THE IMPACT OF MOUTHRINSES ON THE EFFICACY OF FLUORIDE DENTIFRICES IN PREVENTING ENAMEL AND DENTIN EROSION/ABRASION

by

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INTRODUCTION

Erosive tooth wear (ETW) refers to chemical dissolution of mineralized tissues by acids of intrinsic and extrinsic but non-bacterial origin.[1] Epidemiological studies have shown this lesion can be diagnosed in the primary as well as the permanent dentition.[1] Due to its increasing prevalence, tooth wear has amplified the need to preserve the natural teeth and to permanently replace lost tooth structure. Persistent and frequent softening of the dental surfaces by acids will increase the severity of the erosive lesion.[2] In particular to dentin erosion, low pH also activates matrix metalloproteinase (MMPs) enzymes, which have been suggested to contribute to the progression of erosion after extraction of the minerals from tubular dentin.[2]

Tooth abrasion is the loss of tooth structure by mechanical forces from a foreign element. It is commonly associated with incorrect brushing technique,[1] giving rise to notching at the cementoenamel junction.[1] In fact, erosion accelerates toothbrush abrasion due to prior softening of the enamel and dentin by acids.[3] In a study conducted by Eisenburger et al.,[4] it was found that tooth wear increases by 50 percent with the combined effect of erosion and abrasion. The authors concluded that softened enamel is vulnerable and can be easily removed by physical action.

Saliva can modulate erosive/abrasive tooth loss due to its mineral content and by formation of the acquired pellicle, a proteinaceous film covering the tooth surfaces. However, the protection provided by the pellicle is limited.[5]

Proper diagnosis may stop the progression of erosion considering patients comply with advice provided by their dentist. The best approach to prevent erosive tooth wear is primary prevention and elimination of causative factors.[6] Thus, along with cause-related treatment, supplemental measures to minimize tooth tissue loss are also mandatory.[7]

Fluoride has long been recognized for its ability to promote remineralization and help prevent demineralization of tooth surfaces subjected to acids related to the caries process.[8] For this reason, fluoride has been an obvious candidate for assessing its potential to aid in prevention of dental erosion.[9] Fluoride dentifrices have been effective in promoting rehardening of incipient enamel erosive lesions with a secondary consequence being the increased resistance of the remineralized lesions to a subsequent erosive challenge.[10] It has been shown that the presence of 1,100-ppm fluoride as sodium fluoride (NaF) in dentifrices could reduce dentin wear by erosion and erosion plus abrasion; however, the protective effect does not increase with higher fluoride concentration dentifrices.[11] *In situ*, similar findings were observed for enamel.[12] However, a dentifrice with 5,000-ppm fluoride does not appear to prevent enamel erosion in patients who are at risk of developing erosion. For that reason, other preventive measures should be considered.[13] Stabilized stannous fluoride (SnF_2) dentifrices are unique among the fluoride sources used in over-the-counter dentifrices because there are indications that the presence of both ions is relevant for erosion prevention.[14]

The mechanism of the stannous and fluoride ions in erosion prevention seems to be related to the formation of a thin layer on the enamel surface, composed of different precipitates such as Sn_2(PO4)OH, SnF3PO4, Ca($SnF_{3)2,}$ and $CaF_{2.}$ [15]

Habitual toothbrushing with fluoridated toothpaste followed by rinsing with mouthwashes is the most common method to maintain good oral hygiene.[16] Antimicrobial mouthwashes have been used for a long time to augment routine oral care measures by helping the treatment of gingivitis and periodontitis and to favor the reduction of dental caries.[16] A variety of formulations have been made commercially available, such as those containing chlorhexidine gluconate (CHX), essential oils (EO) or cetylpyridinium chloride (CPC). Although some mouthrinses may cause enamel erosion because of their low pH, it is unknown to what extent these mouthrinses can modulate the effect of fluoride derived from toothpaste in an erosive-abrasive model. Mouthrinses may dissolve tooth-bound fluoride and lessen the effect of the toothpaste delivered anti-erosive agents. At the same time, antibacterial agents known to have a high affinity to dental hard tissues, may play a role against erosion/abrasion or modulate the previously deposited fluoride and/or tin-containing layer.

OVERALL AIM

The objective of the present *in-vitro* study was to investigate and compare the impact of CHX, EO and CPC mouthrinses on ETW protection afforded by conventional fluoride toothpastes.

CLINICAL RELEVANCE

To understand the interaction and effect of commercially available mouthrinses predominantly used as anti-plaque or anti-gingivitis agents on the ETW protection afforded by conventional, over-the-counter fluoride toothpastes, and to provide better recommendations to patients at high risk for ETW.

NULL HYPOTHESIS

1) The interplay between different types of mouthrinses and toothpastes does not modulate the protective effect of fluoride compounds on eroded enamel and dentin.

2) The amount of enamel and dentin loss does not depend on the type of fluoride compound in the toothpaste.

3) The type of mouthrinse does not affect the extent of tooth surface loss.

ALTERNATIVE HYPOTHESIS

1) The interplay between different types of mouthrinses and toothpastes does modulate the protective effect of fluoride compounds on eroded enamel and dentin.

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3) The type of mouthrinse does affect the extent of tooth surface loss.

REVIEW OF LITERATURE

“Tooth wear” is recognized as a major problem in both children and adults.[1] It describes a variety of mechanisms that cause dental surface loss as erosion, abrasion, attrition and abfraction.[17] Dental erosion is the irreversible loss of dental hard tissue due to a chemical process of acid dissolution but not involving bacterial acids. Furthermore erosion is not directly associated with mechanical or traumatic factors, or with dental caries.[18] It is a growing public health concern due to an increase in its prevalence. Erosion appears to be unique to modern man. In an anthropologic study of the skulls of humans living in the Copper Age and Middle Ages, no erosion was found in 3927 teeth from 259 individuals.[19] Our fundamental understanding of the etiology and pathology of erosion has been largely informed through *in-vitro* studies that have been conducted over the last few decades.[20] Erosive tooth wear has become a focus of dental researchers and practitioners.[21]

ETIOLOGY OF EROSIVE TOOTH WEAR

In vivo, it was proven that the “critical pH” at which there is net demineralization of enamel and dentine to be below ~5.5 and 6.5, respectively.[22] Ideally, the etiology of erosion should be identified prior to patient management. This is not always possible because of the difficulty in gaining an accurate and relevant history or because the patient may deny important information regarding lifestyle or behavior.[23] However, the identification of risk factors will improve the success of management.

It is important, therefore, to question each patient about his or her medical history, medications and lifestyle.

The etiological factors can be divided into two main categories Intrinsic and Extrinsic factors.[24]

INTRINSIC FACTORS

These are of gastric origin (pH between 1 and 3), mainly composed of digestive enzymes, hydrochloric acid and mucus, and may be associated with significant palatal dental erosion.

a) Chronic Vomiting is the forceful expulsion of gastric content through the mouth and a common manifestation of many organic and psychosomatic disorders. The risk of erosion was up to 18 times higher in patients with chronic vomiting compared with non-vomiting patients.[25]

b) Regurgitation is reflex of gastric content into the pharynx. It is different than vomiting as it lacks abdominal contraction.[25] Also, studies showed that patients who presented gastro-esophageal reflux disease (GERD) are usually diagnosed with dental erosion because of continuous contact of gastric content with the dental tissues.[26]

c) Rumination is a behavior disorder consisting of effortless regurgitation of undigested food within minutes of starting or completing a meal. The food is either held in the mouth or re-chewed and then re-swallowed or expectorated.[25]

Extrinsic Factors

a) Foods: Consumption of carbonated beverages, flavored mineral waters and acidic food and candies are significant factors for erosive tooth wear because of low pH and high frequency of contact with the teeth.

b) Medications: Many acidic medications and food supplements may cause erosion to the dental tissue after long and continuous periods of use (e.g. vitamin C, aspirin, and some iron preparations). Also, medications with side effects of nausea and vomiting are potential risk factors.[27] Furthermore, some acidic oral care products, saliva substitutes and stimulators are classified as causative factors for erosive lesions.[28]

c) Environment: Work related factors like exposure to acids can result in dental erosion. Also, professional wine tasters and competitive swimmers are at a higher risk of erosion.[28]

Interaction Between Erosion and Abrasion

It has been shown in many *in-vitro* and *in-situ* studies that the simultaneous occurrence of different types of tooth wear is not unusual.[29] Erosion usually co-exists with attrition and/or abrasion, but one of these factors may be more significant than the others making the differential diagnosis difficult.[29] 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.[30]

Acids soften the surface of the dental hard tissues, which then become more susceptible to mechanical abrasion due to mineral loss and reduced surface hardness.[31] Many factors are involved in this interaction, e.g. type of food, characteristic of saliva, presence of fluoride in the dental care routine and many more factors.[31] Regarding the toothbrushing process, it is well documented that brushing abrasion is significantly related to the abrasiveness of toothpaste.[30] Toothbrush filament stiffness is considered to be negligible.[32]

Management

The increased prevalence of tooth wear has amplified the need to manage and replace the lost tooth structure. Early diagnosis may stop the progression of erosion providing patients comply with dentists' advice.[6] Careful examination of the most susceptible surfaces (labial & palatal of all upper teeth, occlusal of the lower first molars) under good lighting and on dry teeth facilitates diagnosis.

The main thrust of prevention is to control medical condition, change lifestyle and to record and monitor the erosion lesion.[6]

The early stage of dental erosion, during which there is no significant mineral loss, is reversible as the lost mineral ions can be replaced by those naturally present in saliva[5]. Biologically, saliva and the acquired dental pellicle play important roles in erosion prevention. Saliva provides phosphate and calcium ions that help in decreasing demineralization[33]. It has also been established *in vitro* and *in situ* that acid softened tissue can be remineralized after exposure to saliva by dilution, clearance or buffering of acids.[34]

On the other hand, if the salivary flow is compromised or the erosive challenge is too strong and frequent, saliva alone will be insufficient to protect the teeth from erosion.[35] Because of the irreversible damage caused by erosion, the need for interventions to provide significant protection has increased.[36] Fluoride products are one-approach strategies in the treatment of erosion, and they are effective to reduce its progression.[36] Fluoride is available in the form of dentifrices, mouthrinses, varnishes and gels. The beneficial effect is associated with the formation of CaF_2-like products, which acts as a physical barrier against acid attack.[15]

Fluoridated toothpastes are commonly used in routine oral health care as they claim to act as anticaries, antierosion and antiplaque agents, and have been shown to reduce hypersensitivity.

Mouthrinses

The use of mouthrinses after tooth brushing is a common oral hygiene measure in formal daily practice. Three common organic agents, which can be found in mouthwashes, have been clinically proven to be effective in the treatment of gingivitis and antiplaque when formulated at therapeutic concentrations: chlorhexidine (CHX), essential oils and cetylpyridinium chloride (CPC). The Food and Drug Administration (FDA) classified these agents as safe and allowed their use in over-the-counter medications.

CHX is considered the "gold standard" due to its broad-spectrum antimicrobial action that aids in treatment of gingivitis and periodontitis and to favor the reduction of dental caries.[37] However, prevention of progression of dentin erosion is another benefit that has been proposed with frequent use of CHX.[37] It is safe to use and has very low toxicity.[38]

The ability of CHX to bind to soft and hard tissues enhances its retention to maintain higher concentrations for prolonged periods of time.[39] Moreover, CHX is considered an inhibitor of MMPs - that cause degradation in dentin organic matrix.[38] It has a chelation mechanism that inactivate MMPs, which would otherwise potentiate dentin wear caused by erosion and abrasion.[38]

On the other hand, results of *in-vitro* experiments showed that the fluoride-chlorhexidine association is unfavorable, due to significant decrease in the substantivity of CHX, because of the interaction between its positive charges with the negative charge of the fluoride ion.[40]

A mixture of several essential oils has been shown to have anti-oxidative activity.[41] Thus, EO have a moderate effect on plaque regrowth and some anti-inflammatory effects, which may reduce the severity of gingivitis. However, unlike chlorhexidine, EO have poor oral retention.[42]

Cationic quaternary ammonium compounds such as CPC have been demonstrated in clinical and *in-vitro* studies to inactivate oral bacteria, reducing plaque and gingivitis.[43,44] Although they have greater initial oral retention and equivalent antibacterial activity to chlorhexidine: these compounds are rapidly desorbed from the oral mucosa.[38] A study comparing EO and 0.075 percent CPC concluded that both rinses provide a significant reduction in gingivitis and controlling dental plaque after six weeks of product use.

BOVINE ENAMEL AND DENTIN IN EROSION/ABRASION TESTS

Specimens generated from human teeth are preferred for *in-vitro* dental research because they are more clinical relevant substrate.[45] However; they are more difficult to collect in sufficient quantities with adequate quality. Also, it can be challenging to control the source and age of the collected human teeth, which may lead to larger variation in the outcome measures of the study.[45]

Bovine teeth have been widely used as a substitute for human dental hard tissue in many erosion/ abrasion experiments.[45] It is easier to collect and standardize sufficient number of bovine teeth in comparison to human teeth. Furthermore, bovine incisors have a larger surface area; allowing for more than one specimen to be obtained from one tooth.[46] On the other hand, the difference between human and bovine samples must be taken into consideration, especially as bovine enamel has been shown to be more susceptible to wear than human enamel.[45] However, it has been shown that there is no difference between human and bovine dentin under *in-vitro* erosion/abrasion conditions.[46]

MATERIALS AND METHODS

STUDY DESIGN

In this study, an established erosion/abrasion model was employed[47] to investigate the impact of CHX, EO and CPC mouthrinses on ETW protection afforded by two conventional fluoride toothpastes differing in fluoride compound. The present study followed a 5 (treatment rinses incl. controls) × 2 (fluoride toothpastes) × 2 (erosion with and without toothbrushing abrasion) factorial design. These factors were tested in both enamel and dentin substrates and analyzed independently. Test rinses were CHX, EO, CPC, a fluoride rinse (positive control), and deionized water (negative control); fluoride toothpastes were SnF_2 or NaF-containing ones [Figure 1].

Polished bovine enamel and dentin specimens were subjected to a 5-day pH cycling model with twice-daily treatments, with or without abrasion, with fluoride toothpaste, followed by exposure to mouthrinses. Erosion was performed five times daily. Specimens were exposed to artificial saliva during remineralization periods. After five days, the enamel and dentin surface loss were determined using non-contact profilometry and the efficacy of each treatment combination (toothpaste + rinse) compared.

STUDY TREATMENTS

The present study investigated ETW prevention provided by two fluoride toothpastes in combination with a total of five mouthrinses. Mouthrinses were chosen based on their popularity among dental patients, common availability in the market and likelihood of recommendation by dental professionals.

The two toothpastes were:

- NaF-toothpaste: Crest Cavity Protection; 1100 ppm F (Procter & Gamble, Cincinnati, OH, USA) (Figure 2)
- SnF_2-toothpaste: Crest Pro-Health; 1100 ppm F (Procter & Gamble, Cincinnati, OH, USA) (Figure 3).

The five mouthrinses were:

- CHX: GUM Paroex® Chlorhexidine Gluconate Oral Rinse USP, 0.12 percent (Sunstar Americas Inc., Schaumburg, IL, USA) (Figure 4).
- EO: Original Listerine® Antiseptic Mouthwash. Active Ingredients: Eucalyptol 0.092 percent, Menthol 0.04 percent, Methyl salicylate 0.060 percent and Thymol 0.064 percent (Johnson & Johnson, New Brunswick, New Jersey, USA) (Figure 5).
- CPC: Crest Pro-Health Clinical rinse, Deep Clean Mint. Active Ingredients: Cetylpyridinium Chloride 0.1 percent, (Procter & Gamble, Cincinnati, OH, USA) (Figure 6).
- F: ACT Alcohol Free Anti-cavity Fluoride Rinse, Mint. Active Ingredients: Sodium Fluoride (0.05 percent); 0.026 percent w/v fluoride ion; 226 ppm F), (Chattem, Inc., Chattanooga, TN, USA) (Figure 7).
- D/W: Distilled water as negative control group (Figure 8).

Toothpaste Abrasive Test

The abrasive level of the test toothpastes was determined using the radioactive dentin abrasivity (RDA) method, as described in ISO11690.

In summary, human root dentin specimens were subjected to neutron flux bombardments (Research Reactor Center, University of Missouri, Columbia, MO, USA) resulting in the formation of radioactive phosphorus (^{32}P). They were then brushed in a custom-made automated toothbrushing machine with suspensions (n = 8) prepared with the testing toothpastes (25 g in 40 ml deionized water) or with the standard calcium pyrophosphate ($Ca_2P_2O_7$) abrasive material (RDA standard grade, Odontex, Lawrence, KS, USA) (10 g in 50 ml of an aqueous solution of 0.5 percent carboxymethylcellulose and 10 percent glycerin). The sequence of brushing as well as the brushing procedures was as specified by the ISO11690. After each brushing run, a 1-ml sample of the suspension was collected, weighed, and added to 5 ml of scintillation cocktail (Ultima Gold; PerkinElmer, Waltham, MA, USA). They were thoroughly mixed and immediately put on a liquid scintillation counter (Tri-Carb 2900 TR Liquid Scintillation Analyzer; PerkinElmer, Shelton, CT, USA) for radiation detection, expressed in counts per minutes (cpm)/gram of suspension. The net cpm/gram of the standard abrasive was assigned a value of 100, and the RDA values of the testing dentifrices were calculated considering their cpm/gram values in relation to the standard abrasive.

Specimen Preparation

Enamel and dentin slabs measured (4 mm width × 4 mm length × 2 mm thickness), and stored in 0.1 percent thymol solution pH (7.0) at 4°C were prepared. Enamel slabs were obtained from middle third of bovine mandibular incisors, crowns, and dentin slabs were obtained from bovine mandibular incisors roots.

The bottom and topsides of the enamel and dentin slabs were sequentially ground flat using silicon carbide grinding papers (Struers RotoPol 31/RotoForce 4 polishing unit, USA) (Figure 9).

Next, the slabs were cleaned in an ultrasonic device with deionized water for 5 min (Figure 10). Then, they were embedded in acrylic resin blocks (Varidur acrylic system, Buehler, USA) utilizing a custom-made silicon mold, leaving the enamel and dentin surfaces exposed (Figure 11). The embedded blocks were serially ground and polished up to 4000-grit silicon carbide grinding paper followed by 1-µm diamond polishing suspension (Figure 9). Specimens were examined under (x3) optical magnification loupes, and selected based on the quality of enamel and dentin. Those with cracks or other defects were rejected. Two embedded specimens were glued together to form the study block and remained together throughout the study. During exposure to toothpastes, the entire blocks were submerged in the toothpaste slurry with one side only being exposed to toothbrush abrasion (Figure 12). The study blocks were randomly assigned to 10 experimental groups with eight specimen blocks per group ($n = 8$) (Figure 1 +13). Adhesive unplasticized polyvinyl chloride (UPVC) tapes were placed on the surface of the specimens, leaving an area of 1 × 4 mm exposed in the center of each of the enamel and dentin slabs (Figure 14).

DAILY TREATMENT REGIMEN

The daily treatment regimen comprised two treatments, with or without toothbrushing, with the study toothpastes as aqueous slurries, followed by the assigned rinse treatment after brushing, five acid challenges with a citric acid solution and exposure to artificial saliva at all other times (Table 1).

Erosive Solution

The demineralization solution was composed of 0.3 percent citric acid anhydrous in deionized water (pH 2.6, adjusted, if needed, with 1 N NaOH or HCl; Sigma-Aldrich, St. Louis, MO, USA).

Remineralization Media

Artificial saliva with the following composition was used as remineralization medium: 1.45 mM $CaCl_2$, 5.4 mM KH_2PO_4, 0.1 M Tris buffer, 2.2g/L porcine gastric mucin (adjusted to pH 7.0 with KOH; Sigma- Aldrich, St. Louis, MO, USA).[46]

Brushing Abrasion

Specimens were positioned in an automated brushing machine. They were brushed two times daily for 45 strokes/15s each (OHRI brushing machine) with Oral-B 40 toothbrushes (Procter & Gamble, Cincinnati, OH, USA) using 150 g of load with one of the two types of toothpaste (Figure 15). Toothpaste slurry was prepared by mixing 120 g toothpaste with 360 g distilled water. [47] (Figure 16)

Mouthrinse Treatments

After toothbrushing, specimens were subject to mouthrinse treatments for 1 min under gentle agitation (50 rpm; orbital shaker). After the last cycle each day, the specimens remained in artificial saliva in a closed container at room temperature until the next day (Figure 13).

Profilometry

After completion of the study, surface loss (SL) was measured using an optical profilometer (Proscan 2000, Scantron, United Kingdom). [48] [Figure 17]. The tapes were removed from the specimens, and the specimen was positioned in the optical profilometer with the experimental surface parallel to the horizontal plane. An area of $2 \times 1 \text{ mm}^2$ covering both reference areas (previously protected with UPVC tapes) and treated (exposed) surfaces was scanned [Figure 18] using horizontal resolutions of 0.01 and 0.05 mm, in the x and y directions, respectively. Dentin specimens were allowed to dry for 10 min before scanning, in order to reduce the possible interference caused by the shrinkage of the dentin organic content. Images were analyzed using dedicated software (Proscan 2000; Scantron), which calculates the average height of the two reference areas and subtracts it from the experimental area. The difference in the depth (surface loss), expressed in micrometer, was the response variable in this study [Figure 19].

Statistical Analysis

Separate analyses were performed for the dentin and enamel data. The effects of rinse (5 levels), toothpaste (2 levels), and toothbrushing (2 levels) on surface loss were analyzed using ANOVA. The ANOVA included fixed effects for the three factors and their interactions and a random effect for specimen block to account for erosion with and without abrasion measured within the same specimen block. Pair-wise comparisons between treatment combinations were made using the Sidak method to control the overall significance level at 5 percent.

The distribution of the surface loss measurements were examined and a transformation of the data (e.g. natural logarithm) were used if necessary to satisfy the ANOVA assumptions.

Sample size justification: Based on prior experiments, the coefficient of variation is estimated to be 0.3 per rinse-toothpaste combination, the study had 80 percent power to detect a doubling of the means between any two rinses for each toothpaste with or without abrasion and an 80 percent difference in the ratio of means between toothpastes for each rinse with or without abrasion, assuming two-sided tests conducted at an overall 5 percent significance level. A previous experiment showed the mean surface loss for NaF to be more than twice the mean for SnF_2, so the proposed sample size for this study was sufficient.

The relative dentin abrasiveness data were analyzed using a t-test model (Sigma Plot (12 .0) Software) with the significance level set at 0.05.

RESULTS

The RDA data of the test toothpastes can be found in (Table 2). The SnF_2-containing toothpaste was found to be more abrasive than the NaF-containing toothpaste ($p < 0.0001$). The surface loss of dentin and enamel that was exposed to erosion with abrasion was significantly higher than without abrasion ($p < 0.0001$).

DENTIN RESULTS

There was no interaction among the three factors (type of toothpaste slurries, mouthrinses types and brush/ not brush; $p = 0.0520$). Overall, the data did not show significant interaction between the two factors (type of toothpaste slurries and mouthrinses types; $p = 0.0662$). The mean (SD) dentin surface loss (µm) for NaF toothpaste treated specimens was significantly lower than for SnF_2 toothpaste treated specimens ($p < 0.0001$). The dentin surface loss was not significantly different among rinse types ($p = 0.9927$) (Figure 20).

ENAMEL RESULTS

There was no interaction among the three factors (type of toothpaste slurries, mouthrinses types and brush/ not brush; $p = 0.4720$). Overall, the data did not show significant interaction between the two factors (type of toothpaste slurries and mouthrinses types; $p = 0.1821$). The mean (SD) enamel surface loss (µm) for NaF toothpaste treated specimens was significantly higher than for SnF_2 toothpaste treated specimens ($p < 0.0001$). The enamel surface loss was not significantly different among rinse types ($p = 0.1946$) (Figure 21).

FIGURES AND TABLES

FIGURE 1. Illustration of toothpastes and mouthrinses treatments.

FIGURE 2. Photograph of NaF toothpaste treatment.

FIGURE 3. Photograph of SnF_2 toothpaste treatment.

FIGURE 4. Photograph of CHX treatment.

FIGURE 5. Photograph of EO treatment.

FIGURE 6. Photograph of CPC treatment.

FIGURE 7. Photograph of F treatment.

FIGURE 8. Photograph of deionized water.

FIGURE 9. Photograph of Struers RotoPol.

FIGURE 10. Photograph of Ultrasonic device and solution.

FIGURE 11. Photograph of study block.

FIGURE 12. Photograph of glued study blocks.

FIGURE 13. Photograph of the experimental groups.

FIGURE 14. Photograph of UPVC tape on the surface of the specimens.

FIGURE 15. Photograph of the brushing machine.

FIGURE 16. Photograph of slurry preparation.

FIGURE 17. Photograph of optical profilometer.

FIGURE 18. Photograph of specimen after tape removal.

FIGURE 19. Photograph of an output screen from optical profilometer analysis software.

FIGURE 20. Bar graph showing the mean (± standard deviation) dentin surface loss for all experimental groups.

FIGURE 21. Bar graph showing the mean (± standard deviation) enamel surface loss for all experimental groups.

TABLE I

Daily pH cycling regimen

	Treatment	**Duration**
Step 1	Erosion with citric acid (1of 5)	5 min
Step 2	Remineralization in artificial saliva (1 of 7)	60 min
Step 3	Exposure to fluoride toothpaste slurry in brushing machine (one side brushed [abrasion] and one side not brushed). (1 of 2)	15s (45 strokes)
Step 4	Exposure to treatment rinse (1 of 2)	1 min
Step 5	Remineralization in artificial saliva (2 of 7)	60 min
Step 6	Erosion with citric acid (2 of 5)	5 min
Step 7	Remineralization in artificial saliva (3 of 7)	60 min
Step 8	Erosion with citric acid (3 of 5)	5 min
Step 9	Remineralization in artificial saliva (4 of 7)	60 min
Step 10	Erosion with citric acid (4 of 5)	5 min
Step 11	Remineralization Treatment (5 of 7)	60 min
Step 12	Erosion with citric acid (5of 5)	5 min
Step 13	Remineralization in artificial saliva (6 of 7)	60 min
Step 14	Exposure to fluoride toothpaste slurry in brushing machine (one side brushed [abrasion] and one side not brushed). (2 of 2)	15s (45 strokes)
Step 15	Exposure to treatment rinse (2 of 2)	1 min
Step 16	Remineralization in artificial saliva (7 of 7)	Overnight

TABLE II

Radioactive dentin abrasion mean values

Test Article	**Relative Dentin Abrasion**
Crest Pro - Health	146.56 ± 10.35
Crest Cavity Protection	100.93 ± 2.16

TABLE III

Results of the statistical analysis for surface loss of dentin – study factors and their interaction (1/2)

Effect	NumDF	DenDF	FValue	ProbF	sig
TP	1	140	23.92	<.0001	*
Rinse	4	140	0.06	0.9927	
TP*Rinse	4	140	2.25	0.06	
Brush_not	1	140	45.42	<.0001	*
TP*Brush_not	1	140	0.01	0.9430	
Rinse*Brush_not	4	140	0.73	0.5752	
TP*Rinse*Brush_not	4	140	2.41	0.0520	

TABLE IV

Results of the statistical analysis for surface loss of dentin – toothpaste and brushing effects (2/2)

Comparison	Result	Estimate	StdErr	Probt	Sig
TP	NaF < SnF	-1.2239	0.2503	<.0001	*
Brush_not	No < Yes	-1.6867	0.2503	<.0001	*

TABLE V

Summary of the statistical results for dentin surface loss

Analysis Variable: Result							
TP	Rinse	Brush/not	N	Mean	Std Dev	Minimum	Maximum
NaF	CHX	No	8	-3.810	1.319	-6.197	-1.490
		Yes	8	-4.380	2.376	-7.447	-0.224
	CPC	No	8	-3.956	1.735	-6.949	-1.380
		Yes	8	-5.824	0.994	-7.563	-4.766
	D/I	No	8	-3.654	1.710	-5.602	-0.235
		Yes	8	-4.557	1.307	-7.042	-2.489
	EO	No	8	-3.408	1.333	-5.160	-0.434
		Yes	8	-6.423	1.452	-8.084	-4.169
	F	No	8	-4.034	1.568	-6.714	-2.160
		Yes	8	-6.023	1.562	-8.890	-4.034
SnF_2	CHX	No	8	-4.468	1.226	-6.280	-2.792
		Yes	8	-7.906	2.113	-10.116	-3.965
	CPC	No	8	-4.903	0.956	-5.881	-2.934
		Yes	8	-6.425	1.906	-9.091	-2.512
	D/I	No	8	-5.829	1.047	-7.216	-3.735
		Yes	8	-6.683	1.500	-8.632	-5.124
	EO	No	8	-5.136	1.819	-9.194	-2.905
		Yes	8	-6.259	1.930	-9.500	-3.178
	F	No	8	-4.556	1.199	-7.069	-2.960

Analysis Variable: Result							
TP	Rinse	Brush/not	N	Mean	Std Dev	Minimum	Maximum
		Yes	8	-6.143	1.725	-8.124	-2.786

TABLE VI

Results of the statistical analysis for surface loss of enamel– study factors and their interaction (1/2)

Effect	NumDF	DenDF	FValue	ProbF	sig
TP	1	140	42.66	<.0001	*
Rinse	4	140	1.54	0.1946	
TP*Rinse	4	140	1.58	0.1821	
Brush_not	1	140	292.85	<.0001	*
TP*Brush_not	1	140	0.13	0.7229	
Rinse*Brush_not	4	140	0.66	0.6179	
TP*Rinse*Brush_not	4	140	0.89	0.4720	

TABLE VII

Results of the statistical analysis for surface loss
of enamel – toothpaste and brushing effects (2/2)

Comparison	Result	Estimate	StdErr	Probt	Sig
TP	NaF > SnF	1.6430	0.2516	<.0001	*
Brush_not	No < Yes	-4.3049	0.2516	<.0001	*

TABLE VIII

Summary of the statistical results for enamel surface loss

Analysis Variable: Result							
TP	Rinse	Brush/not	N	Mean	Std Dev	Minimum	Maximum
NaF	CHX	No	8	-2.319	0.523	-3.162	-1.562
		Yes	8	-8.041	1.491	-9.881	-5.589
	CPC	No	8	-2.835	1.370	-4.780	-0.761
		Yes	8	-6.140	3.018	-11.733	-2.440
	D/I	No	8	-3.438	1.146	-5.306	-1.311
		Yes	8	-7.417	1.247	-9.402	-5.837
	EO	No	8	-3.738	0.420	-4.390	-3.366
		Yes	8	-7.762	2.353	-10.685	-3.975
	F	No	8	-3.133	0.611	-3.957	-2.153
		Yes	8	-7.180	1.949	-10.630	-5.070
SnF_2	CHX	No	8	-1.381	0.300	-2.009	-1.040
		Yes	8	-5.562	1.789	-8.293	-3.420
	CPC	No	8	-1.052	0.966	-2.483	-0.184
		Yes	8	-5.358	2.606	-8.603	-0.797
	D/I	No	8	-1.790	0.539	-2.406	-0.906
		Yes	8	-6.260	2.096	-8.452	-2.706
	EO	No	8	-0.953	0.363	-1.623	-0.388
		Yes	8	-4.899	2.184	-7.633	-1.539
	F	No	8	-1.625	0.733	-2.370	-0.449
		Yes	8	-6.693	1.804	-8.794	-4.177

DISCUSSION

Fluoridated toothpastes are widely used for routine oral hygiene habits and have beneficial effects in the prevention of tooth demineralization.[36] Various factors influence the efficacy of fluoride, such as the type of fluoride compound, concentration, and amount of toothpaste applied to the toothbrush, frequency of brushing and post-brushing rinsing behavior.[49] After toothbrushing, it is a common practice to rinse with a mouthrinse to augment the routine dental care. Three common organic agents, which can be found in mouthrinses, have been clinically proven to be effective in the treatment of gingivitis and antiplaque when formulated at therapeutic concentrations: chlorhexidine, essential oils and cetylpyridinium chloride. The United States Food and Drug Administration (FDA) classified these agents as safe and allowed their use in over-the-counter medications.

The aim of the present study was to investigate and compare the impact of chlorhexidine; essential oils and cetylpyridinium chloride mouthrinses on erosive tooth wear protection afforded by conventional fluoride toothpastes. To answer the study questions, an established five-day erosion/abrasion cycling protocol was employed, involving episodes of erosion challenges, remineralization in artificial saliva, brushing abrasion and mouthrinse treatments.

For the erosive challenge, we used 0.3 percent citric acid (pH 2.6) five times per day for five minutes each time. Artificial saliva containing mucin was applied between erosive and abrasive challenges for one hour as well as for overnight storage.

This allowed for the adsorption of mucin onto the eroded specimen surfaces, thus modulating the remineralization process in a similar manner as human saliva.[48]

The 1-hour saliva storage was designed to simulate the pellicle layer that remains on tooth surfaces just after brushing, and constant bathing in artificial saliva enabled the maturation of the pellicle over time.

In this study, we attempted to simulate the recommended brushing time of nearly two minutes per session. Each specimen was brushed for 30 seconds, the equivalent of 15 seconds or 45 brushing strokes for each surface. The 45 brushing strokes equate to 450 brushing strokes at the end of each cycle and represent five days of brushing. This experimental approach is more representative of the everyday clinical situation since most people brush their teeth twice daily rather than after each contact with erosive foodstuff.

The toothbrushes were attached to brushing machine in order to standardize movement of the toothbrush and to ensure that the surfaces of all the specimens were brushed under consistent load. The use of 150 g brushing load for testing the abrasiveness of toothpastes is in agreement with previous recommendations by Wiegand et al. [31] as well as the International Standards Organization (ISO 11609).

The toothpaste slurries in this experiment were prepared using commercially available fluoridated toothpastes by adding one part (120g) of toothpaste to three parts (360g) deionized water. [47] The slabs were immersed in these solutions for one minute, two times per day.

One half of enamel and dentin samples received NaF toothpaste (1100 ppm F), the other received SnF_2 toothpaste (1100 ppm F). Then the specimens were subjected to mouth rinse treatments for one minute.

Non-contact surface profilometry was used in this study for two reasons. [48] Firstly, the profilometer is useful 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. Secondly, by using a non-contact profilometer, we eliminated any possible interference that may be caused by a contact profilometer device due to direct contact between the device and tested specimen.

Although clinical investigations can reflect the actual erosive challenges and oral environments, it is more difficult to standardize study parameters and control study conditions. The advantage of *in-vitro* models is standardization of erosive and toothbrush abrasion as well as saliva properties to provide a better understanding of the tested variables and to provide a close view of their interaction.

The Effect of Brushing and Toothpaste on Surface Loss

The surface loss was statistically different ($p < 0.0001$) between enamel and dentin specimens that were subjected to the brushing process in comparison to the non-brushed groups. Toothbrush abrasion is considered three-body wear because of the presence of abrasive particles from the toothpaste, which are considered a contributing factor for tooth surface loss.[3]

Furthermore, the presence of fluoride in dentifrices is important to lessen its abrasiveness and in modulating the erosive-abrasive lesion in enamel and dentin. In a previous study, it was shown that toothpaste abrasiveness caused pronounced dentin surface loss, and it proved the importance of fluoride to provide sufficient surface protection.[47]

In our experiment, brushing process was accomplished using an automated brushing machine with nylon toothbrushes. It has been established previously that nylon toothbrushes alone have negligible effects on the dental hard tissues.[50] In another study, it was found that the correlation between toothbrush filament stiffness and surface loss of previously eroded enamel and dentin to be very low.[51] However, filament stiffness may indirectly influence the abrasion process by modulating the action of toothpaste abrasive particles.[5]

However, the efficacy of toothpastes in preventing surface loss is modulated when combined with toothbrushing. In the present study, it can be seen that the tested dentifrices provided a degree of protection against erosive challenges when applied as slurries. SnF_2 slurries showed statistically significant enamel protection against erosive and abrasive challenges compared to NaF paste ($p < 0.0001$).

This is in agreement with previous findings, which showed that after five erosive cycles, SnF_2 offered more protection to enamel surfaces in comparison to NaF and sodium monofluorophosphate (SMFP).[52,53] Also, other investigators concluded that the marketed dentifrice formulated with stabilized SnF_2 might provide enhanced protection of exposed tooth surfaces against dietary acid attack compared to NaF and SMFP/arginine-containing dentifrices.[36]

Stannous fluoride has been demonstrated to enhance acid protection due to formation of amorphous deposits on the enamel surface, and incorporation of Sn ions into eroded enamel.[15]

In contrast to enamel, dentin was afforded more protection against surface loss by NaF compared to SnF_2. Our findings are different than those of Diamonti et al., who concluded that there was no significant difference between 1450 ppm F as NaF and 1100 ppm F as stabilized SnF_2.[54]Also, in an in-situ study NaF toothpaste was found to be more effective than SnF_2 in preventing dentin surface loss after erosive cycles.[55] However, Ganss et al. showed that SnF_2 has more potential to reduce erosion/ abrasion in dentin.[56]

Dentin erosion is more complex than enamel erosion because the organic matrix plays an important role in the progression of wear and prevents further demineralization especially in the presence of fluoride.[2]

The potential of sodium fluoride to inhibit dentin erosion is attributed to the formation of F rich layer that acts as a physical barrier against acidic challenges.[57]

Interestingly, this layer forms more easily on dentin than on enamel and acts as a mineral reservoir, buffers acids, enhances fluoride adsorption, and therefore the overall stability of the hard tissue will tend to increase.[58] One reason for this is the smaller hydroxyapatite crystals in dentine, which results in a larger surface area to crystallite volume ratio and therefore a more reactive mineral phase.[59]

Conversely, the findings of another *in-vitro* studies showed that fluoride concentration is more important than the type of fluoride compound (NaF or SnF_2) in the presence of the demineralized organic matrix.[60]

Actually, it appears difficult to identify specific active agents in toothpaste formulations to reduce enamel and dentin surface loss. Differences in experimental settings, type and concentration of fluoride compounds tested, and differences in dentifrice formulations have led to variability between studies and difficulty in generalizing certain outcomes.

The Effect of Mouthrinses on Modulating ETW Protection Afforded by Fluoride Toothpastes

The main result of the present study is that there was no statistically significantly difference between CHX, EO, CPC, F and D/W rinses in modulating the effect of the tested fluoride compounds in their ability to prevent erosive tooth wear. There was no statistical difference among all tested rinses in the surface loss results.

The tested rinses were used immediately after the brushing procedure with fluoride slurry, which may have accelerated the clearance of fluoride from the tooth surface and reduce its efficacy. Many factors influence F substantivity on dental hard tissues, such as rinsing behavior, time of rinsing, and volume of the rinsing liquid, which may also have a major impact on fluoride retention. One way for fluoride retention on the tooth surface is association with amine groups in mucin that can link to the negative charges available in the acquired pellicle, which leads to increased substantivity of fluoride and prolongs its availability on the dental surfaces. [40] In the present study, mouthrinse applications were conducted under 50 rpm agitation, which can lead to partial removal of loosely bound fluoride on the tooth surface. In previous studies, it was concluded that post-brushing rinsing should be kept to a minimum in order to reduce rapid intra-oral fluoride clearance. [61]

A study by Attin et al. found that rinsing after brushing with fluoridated dentifrices reduces the salivary fluoride concentration. [62] Moreover, an interesting observation by Nordstrom et al. was that the difference between 5,000-ppm F toothpaste with rinsing and 1,450-ppm F toothpaste without rinsing was minor in terms of fluoride salivary retention. [63]

Sodium fluoride mouthwash was used as a positive control in this study. It contained 225 ppm F, which is commonly employed in commercial mouthrinses.

The tested sodium fluoride mouthrinse was not statistically significant different compared to D/I water ($p = 0.9927$ for dentin, and $p = 0.1946$ for enamel).

The explanation of this may be twofold: a) the low fluoride concentration of this rinse does not afford protection against erosion, and/or b) the specimens had little capacity to accumulate further fluoride after treatment with toothpaste slurries. In a review of literature published in 2010, the authors concluded that F rinse with elevated concentration (at least 450 ppm F) is important in prophylaxis and management of dietary acid-mediated enamel erosion. [64] Also, an *in-vitro* study conducted by O'Toole et al. found that a NaF mouthrinse with 225 ppm F was effective in reducing enamel surface loss after the first cycle of the study, but the result was not promising after the fifth cycle of erosive challenge. [52] However, another study showed erosion was reduced using a 225 ppm F rinse, however discrepancies in study design between this and the present study make do not necessarily justify a comparison.[65]

Chlorhexidine is a well-known antibacterial agent that has prolonged bacteriostatic action, which reduces plaque accumulation and oral bacteria counts in general.

The present results showed no effect of CHX on the anti-erosive action of fluoride dentifrices. This is probably because of fluoride clearance from the enamel and dentin surfaces due to rinsing action, which reduce the F retention. Another *in-vitro* study showed that the fluoride-chlorhexidine interaction was unfavorable due to significant decrease in the substantivity of CHX, due to its positive charge associating with the negative charge of fluoride, [40] which in turn may affect substantivity of fluoride.

On the other hand, studies on caries and using inherently different outcome measures contradict the present findings: an *in-vivo* study conducted in 1994 found that the combination of CHX and fluoride was significantly more effective in reducing both lesion depth and mineral loss. [66]

EO and CPC are commonly used anti-plaque agents. However, the evidence supporting the effectiveness of antiplaque agents in preventing dental caries is very limited.[42, 43] The present findings for EO and CPC rinses match those for CHX in that no significant difference was found between these mouthwashes and other controls in their ability to modulate the effect of fluoride dentifrices in ETW prevention. Our results are in agreement with a previous study that showed no statistically significant difference between EO and water after the fifth cycle of erosion. [65]

Lastly, the present study was conducted *in vitro* and did not take into account the soft tissue and oral mucosa, in a vivo environment, which reflects the actual erosive conditions. Fluoride and other actives, such as CHX, CPC and EO, may be retained on the tongue. Due to its large surface area, this may not only increase their retention but also alter their interaction, which warrants further research.

Furthermore, the time interval between brushing and rinsing was kept constant which may not necessarily be representative as some rinses (CHX) are recommended to be used at least 1 h after toothbrushing. In future studies, different waiting times between brushing and rinsing should be considered. Further research may also include studies on the effect of the abrasive level and pH of the toothpaste slurries.

SUMMARY AND CONCLUSION

Toothbrushing with fluoride toothpaste followed by rinsing with mouthwash is a routine procedure to maintain good oral hygiene. The objective of the present *in-vitro* study was to investigate and compare the impact of chlorhexidine (CHX), essential oils (EO), and cetylpyridinium chloride (CPC) rinses in comparison to deionized water and sodium fluoride rinses on the erosive tooth wear protection afforded by conventional fluoride toothpastes. A clinical relevant *in-vitro* erosion/abrasion pH cycling model was employed to test the effect of the aforementioned rinses on modulating the ability of NaF and SnF_2 toothpastes.

The results showed that the mean dentin surface loss associated with NaF toothpaste was significantly lower than for SnF_2 toothpaste. On the other hand, enamel surface loss with SnF_2 toothpaste was significantly lower than for the NaF toothpaste. Also, the surface loss of erosion when associated with abrasion was significantly higher than without brushing and for both enamel and dentin. The interesting finding was that there was no significant difference in the surface loss among all rinse types.

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

1) Commonly used mouthrinses containing antimicrobial agents or additional fluoride, do not impact fluoride toothpaste action on erosion/abrasion.

2) Considering erosion only, the tested SnF_2 dentifrice offered greater protection against enamel surface loss than the tested NaF dentifrice.

3) For dentin, considering erosion only, the tested NaF dentifrice offered greater protection against surface loss than SnF_2 dentifrice.

4) Toothbrushing abrasion of previously eroded enamel and dentin significantly increased surface loss.

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ABSTRACT

THE IMPACT OF MOUTHRINSES ON THE EFFICACY OF FLUORIDE DENTIFRICES IN PREVENTING ENAMEL AND DENTIN EROSION/ABRASION

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Objective: Toothbrushing with fluoride toothpaste followed by rinsing with mouthwash is a routine procedure to maintain good oral hygiene. It is unknown to what extent these rinses can modulate the effect of fluoride in its ability to prevent erosion/abrasion.

The aim of this *in-vitro* study was to investigate and compare the impact of chlorhexidine (CHX), essential oils (EO) and cetylpyridinium chloride (CPC) mouthrinses on erosive tooth wear protection afforded by conventional fluoride toothpastes.

Materials and Methods: The following experimental factors were considered: five rinses: CHX, EO, CPC, a fluoride rinse, and deionized water, two fluoride toothpastes: stannous fluoride (SnF_2) or sodium fluoride (NaF) and two models: (erosion/ erosion+abrasion). Slabs of bovine enamel and dentin were prepared and embedded in resin blocks and generated 10 enamel and dentin testing groups ($n = 8$). UPVC tapes were placed on the sides of each slab leaving 1mm area exposed in the center. The blocks were subjected to a five-day cycling model. Then, the blocks were placed in a brushing machine and exposed to fluoride toothpaste slurry (one side was brushed and the other wasn't). The blocks were then exposed to rinse treatments. Artificial saliva was used to remineralize the specimens after erosions and treatment challenges, and as storage media. After the fifth day of cycling, surface loss (in micrometers) was determined by profilometer. Data were analyzed using ANOVA ($\alpha = 0.05$).

Results: There was no interaction among the three factors (type of toothpaste, mouthrinse and abrasion or not (dentin $p = 0.0520$, enamel $p = 0.4720$). There were no significant two-way interactions as SL was only affected by toothpaste and mouthrinse.

NaF caused less SL than SnF_2 (4.60 vs. 5.83 µm; $p < 0.0001$) in dentin, whereas the opposite was found in enamel (5.20 vs. 3.56 µm; $p < 0.0001$). Toothbrushing abrasion caused comparatively more SL in enamel (6.53 vs. 2.23 µm; $p < 0.0001$) than in dentin (6.06 vs. 4.38 µm; $p < 0.0001$). None of the tested mouthrinses affected SL.

Conclusion: Commonly used mouthrinses containing antimicrobial agents or additional fluoride, do not impair the erosion/abrasion protection afforded by fluoride toothpastes. Tested SnF_2 dentifrice offered greater protection against enamel surface loss and NaF dentifrices showed more protection for the dentin surface.

Clinical relevance: The understanding of the interaction between commonly used rinses and fluoride dentifrices will help dentists provide better recommendations to patients with erosive lesions.

CURRICULUM VITAE

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