovine enamel shows more susceptibility to wear than human enamel.[61] However, it has been shown that there is no difference in performance between human dentin substrate and bovine dentin substrate under the *in-vitro* erosion/abrasion model.[62]

Different erosion challenges have been used in erosion/abrasion laboratory studies, including commercial beverages, citric acid, and hydrochloric acid. The use of specific erosive media depends on the objective of the study. For instance, to simulate the extrinsic source of acid, soft drinks, sport beverages, and citric acid have been used. However, citric acid (pH 2.3 to pH 3.8) is generally used because it is in wide use as an additive to foodstuff and drink.[7] To simulate intrinsic sources of acid, hydrochloric acid has been used. The duration of erosion challenges varied from between 15 s to 40 min per cycle. However, 1 min to 5 min was the most common erosion duration used in the studies.[19]

Several *in-vitro* erosion/abrasion cycling models have included remineralization phases. Both artificial and human saliva have been used. However, artificial saliva was used more often than human saliva, because artificial saliva can be prepared in constant composition, creating a high degree of standardization. In addition, high concentrations of calcium and phosphate in artificial saliva exhibit supersaturation with respect to different calcium and phosphate minerals, such as hydroxyapatite.[19,63]

MATERIALS AND METHODS

STUDY DESIGN

In this study, an established erosion/abrasion model was used.[20,64] Three experimental factors were tested in a 2 × 3× 2 factorial design: abrasive, at two levels (high (Z103) and low (Z113)), toothbrushes at three levels determined by bristle stiffness (soft, medium, and hard), and cycling time (third vs fifth days of erosion/abrasion cycles). The response variables were enamel and dentin surface loss, in microns, as measured by optical profilometry following the third and fifth erosion/abrasion cycles.

Specimen preparation

Enamel and dentin slabs (4 mm width × 4 mm length × 2 mm thickness) obtained from bovine incisors stored in 0.1-percent thymol solution were prepared. The bottom and top of the enamel and dentin sides of the slabs were sequentially ground flat using silicon carbide grinding papers (Struers RotoPol 31/RotoForce 4 polishing unit, USA) (Figure 1). Uniform thicknesses of approximately 2 mm have been created. Both slabs of enamel and dentin have been embedded in acrylic resin (Varidur acrylic system, Buehler, USA) utilizing a custom-made silicon mold, leaving the enamel and dentin surfaces exposed (Figure 2). The embedded blocks have been serially ground and polished up to 4000-grit grinding paper followed by a 1-µm diamond polishing suspension. Specimens have been selected based on the quality of enamel and dentin and randomized into the 6 experimental groups (n = 8) (Table I).

Surface area delimitation

UPVC tapes have been placed on the surface of the specimens, leaving an area of 1 mm x 4 mm exposed in the center of the each enamel/dentin slab (Figure 3).

Erosive Solution

A solution of 0.3-percent citric acid anhydrous (Sigma C1857) in DI water (pH approx. 3.75) has been used as an erosive challenge in this study.

Remineralization Media

The artificial saliva (pH adjusted to 7.0 with HCl) formulation has been prepared and used as the remineralization medium (Table II).[64]

Abrasive Slurries

Two abrasive slurries have been prepared, as described in Table III with two levels of abrasives (low, REA = 4.01±0.79/RDA = 69.24 and high, REA = 7.14±1.96/RDA = 208.03±26.57). Two hundred and seventy-five ppm fluoride (as NaF) has been added to simulate the fluoride concentration in toothpaste after dilution during brushing. The slurries have been prepared by mixing the ingredients mentioned above with an aqueous suspension containing 0.5% (w/w) Blanose 7MF carboxymethylcellulose (CMC) and 10% (w/w) glycerol. Sixty grams of the slurry have been used in each slot of the brushing machine.

Brushing abrasion

Specimens have been positioned in an automated brushing machine (Figure IV). They have been brushed for 15 s (45 strokes, OHRI brushing machine) with one of three test toothbrushes - soft, medium and hard (Lactona, Dental Care Clinic) (Figure 5, Figure

6), using 150 g of load with their respective abrasive slurries. Additional information about the toothbrushes is given in Table IV, and Figure 7.

Daily Treatment Regimen

The daily treatment regimen consisted of four acid challenges and two tooth-brushing treatments with the abrasive slurries. At the end of the cycle each day, specimens were stored in a closed container with a humid environment at 5ºC, until the next test day. The daily treatment schedule is highlighted in Table V.

Experimental setup

The samples have been subjected to five treatment days each consisting of 4 x 5 min of erosion, 5 x 1 h storage in artificial saliva, 2x tooth brushing abrasion followed by slurry exposure without brushing, and storage in artificial saliva overnight. Enamel and dentin surfaces have been eroded by unstirred storage in 0.3-percent citric acid for 5 min and were then rinsed for 10 s with distilled water. Thereafter, the samples were brushed in an automatic brushing machine with a load of 150 g using manual toothbrushes with different filament stiffness and slurries with different abrasivity. After the 3rd and 5th day of cycling, enamel and dentin surface loss were measured by profilometry.

Profilometry

Surface loss (SL) was measured using an optical profilometer (Proscan 2000, Scantron, England; (Figure 8) after 3 days and 5 days of cycling. Tapes were removed from the specimens and an area of 1 mm x 4 mm in the center of the specimen (including both exposed and tape-covered areas) was scanned (Figure 8). Dedicated software (Proscan 2000, Scantron) was used to analyze surface loss (Figure 9).

Statistical Analysis

The effects of cycling time, slurry abrasiveness, and toothbrush filament hardness on surface loss have been examined using ANOVA. Separate analyses have been performed for enamel and dentin surfaces. The ANOVA has included main effect terms for each of the three factors, all interactions among the factors, and a random effect to correlate the results from the two cycles within a sample. Fisher's Protected Least Significant Differences has been used to control the overall significance level of the tests. A 5-percent significance level will be used.

Sample size

Based on a prior study the within-group standard deviation of the surface loss is expected to be 1.5 µm. With a sample size of 8 specimens per abrasiveness-hardness combination, the study will have 80-percent power to detect differences of 2.3 µm between any two abrasiveness-hardness combinations for each cycling time, assuming two-sided tests conducted at a 5-percent significance level.

RESULTS

DENTIN RESULTS

The Effect of Abrasivity of Toothpaste Slurries

Mean (SD) dentin loss (µm) by brushing of eroded dentin specimens in different group is presented in Table VI and Figures 12, 14. ANOVA showed that dentin loss increases along with the RDA-value of toothpaste slurries; high abrasive (RDA) had significantly higher dentin surface loss than low abrasive (RDA) in all the groups (A, B, C, D, E, and F) ($p < 0.0001$) (Table VII).

The Effect of Toothbrush Filament Stiffness

Only the Hard toothbrush had significantly higher surface loss than the medium toothbrush for the high abrasive at Cycle 5 ($p = 0.0088$) with no other significant toothbrush differences ($p > 0.18$).

The Effect of Cycling Time

The fifth Cycle had significantly higher dentin surface loss than Cycle 3 overall ($p < 0.0001$), with particularly large differences for Group F (hard toothbrush/high abrasive, $p < 0.0001$) and Group C (medium toothbrush/low abrasive, $p = 0.0001$) (Table VIII).

Overall, the data did not show significant interaction between the two factors (abrasivity of toothpaste slurries and filament stiffness of toothbrushes) ($p = 0.1948$). However, the data showed that the impact effect of all factors (abrasivity, toothbrush filament stiffness, and cycling time) had a strong effect on dentin loss, except for the medium toothbrush on fifth cycle (Table IX).

ENAMEL RESULT

The Effect of Abrasivity of Toothpaste Slurries

Mean (SD) enamel loss (µm) by brushing of the eroded enamel specimens in the different groups is presented in Table VI and Figures 13, 15. This data showed that the abrasivity of toothpaste slurries did not affect enamel surface loss, there is no significant difference between low and high abrasive on enamel wear ($p = 0.2380$).

The Effect of Cycling Time

The fifth cycle had significantly higher enamel surface loss than the third cycle ($p = 0.0003$) (Table 9).

The Effect of the Toothbrush

Different toothbrushes (high, medium, and low) did not significantly affect enamel surface loss ($p = 0.6204$).

TABLES AND FIGURES

TABLE I

Experimental groups

Toothbrush	Low Abrasive	High Abrasive
Soft toothbrush	A	B
Medium toothbrush	C	D
Hard toothbrush	E	F

TABLE II

Artificial saliva composition

Chemicals	Quantity (in g/l)
$CaCl_2*2H_2O$	0.213g
H_2PO_4	0.738g
KCl	1.114g
NaCl	0.381g
Tris buffer	12g

TABLE III

Abrasive slurry compositions

Abrasive	AbrasiveLoad (%)	Fluoride(ppm NaF)	Abrasive Amount (g)
Zeodent 113(Low)	5	275	3
Zeodent 103(High)	15	275	9

TABLE IV

Characteristics of the experimental toothbrushes, the measurements have been taken at OHRI

Parameter	Lactona/ Soft	Lactona/ Medium	Lactona/ Hard
Filament Diameter	212.8 µm	228.6 µm	310.4 µm
Bristle Length	11 mm	11mm	11mm
Tufts	43	43	43
Bristles/tuft (no)	50	36	16

TABLE V

Daily treatment schedule

Treatment	Duration
Erosion (1/4)	5 min
Remineralization (1/6)	1 hour
Treatment/abrasion (1/2)	Brushing: 15s (45 stk) + 45s slurry exposure
Remineralization (2/6)	1 hour
Erosion (2/4)	5 min
Remineralization (3/6)	1 hour
Erosion (3/4)	5 min
Remineralization (4/6)	1 hour
Erosion (4/4)	5 min
Remineralization (5/6)	1 hour
Treatment/abrasion (2/2)	Brushing: 15s (45 stk) + 45s slurry exposure
Remineralization (6/6)	Overnight

TABLE VI

Summary of group means in µm

Group	Cycle	Dentin Mean (SE)	Enamel Mean (SE)
A [soft toothbrush+low abrasive]	3	-2.64 (0.25)	-0.76 (0.21)
	5	-2.97 (0.22)	-1.40 (0.39)
B [soft toothbrush+high abrasive]	3	-4.21 (0.17)	-0.81 (0.21)
	5	-5.01 (0.18)	-1.76 (0.17)
C [medium toothbrush+low abrasive]	3	-1.87 (0.28)	-0.80 (0.25)
	5	-3.91 (0.33)	-1.85 (0.57)
D [medium toothbrush+high abrasive]	3	-3.54 (0.15)	-1.27 (0.37)
	5	-4.37 (0.43)	-1.64 (0.30)
E [hard toothbrush+low abrasive]	3	-2.46 (0.40)	-1.02 (0.19)
	5	-3.33 (0.27)	-1.74 (0.61)
F [hard toothbrush+ high abrasive]	3	-3.72 (0.46)	-1.04 (0.18)
	5	-5.91 (0.74)	-1.71 (0.27)

TABLE VII

Results of statistical analysis for surface loss of dentin showing p-value for abrasive levels

Abrasive	Mean (SE)	p-value
Low	-2.86 (0.15)	<0.0001
High	-4.46 (0.20)	

TABLE VIII

Results of statistical analysis for surface loss of dentin showing p-value for cycling time

Cycle	Mean (SE)	p-value
3	-3.07 (0.17)	<0.001
5	-4.25 (0.21)	

TABLE IX

Statistical analysis for the surface loss of eroded dentin resulted from the interaction between study variables

Toothbrush	Abrasive	Cycle	Mean (SE)	p-Value
Hard	High	3	-3.72 (0.46)	<0.0001
		5	-5.91 (0.74)	
Hard	High	3	-3.72 (0.46)	0.0048
	Low		-2.46	
			(0.40)	
Hard	High	5	-5.91 (0.74)	0.0001
	Low		-3.33 (0.27)	
Medium	Low	3	-1.87 (0.28)	0.0001
		5	-3.91 (0.33)	
Medium	High	3	-3.54 (0.15)	0.0002
	Low		-1.87 (0.28)	
Soft	High	3	-4.21 (0.17)	0.0005
	Low		-2.64 (0.25)	
Soft	High	5	-5.01 (0.18)	0.0006
	Low		-2.97 (0.22)	
Hard	High	5	-5.91 (0.74)	0.008
Medium			-4.37 (0.43)	

TABLE X

Results of statistical analysis for the surface loss of enamel showing p-value for cycling time

Cycle	Mean (SE)	p-Value
3	-0.95 (0.10)	0.0003
5	-1.68 (0.16)	

FIGURE 1. Photographs of the Struers RotoPol 31/RotoForce (polishing machine).

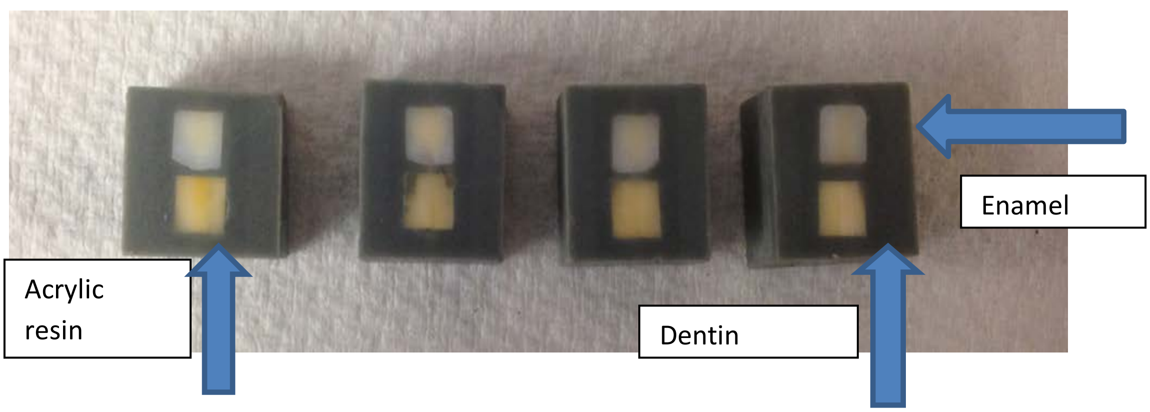


FIGURE 2. (Top) Photograph of dentin slabs mounted in acrylic resin. (Bottom) Photograph of slabs of enamel and dentine embedded in acrylic resin.

UPVC tapes

FIGURE 3. A photograph of UPVC tapes placed on the surface of the specimens, leaving an area of 1 x 4 mm exposed in the center of the each enamel and dentin slab.

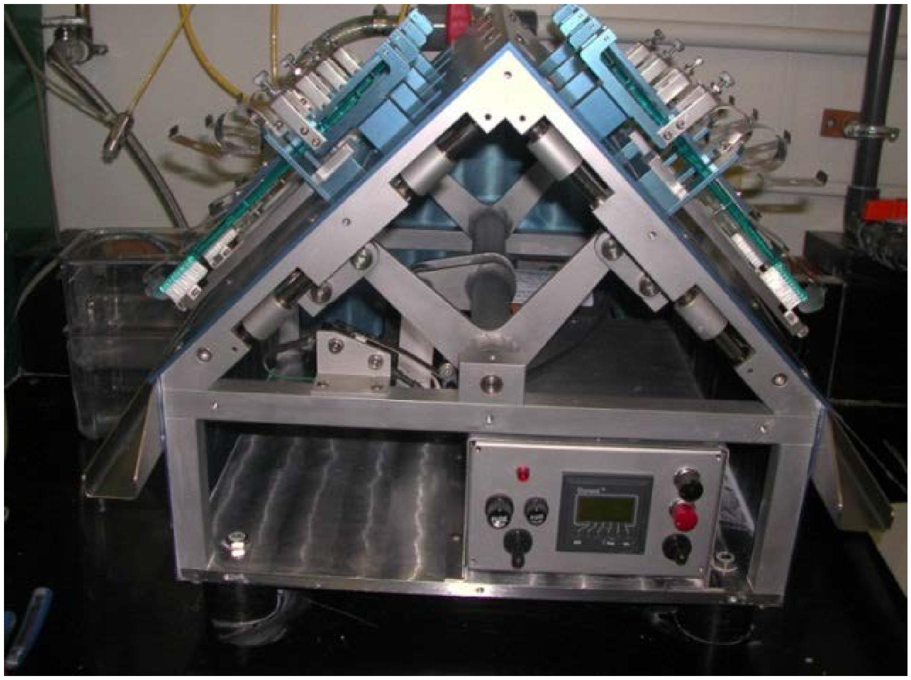
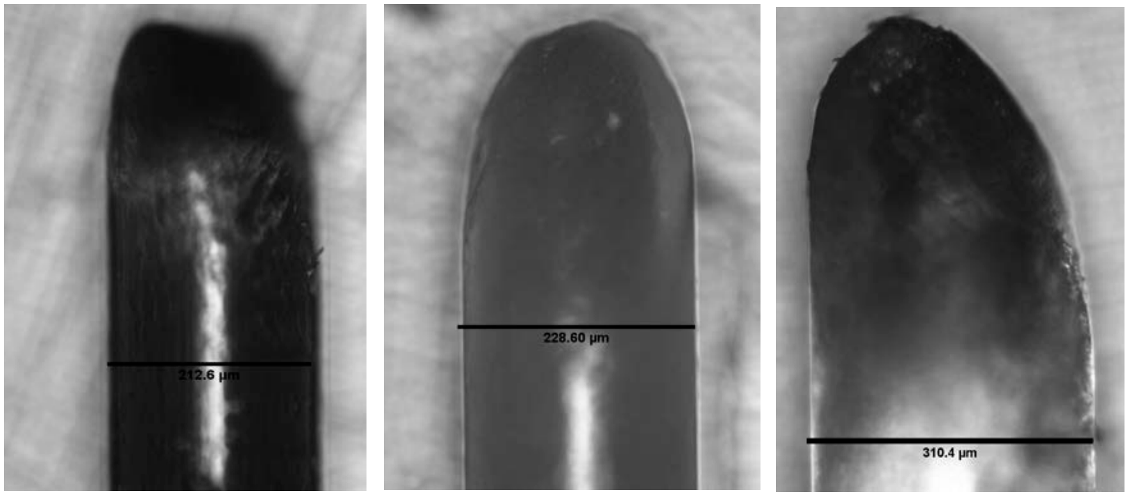


FIGURE 4. A photograph of brushing machine.

FIGURE 5. A photograph of experimental toothbrush (Lactona Dental Care).

Soft toothbrush Medium toothbrush Hard toothbrush

FIGURE 6. Photographs of bristles of different types of toothbrushes used in the project.



Soft toothbrush Medium toothbrush Hard toothbrush

FIGURE 7. Photographs of different types of toothbrush bristles with different diameter

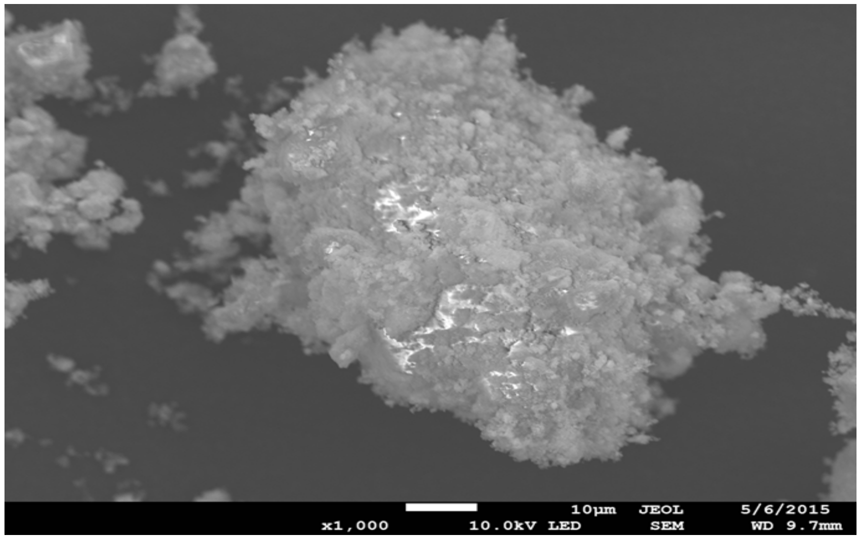


FIGURE 8. Electromicroscopic image of low abrasive (Zeodent 113) used in the project.

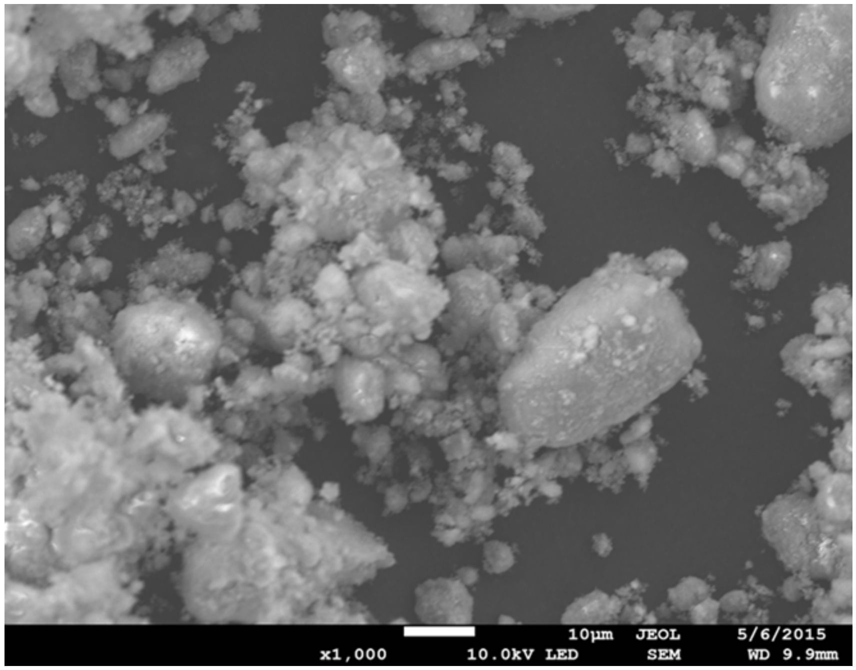


FIGURE 9. Electromicroscopic image of high abrasive (Zeodent 103) used in the project.

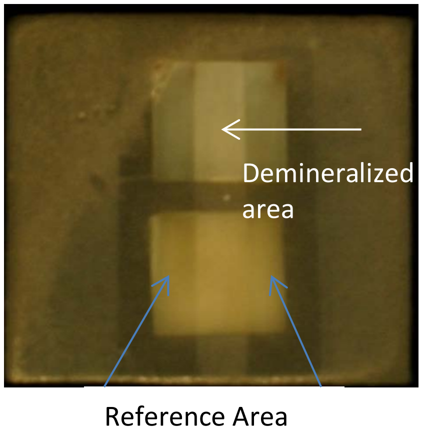


FIGURE 10. A photograph shows the specimen after removing tape.

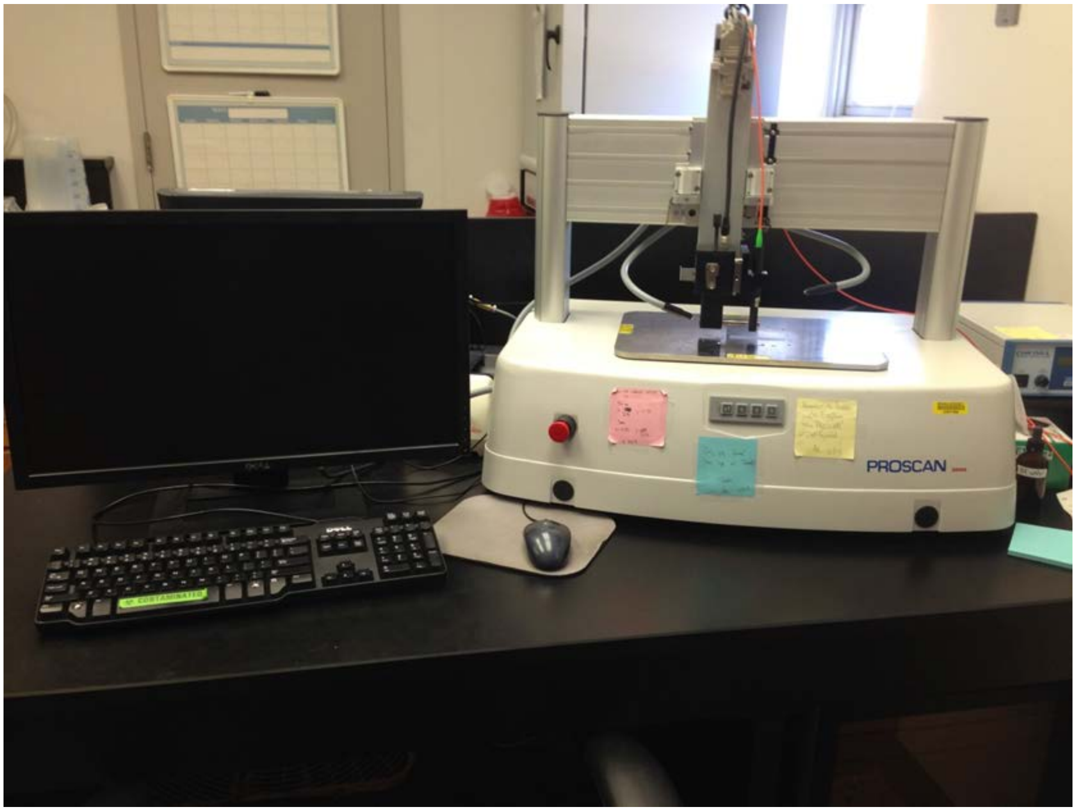


FIGURE 11. A photograph shows the optical profilometer used in the project.

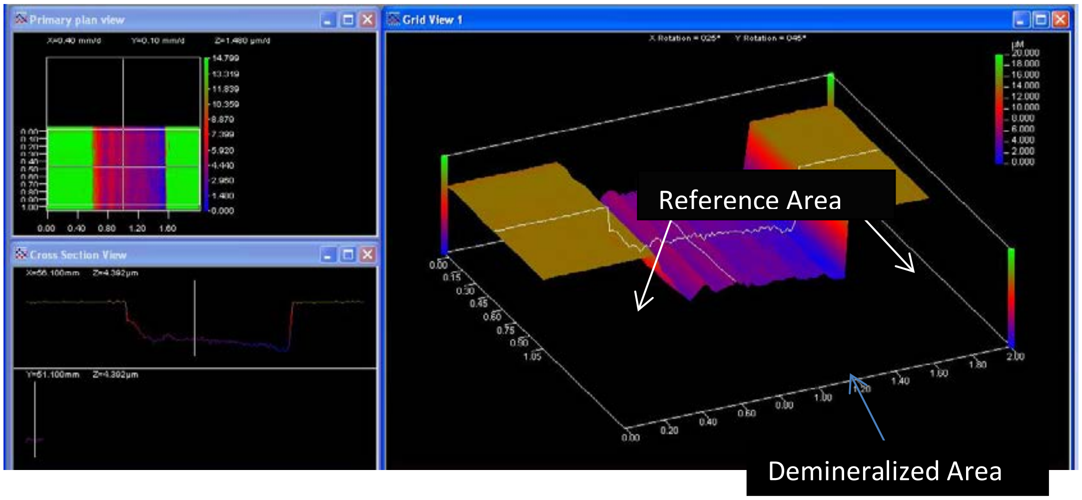


FIGURE 12. An output screen from the optical profilometer analysis software.

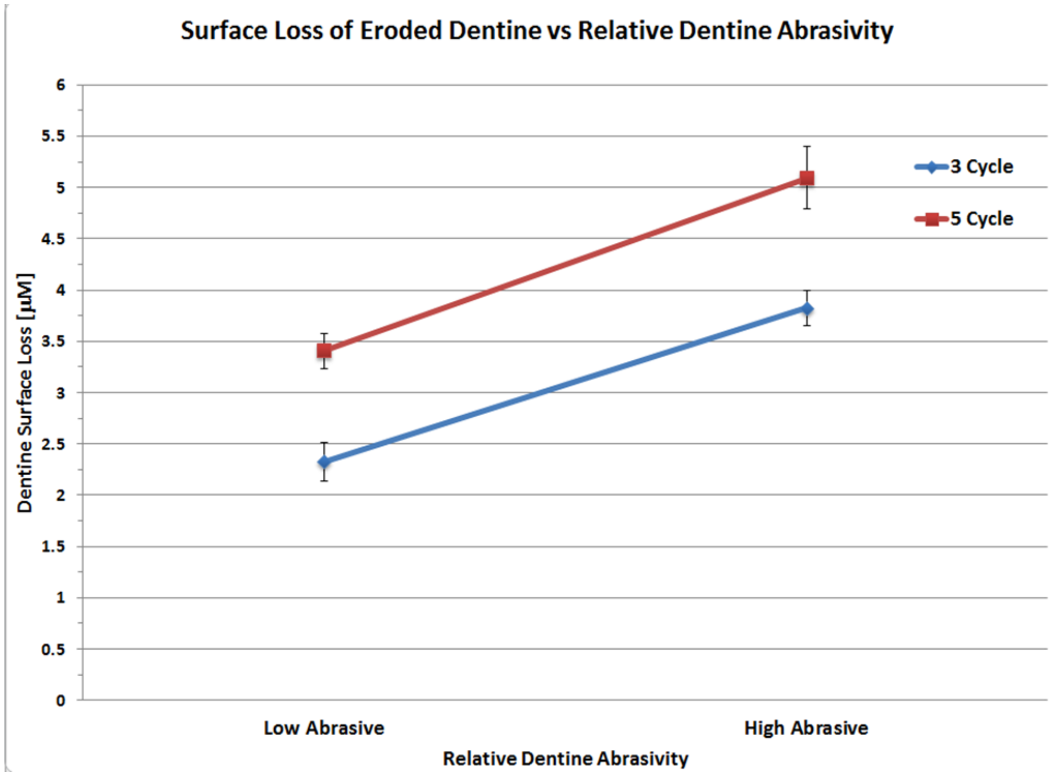


FIGURE 13. Line graphs showing means of surface loss of eroded dentin for different experimental groups brushed with two different abrasive slurries (high and low).

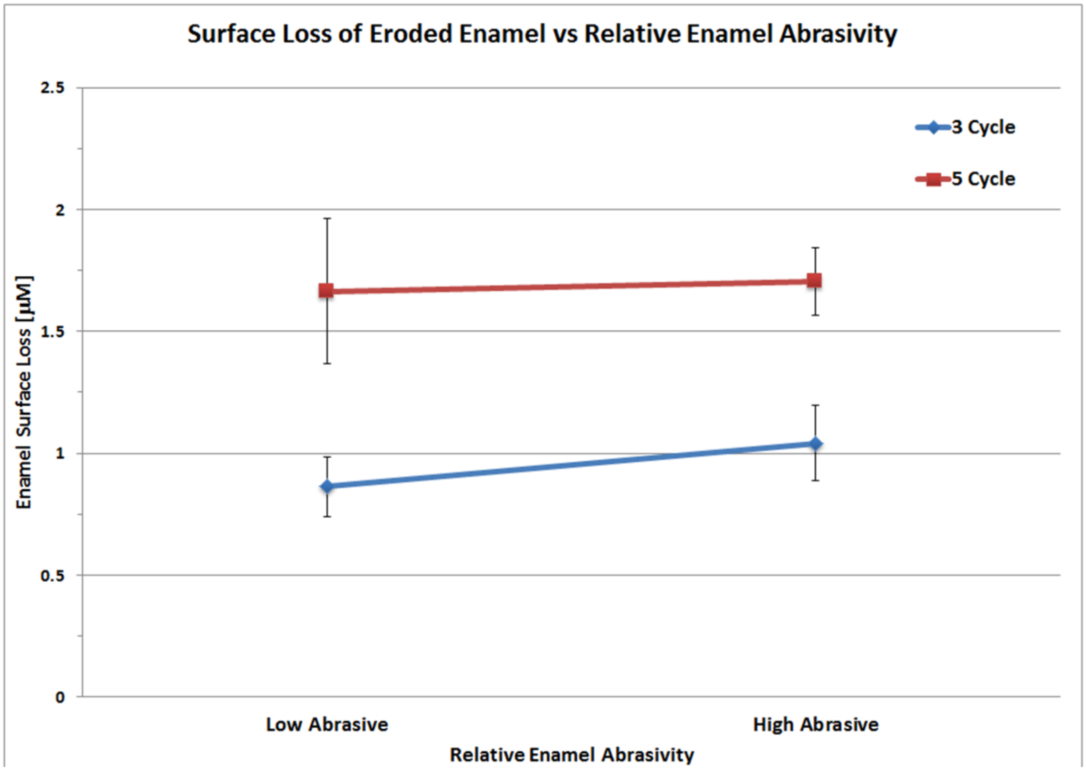
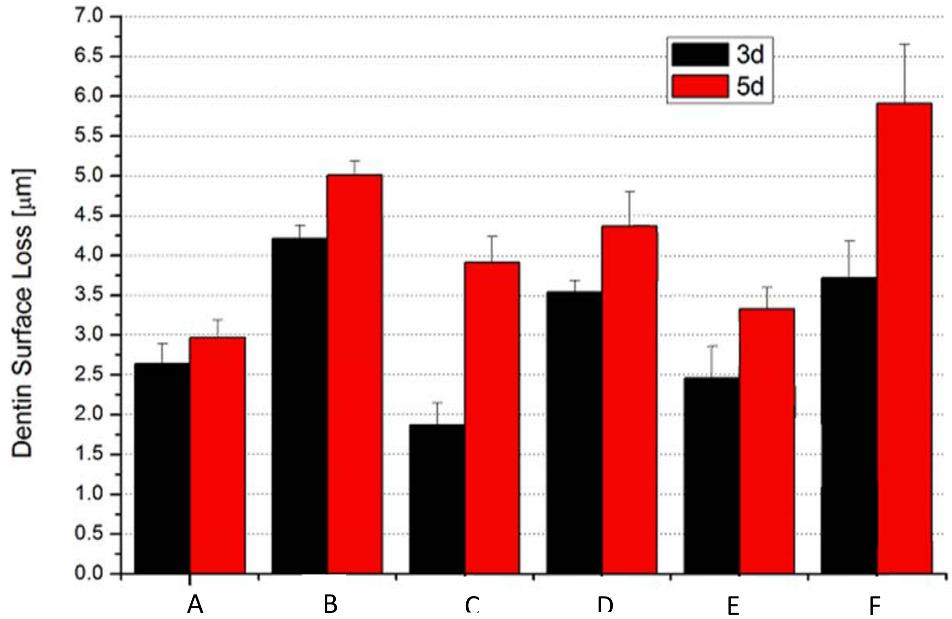
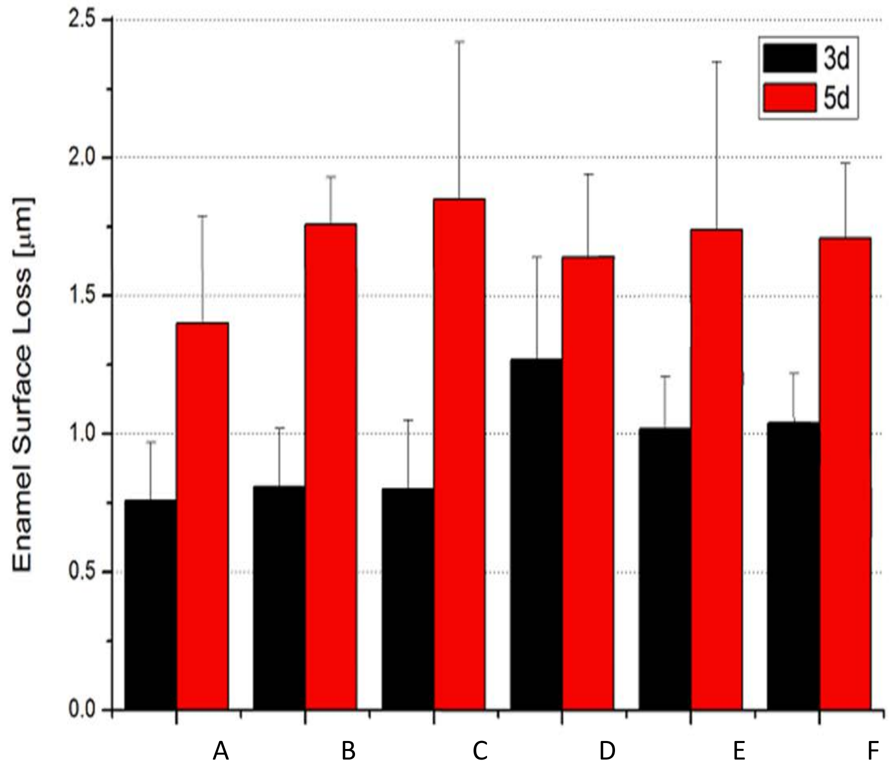


FIGURE 14. Line graphs showing means of surface loss of eroded enamel for different experimental groups brushed with two different abrasive slurries (high and low).



A: Low abrasive/Soft toothbrush. B: High abrasive/soft toothbrush. C: Low abrasive/soft toothbrush D: High abrasive/soft toothbrush. E: Low abrasive/soft toothbrush. F: High abrasive/ hard toothbrush

FIGURE 15. Bar graphs showing the mean of surface loss of eroded dentin for different groups.



A: Low abrasive/Soft toothbrush. B: High abrasive/soft toothbrush. C: Low abrasive/soft toothbrush. D: High abrasive/soft toothbrush. E: Low abrasive/soft toothbrush. F: High abrasive/ hard toothbrush

FIGURE 16. Bar graphs showing the mean of surface loss of eroded enamel for different groups.

DISCUSSION

Justifications for Experimental Parameters

The present study was aimed at investigating the surface loss of eroded enamel and dentin resulting from the interaction between toothpaste abrasivity and toothbrush filament stiffness. To investigate the study questions, a five-day established erosion/abrasion cycling protocol was used, involving episodes of erosion challenges (five times a day, for five min each), remineralization in artificial saliva (six times a day, for 1 h each), and brushing abrasion (two times a day, for 15 s each).[64,65] The brushing of eroded enamel and dentin occurred after storage of the samples in artificial saliva. In addition, brushing was conducted two times in each cycle instead of after each erosion treatment. This experimental approach is more representative of the everyday clinical situation than previous studies, because most people brush their teeth twice daily rather than after each contact with erosive food stuff.

In this study, we attempted to simulate the recommended brushing time of nearly two min. Each specimen was brushed for 30 s, the equivalent of 15 s, or 45 brushing strokes for the facial/buccal surface, and the palatal/lingual surface.[66] The 45 brushing strokes equated to 450 brushing strokes at the end of each cycle and represented 5 d of brushing. The majority of *in-vitro* and *in-situ* studies exaggerate the clinical situation by applying a high number of brushing strokes ranging from 300 strokes to 400 strokes in their brushing treatment.[67] This can easily lead to the removal of the fragile outer layer of softened dental hard tissue, regardless of the effects of different abrasivity values of toothpaste and toothbrush filament stiffness.

In the majority of studies, the brushing was performed with manual toothbrushes.[19] The toothbrushes were attached to a brushing machine in order to standardize the movement of the toothbrush and to ensure that the surfaces of all the specimens were brushed under constant load. The use of 150-g brushing load for testing the abrasivity of toothpaste is in agreement with previous recommendations of Wiegand et al.[19]as well as the International Standards Organization (ISO11609). The toothpaste slurries in this experiment were prepared with fluoride because most toothpaste available internationally contain fluoride.

In order to control variability among toothpaste slurries, we have prepared our own slurries in this experiment. Under clinical conditions, toothpaste will be diluted by saliva during brushing. Therefore, this investigation used one part of toothpaste to three parts of artificial saliva (1:3).[38] For this reason, 275 ppm of fluoride was used, representing 1100 ppm fluoride of regular toothpaste after saliva dilution.[68]

Non-contact surface profilometry was used in this study for two reasons. First, the profilometer offers good flexibility for analyzing combined erosion-abrasion tissue loss. In this study, the specimens were polished and flattened to obtain a profilometer measurement with maximum sensitivity and accuracy.[69] Secondly, it has been suggested that the contact profilometers may cause damage to the eroded dental surfaces.[70] Therefore, by using a non-contact profilometer we eliminated any possible interference that may cause damage to the eroded surfaces of enamel and dentin.[69,71]

The Effect of Abrasivity Levels on the Abrasion of Eroded Dentin

In this study, the high abrasive slurries caused more surface loss of eroded dentin than the low abrasive one. These results are in agreement with previous findings of

Wiegand et al., Hooper et al., and Dyer et al., who found that abrasion of eroded dentin increased along with the RDA-value of the toothpaste slurry.[2,11,15]

From the present data, it can be assumed that low abrasive slurries may only abrade the superficial layer of softening dentin, whereas their high abrasive counterpart may probably wear the deeper part of the eroded dentine. Under this assumption, and taking into consideration that Addy et al. considered that abrasivity degree of toothpastes found *in-vitro* studies could be applied to clinical settings,[72] patients with dentine erosive lesions should be advised to use toothpaste with low RDA.

Although there is a remineralization period before and after brushing abrasion, and considering the abrasive slurries were prepared with fluoride (which has been shown to decrease brushing abrasion of eroded dentine),[73] eroded dentine is still susceptible to wear. This may be related to the fact that dentine is vulnerable and difficult to protect.[45,74] Vanuspong et al. indicated that re-hardening of dentine after acid attacks may not occur.[74] Furthermore, Hara et al. have concluded that 60 min of remineralization between the erosion and the abrasion treatments has no effect on the prevention of surface loss of eroded dentine.[75] However, in this study we did not aim to study the effect of remineralization on eroded dentine.

The Effect of Toothbrush Stiffness on the Abrasion of Eroded Dentin

It has been established previously that nylon toothbrushes alone have negligible effects on dental hard tissue,[76] but might indirectly influence the abrasion process by modulating the action of toothpaste. This is related to the previous indication that different types of toothbrushes probably differ in their capacity to hold toothpaste abrasives, which may result in differences in abrasion of the dental substrate.[77] Dyer et al.

suggested two reasons related to this indication: filament stiffness and density of the brush, and second, the filament area of the brush head.[15]

The present study involved one brand of three different types of toothbrush (soft, medium, and hard) with nylon bristles and end rounded tips. Only one brand of toothbrush was used in this experiment, because stiffness (soft, medium, hard) among the brands of toothbrushes is not constant.[37]

In a study by Wiegand et al., toothbrushes with a filament diameter of 0.15 mm, 0.20 mm, and 0.25 mm were applied on eroded dentine using non-fluoridated toothpaste slurries. Wear of the eroded dentin increased along with the decreased diameter of toothbrushes. However, from our data, only with the high-abrasive toothpaste slurries did the hard toothbrush cause more dentin wear than the medium toothbrush at the fifth cycle; there were no other significant toothbrush differences. This finding is somewhat similar to the previous study of Manly and Harte, who showed that hard toothbrushes cause more surface loss than medium ones on sound dentine.[9]

Surprisingly, our data showed that toothbrush by itself was not significant nor by interacting with abrasives and the highest surface loss resulted only when all variables interacted together (abrasives, toothbrushes, and time). This is related to the effect of time, since Addy and Hunter have indicated that abrasion process is time-dependent.[3]

Our data confirmed a previous finding that the correlation between dentin wear and toothbrush stiffness is low, and dentine wear mainly depends on the abrasivity of the toothpaste.[11]

The Effect of Abrasivity Levels on the Abrasion of Eroded Enamel

In the comparison between high and low toothpaste slurries, the wear of eroded enamel specimens was not statistically different. These results are in agreement with those reported by Hooper et al.,[2] who investigated the interplay between erosion and abrasion of dental hard tissue using toothpaste with different abrasivities. They have stated that fluoridated toothpastes with differing RDA values display similar abrasiveness on previously eroded enamel. The authors speculated that any mechanical force may remove the softened layer of enamel, regardless of different abrasivity levels (RDA). In addition, they have calculated the lifetime brushing (100 years) based on the highest mean of enamel abrasion in their studies, which would be equivalent to 38 µm. Since the thickness of enamel at the cervical area is equal to 130 µm, a result of less than 100 µm enamel wear is considered to be clinically irrelevant. This might be an explanation of our results.[2] However, our data is in contrast to the previous findings of Wiegand et al.,[12] who showed that the abrasivity of toothpaste slurries is considered to be the major factor for eroded enamel wear. Wiegand et al. attempted to simulate the worst case scenario in evaluating the effects of slurries exhibiting different abrasivity levels on softened enamel. Their samples were subjected to 60 cycles each consisting of 15 s of erosion in 1 ml of hydrochloric acid , and toothbrushing abrasion performed at 250-g load. This is in contrast to the less aggressive nature of our model. Furthermore, in their study, the brushing was performed with non-fluoridated toothpaste to focus on the abrasivity of the toothpaste itself. On the other hand, in our model, we brushed our specimens with fluoridated toothpaste slurries, given that most available toothpaste contains fluoride. Hara et al. reported that fluoridated toothpaste reduces the wear of erosive-abrasive lesions.[64] Magalhaes et al. had suggested in their *in situ/ex vivo* study, which assessed the effect of low-dosage fluoridated dentifrices on the abrasion of eroded enamel, that brushing with fluoridated toothpaste had a protective effect on eroded enamel surface.[78] Moreover, it has been demonstrated that there is a tendency toward differences between toothpastes of different abrasivities in the absence of fluoride.[79] Although Hara et al. in 2009 indicated that the presence of fluoride in toothpaste increased the difference between high and low abrasives,[64] we assumed in our experiment the presence of fluoride could mask the abrasiveness of toothpaste slurries. This could explain our results compared with those of Wiegand.

However, it is important to point out that we did not intend to investigate whether fluoridated toothpaste is able to promote the complete rehardening of eroded enamel.

Further studies should be carried out to compare fluoridated toothpaste of different abrasivities with non-fluoridated toothpaste to confirm the present data.

It has been shown that saliva can remineralize the acid-softened tooth surface.[79] Furthermore, Attin et al. and Jaeggi found significant differences between brushing immediately compared with brushing after 60 min of exposure to saliva.[22,80] For this reason, the majority of *in-vitro* studies did not include a remineralization period in-between erosion and abrasion treatments in order to avoid rehardening of eroded enamel. However, a remineralization treatment was used in this investigation to simulate clinical conditions, which resulted in decreased eroded enamel wear.

The brushing load of 150 g may have affected negatively the extent of enamel wear in our investigations. Parry et al. have suggested that using 150-g brushing load had an influence on the abrasion level of the enamel in his investigation. They also indicated that, using a greater load is more appropriate in assessing the toothpaste abrasivity on enamel.[81]

Finally, by using a clinically relevant brushing time, the softened layer is unlikely to be completely removed. This probably confirms Wiegand et al., who assumed the outermost layer of softened enamel may behave differently compared with deeper layers.

The Effect of Toothbrush Stiffness on Abrasive Wear of Eroded Enamel

Regarding the abrasion of eroded enamel, Wiegand et al. have indicated that toothbrush filament stiffness is of secondary importance, because only a medium toothbrush and toothpaste with REA 6 caused more abrasion of the eroded enamel than did either soft or hard toothbrush.[12] In our study, toothbrush stiffness did not significantly affect enamel surface loss. This is not surprising because many investigators reported that filament stiffness was not a factor affecting toothpaste abrasivity. Voronets et al. have compared abrasion of softened human enamel brushed with two different types of toothbrushes (hard and soft). They stated that there was not any significant difference between hard and soft toothbrushes on the abrasion of eroded enamel.[82]

From these data we suggest that the choice of soft, medium, or hard toothbrush is of lesser relevance to enamel than to dentin abrasion.

The Effect of Cycling Time on the Abrasion of Eroded Enamel and Dentin

Regarding the variable of time, the measurement of surface loss was taken at two different times (third and fifth cycles). This allowed us to see the significant increase of surface loss of eroded enamel and dentin in the fifth cycle versus the third cycle. This was not surprising because the total exposed time to erosion in the third cycle was 1 hour, and in the fifth cycle, 1 hour and 40 min. Based on this, we may consider that the depth of softening of the surface of enamel and dentin specimens in the fifth cycle was probably more than that of the third cycle. As result, significantly greater surface loss was observed in the fifth cycle compared with the third.

SUMMARY AND CONCLUSIONS

The surface loss of eroded enamel and dentin has been assessed using a clinically relevant *in-vitro* cycling model for two surrogate toothpastes of varying abrasivity (low, high) and three different types of toothbrushes (soft, medium, hard). As for dentin, the results of our data showed the surface loss of softened dentin is mainly affected by toothpaste abrasivity, because more abrasive toothpaste presents greater surface loss. For enamel, neither abrasive levels nor toothbrushes affected the surface loss of softened enamel under the conditions of the present study.

Within the limitations of the present study, the following conclusion can be drawn:

1. The surface loss of eroded dentine is strongly correlated to relative dentine abrasivity (RDA).

2. The correlation between toothbrush filament stiffness and eroded dentine surface loss is very low.

3. The surface loss of eroded enamel is not correlated to the RDA. In other words, the wear of superficial, acid-softened enamel is not dependent on the abrasivity of toothpaste.

4. The toothbrush stiffness was not a factor affecting eroded enamel surface loss.

5. The extent of enamel and dentin loss is affected by the cycling time.

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ABSTRACT

INTERACTION BETWEEN TOOTHPASTE ABRASIVITY AND TOOTHBRUSH FILAMENT STIFFNESS ON THE DEVELOPMENT OF EROSIVE-ABRASIVE LESIONS

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Background: Toothpaste abrasivity is considered the major contributor in toothbrushing abrasive wear, while toothbrush stiffness can be considered a secondary factor that may modify the abrasivity of toothpaste.

Objectives: To investigate the longitudinal enamel and dentin surface loss caused by the interaction between the abrasives in toothpaste and toothbrush filament stiffness.

Study Hypothesis: The amount of enamel and dentin loss depends on the abrasivity of the toothpaste and the filament stiffness of toothbrush.

Materials and Methods: The following experimental factors were considered: abrasive suspension, at two levels (L-low: Z113 and H-high: Z103); and toothbrushes at three levels determined by bristle stiffness (soft, medium, and hard) generating 6 testing groups ($n = 8$). Slabs of bovine enamel and dentin were cut, embedded in acrylic resin, and polished. UPVC tapes were placed on the surface of the specimens, leaving an area of 1 × 4 mm exposed in the center of the each enamel slab. Specimens ($n = 8$) were subjected to 5 d of erosion/abrasion cycling: erosion (5min, 4×/d, 0.3% citric acid, pH 3.75), abrasion (15 s, 2×/d, 45 strokes each, 150-g load, automated brushing machine), fluoride treatment (15 s with abrasion and 45 s without abrasion; 275 ppm F as NaF in abrasive slurry) with exposure to artificial saliva between erosion and abrasion (1h) and all other times (overnight). Surface loss (SL, in micrometers) was determined by optical profilometry, after the third and fifth days of cycling. Data were analyzed using three-way ANOVA (alpha = 0.05). For enamel, only cycling time was found to affect surface loss with 5 d > 3 d. Overall, there was little SL (mean range: 0.76 µm to 1.85 µm). For dentin (mean SL range: 1.87 µm to 5.91 µm), significantly higher SL was found for 5 d vs. 3 d, with particularly large differences for hard toothbrush high abrasive, and medium toothbrush/low abrasive. Hard toothbrush resulted in significantly higher SL than medium toothbrush for high abrasive after 5 d, with no other significant stiffness differences. High abrasive had significantly higher SL than low abrasive overall with strong effects for all combinations, except medium stiffness after 5 d. In conclusion, the interplay between abrasivity and filament stiffness appears to be more relevant for dentin than enamel.

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