MEDICAL SCIENCE

The impact of mouth rinses on the efficacy of fluoride dentifrices in preventing enamel and dentin erosion/abrasion

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ABSTRACT

Purpose: Toothbrushing followed by is a method to maintain good oral hygiene. It is unknown to what extent mouth rinses can modulate the effect of fluoride in its ability to prevent erosion/abrasion. The aim of this in vitro study was to investigate the impact of chlorhexidine (CHX), essential oils (EO), and cetylpyridinium chloride (CPC) mouth rinses on erosive tooth wear protection afforded by conventional fluoride toothpaste. *Methodology:* The following experimental factors were considered: Five rinses: CHX, EO, CPC, a fluoride rinse, and water, two fluoride toothpaste: stannous fluoride (SnF_2), sodium fluoride (NaF), and two models: erosion only and erosion + abrasion. Bovine enamel and dentin slabs were embedded in resin blocks (n=8). Specimens were subjected to a five-day cycling regimen consisting of twice-daily treatments, with or without abrasion, with fluoride toothpaste, followed by mouth rinse exposure. Erosion (0.3% citric acid) was performed 5×/d. Specimens were exposed to artificial saliva during remineralization periods. Surface loss (SL) was determined using non-contact profilometry. Data were analyzed using ANOVA ($\alpha=0.05$). *Results:* There was no interaction among the three factors (type of toothpaste, mouth rinse, and abrasion or not). There were no significant two-way interactions, as SL was only affected by toothpaste and mouth rinse. NaF caused less SL than SnF_2 ($p<0.0001$) in dentin, whereas the opposite was found in enamel ($p<0.0001$). Erosion + abrasion caused more SL than erosion only ($p<0.0001$). None of the tested mouth rinses affected SL. *Conclusion:* Commonly used mouth rinses do not impair the erosion/abrasion protection fluoride toothpaste provides.

Keywords: Dental, erosion, abrasion, fluoride, mouth rinse.

1. INTRODUCTION

Dental erosion refers to the chemical dissolution of mineralized tissues by acids of intrinsic and extrinsic but non-bacterial origin. Tooth abrasion is the loss of tooth structure by mechanical forces from a foreign element. It is commonly associated with incorrect brushing technique, giving rise to notching at the cementoenamel junction (Imfeld, 1996). Erosion accelerates toothbrush abrasion due to prior softening of the enamel and dentin by acids, increasing the severity of erosive tooth wear (Attin, 2006). The literature found tooth wear to increase by 50% with the combined effect of erosion and abrasion (Eisenburger et al., 2003).

Proper diagnosis may stop erosion progression, assuming patients also comply with advice provided by their dentist (Hannas et al., 2016). The best approach to prevent erosive tooth wear is primary prevention and elimination of causative factors (Lussi and Hellwig, 2006). Thus, along with cause-related treatment, supplemental measures to minimize tooth tissue loss are also recommended (Attin et al., 2009). 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 (White et al., 1994). For this reason, fluoride has been an obvious candidate for assessing its potential to aid in the prevention of dental erosion (Huysmans et al., 2014).

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 appear to increase with higher fluoride concentration dentifrices (Magalhães et al., 2008). 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 (Eversole et al., 2015). Habitual toothbrushing with fluoridated toothpaste followed by rinsing with mouth rinses is a method to maintain good oral hygiene. 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 (Schlueter et al., 2009).

A variety of such mouth rinses are commercially available, such as those containing chlorhexidine gluconate (CHX), essential oils (EO), or cetylpyridinium chloride (CPC). CHX is considered the "gold standard" due to its broad-spectrum antimicrobial action that aids in treating periodontal diseases (Lorenz et al., 2006). Moreover, prevention of progression of dentin erosion and inhibition of MMPs are other benefits that have been proposed with frequent use of CHX (Magalhães et al., 2009). Although some mouth rinses may cause enamel erosion because of their low pH, it is unknown to what extent they can modulate the effect of fluoride derived from toothpaste in preventing erosion/abrasion. Therefore, the objective of the present in vitro study was to investigate and compare the impact of CHX, EO, and CPC mouth rinses on erosive toothwear (ETW) protection afforded by conventional fluoride toothpaste.

2. MATERIALS AND METHODS

Study design

In this study, an established erosion/abrasion model was employed (Hara et al., 2009) to investigate the impact of CHX, EO, and CPC mouth rinses on ETW protection afforded by two conventional fluoride toothpastes differing in fluoride compound. The present study followed a 5 (treatment rinses: CHX, EO, CPC, a fluoride rinse (positive control), and deionized water (negative control)) × 2 (fluoride toothpaste: SnF_2 or NaF) × 2 (erosion with and without toothbrushing abrasion) factorial design. These factors were tested in both enamel and dentin substrates and analyzed independently.

Study treatments

The present study investigated ETW prevention provided by two fluoride toothpaste combined with five mouth rinses. Mouthrinses were chosen based on their popularity among dental patients, common availability in the market, and likelihood of recommendation by dental professionals (Figure 1).

The two toothpastes were

NaF - toothpaste: Crest Cavity Protection; 1100 ppm F (Procter & Gamble, Cincinnati, OH, USA).

SnF_2 - toothpaste: Crest Pro-Health; 1100 ppm F (Procter & Gamble, Cincinnati, OH, USA).

The five mouth rinses were

CHX: GUM Paroex® Chlorhexidine Gluconate Oral Rinse USP, 0.12%, (Sunstar Americas Inc., Schaumburg, IL, USA).

EO: Original Listerine® Antiseptic Mouthwash.

Active Ingredients: Eucalyptol 0.092%, Menthol 0.042%, Methyl salicylate 0.060%, and Thymol 0.064%, (Johnson & Johnson, New Brunswick, New Jersey, USA).

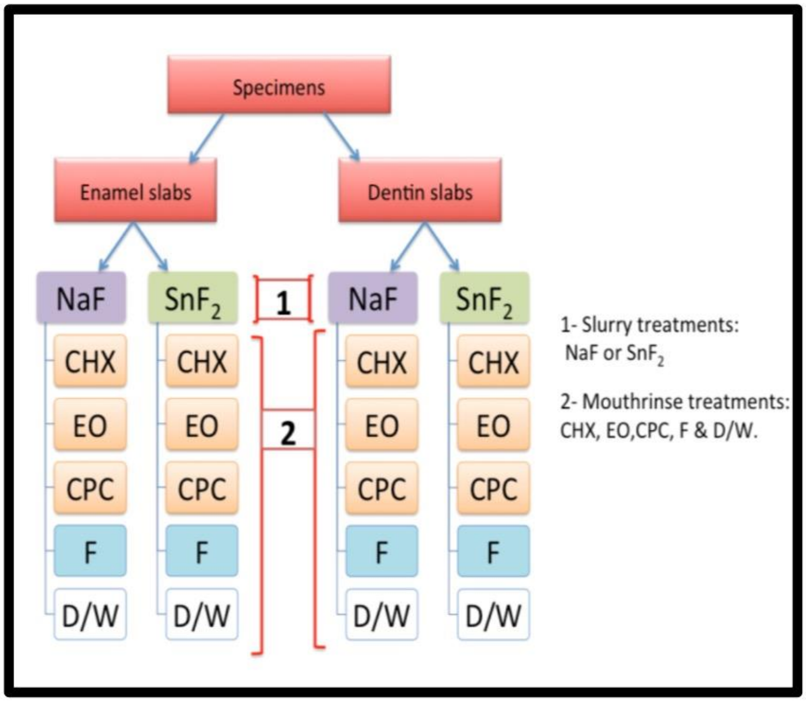

Figure 1 Illustration showing the toothpaste and mouth rinse treatments used in the study.

CPC: Crest Pro-Health Clinical rinse, Deep Clean Mint. Active Ingredients: Cetylpyridinium Chloride 0.1% (Procter & Gamble, Cincinnati, OH, USA).
F: ACT Alcohol-Free Anti-Cavity Fluoride Rinse, Mint. Active Ingredients: Sodium Fluoride (0.05%); 0.026% w/v fluoride ion; 226 ppm F), (Chattem, INC. Chattanooga, TN, USA).
D/W: Distilled water as a negative control group.

Toothpaste abrasive test

The abrasive level of the test toothpaste 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 (32P). They were then brushed in a custom-made automated toothbrushing machine with suspensions (n=8) prepared with the testing toothpaste (25 g in 40 ml deionized water) or with the standard calcium pyrophosphate (Ca2P2O7) abrasive material (RDA standard grade, Odontex, Lawrence, KS, USA) (10 g in 50 ml of an aqueous solution of 0.5% carboxymethylcellulose and 10% glycerin).

The sequence of brushing, as well as the brushing procedures, was as specified by 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 (4 mm width × 4 mm length × 2 mm thickness) obtained from bovine mandibular incisors, stored in 0.1% thymol solution pH (7.0) at 4°C, were prepared, ground flat using silicon carbide grinding papers (Struers RotoPol 31/RotoForce 4 polishing unit, USA). Then, they were embedded in acrylic resin blocks (Varidur acrylic system, Buehler, USA) utilizing a custom-made silicon mold, exposing the enamel and dentin surfaces. The embedded blocks were serially ground and polished up to 4000-grit silicon carbide grinding paper followed by 1-μm diamond polishing suspension.

Specimens were 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. The study blocks were randomly assigned to 10 experimental groups with eight specimen blocks per group (n=8). 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 enamel and dentin slab.

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).

Table 1 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 (5 of 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

Erosive Solution

The demineralization solution was composed of 0.3% 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) (Hara et al., 2009).

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. Toothpaste slurry was prepared by mixing 120 g toothpaste with 360 g distilled water (Hara et al., 2009).

Mouthrinse Treatments

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

Profilometry

After completion of the study, surface loss (SL) was measured using an optical profilometer (Proscan 2000, Scantron, United Kingdom). The tapes were removed from the specimens, and they were positioned in the optical profilometer with the experimental surface parallel to the horizontal plane. An area of 2 ×1 mm^2 covering both reference areas (previously protected with UPVC tapes) and treated (exposed) surfaces was scanned using horizontal resolutions of 0.01 and 0.05 mm in the x and y directions, respectively. 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 a micrometer was the response variable in this study.

Statistical Analysis

Separate analyses were performed for the dentin and enamel data. The effects of rinse (five levels), toothpaste (two levels), and toothbrushing (two levels) on surface loss were analyzed using ANOVA. The ANOVA included fixed effects for the three factors and their interactions. Pair-wise comparisons between treatment combinations were made using Fisher'sFisher's Protected Least Significant Differences to control the overall significance level at 5%. The relative dentin abrasiveness data were analyzed using a two-sample t-test. Sample size justification: Based on prior experiments, the coefficient of variation was estimated to be 0.3 per rinse-toothpaste combination. The study had 80% power to detect a doubling of the means between any two rinses for each toothpaste with or without abrasion and an 80% difference in the ratio of means between toothpastes for each rinse with or without abrasion, assuming two-sided tests conducted at an overall 5% significance level.

3. 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 exposed to erosion with toothbrushing abrasion was significantly higher than without abrasion ($p<0.0001$).

Table 2 Radioactive dentin abrasion means values

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

Dentin Results

The surface loss data for dentin can be found in (Figure 2). There was no interaction among the three factors (type of toothpaste, mouth rinse, and abrasion or not; $p=0.0520$). The data did not show a significant interaction between the two factors (type of toothpaste slurries and mouth rinse 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 ($p=0.9927$).

Enamel Results

The surface loss data for enamel can be found in (Figure 3). There was no interaction among the three factors (type of toothpaste, mouth rinse, and abrasion or not; $p=0.4720$). Overall, the data did not show a significant interaction between the two factors (type of toothpaste slurries and mouth rinses 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 ($p=0.1946$).

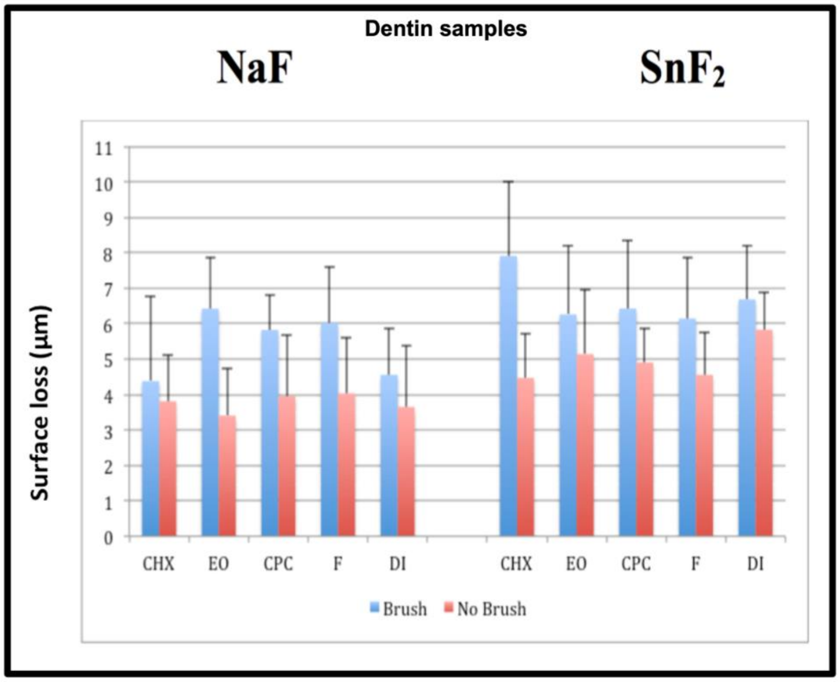

Figure 2 Bar graph showing the mean (± standard deviation) dentin surface loss for all experimental groups.

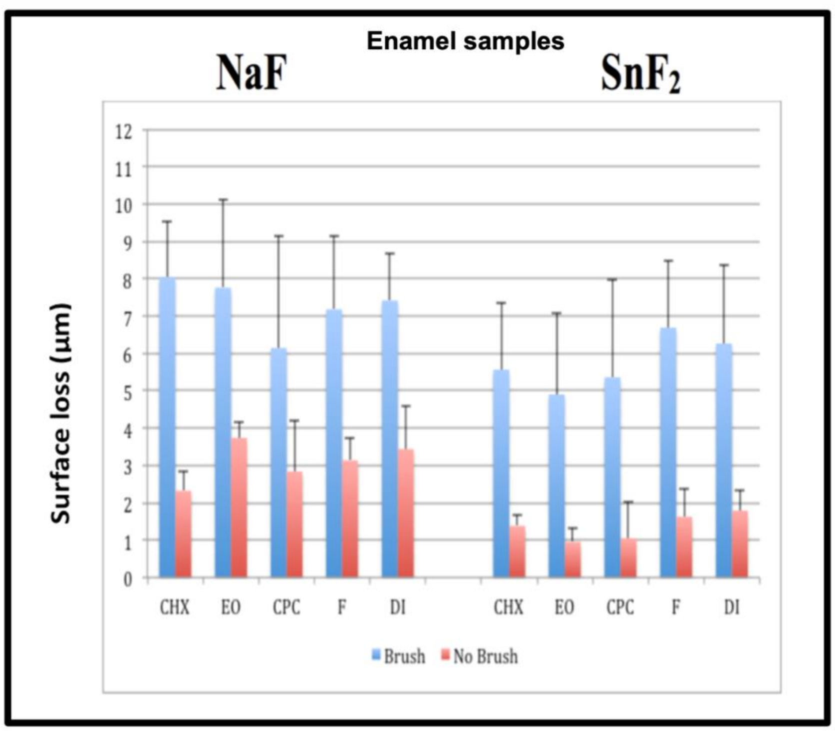

Figure 3 Bar graph showing the mean (± standard deviation) enamel surface loss for all experimental groups.

4. DISCUSSION

Fluoridated toothpastes are widely used for routine oral hygiene habits and can prevent tooth demineralization (Sandra et al., 2014). Various factors influence the efficacy of fluoride, such as the type of fluoride compound, concentration, amount of toothpaste applied to the toothbrush, frequency of brushing, and post-brushing rinsing behavior (Davies et al., 2003). After toothbrushing, it is a common practice to rinse with a mouth rinse to augment routine dental care. Three common organic agents, which can be found in mouth rinses, 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 (Lorenz et al., 2006). The United States Food and Drug Administration (FDA) classified these agents as safe and allowed their use in over-the-counter medications.

Although clinical investigations can reflect the actual erosive challenges and oral environments, they can be limited by the difficulty of standardizing study parameters and controlling study conditions. On the other hand, in vitro models allow for the standardization of erosive and toothbrush abrasion challenges and saliva remineralization action to provide a better understanding of the tested variables and how they interact with each other. In this study, we attempted to simulate the recommended brushing time of 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 represents the everyday clinical situation since most individuals brush their teeth twice daily rather than after each contact with erosive foodstuff.

The surface loss was statistically different ($p<0.0001$) for enamel and dentin specimens, respectively, that were subjected to the brushing process compared to the non-brushed groups. Toothbrush abrasion is a three-body wear process due to the presence of abrasive particles from the toothpaste, which contribute to tooth surface loss (Attin, 2006). Furthermore, the presence of fluoride in dentifrices is vital to lessen their abrasiveness and in modulating the erosive-abrasive lesion in enamel and dentin (Hara et al., 2009). Toothbrushes have been shown to have negligible effects on the dental hard tissues on their own. However, toothbrushes modulate the interaction of abrasives with the tooth surface (Wiegand et al., 2009; Manly and Brudevold, 1957).

The present study demonstrated that the tested dentifrices provided a degree of protection against erosive challenges. The SnF2–containing dentifrice showed statistically significant superior enamel protection against erosive and abrasive challenges compared to the NaF-containing dentifrice. This agrees with previous findings, which showed that after five erosive cycles, SnF2 offered more protection to enamel surfaces in comparison to NaF and sodium monofluorophosphate (SMFP) (O'Toole et al., 2016; Paepegaey et al., 2013). Furthermore, other investigators concluded that the marketed dentifrice formulated with stabilized SnF2 might provide enhanced tooth protection against dietary acid attack compared to NaF and SMFP/arginine-containing dentifrices (Sandra et al., 2014). Stannous fluoride has been demonstrated to enhance acid protection due to the formation of amorphous deposits on the enamel surface and the incorporating of Sn ions into eroded enamel (Schlueter et al., 2009).

In contrast to enamel, dentin was afforded more protection against surface loss by the NaF-containing dentifrice than the SnF2-containing one. An in-situ study demonstrated similar results as a NaF toothpaste was found to be more effective than a SnF2 one in preventing dentin surface loss after erosive cycles (West et al., 2012). However, Ganss et al., (2014) showed that SnF2 has more potential to reduce erosion/abrasion in dentin. The potential of sodium fluoride to inhibit dentin erosion is attributed to forming a fluoride-rich layer that acts as a physical barrier against acidic challenges (Ganss et al., 2001). Interestingly, this layer forms more easily on dentin than on enamel and acts as a mineral reservoir, buffers acids, and enhances fluoride adsorption; therefore, the overall stability of the hard tissue will tend to increase (Ganss et al., 2007).

One reason for this is the smaller hydroxyapatite crystals in dentin, which results in a larger surface area to crystallite volume ratio and, therefore, a more reactive mineral phase (Cate et al., 1998). Conversely, the findings of another in vitro study showed that fluoride concentration is more important than the type of fluoride compound (NaF or SnF2) in the presence of the demineralized organic matrix (Ganss et al., 2010). 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 specific outcomes. The most important finding of the present study is that there was no statistically significant 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.

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 reduced its efficacy. Many factors influence F substantivities on dental hard tissues, such as rinsing behavior, time of rinsing, and volume of the rinsing liquid, which also have a significant impact on fluoride retention. In the present study, mouth rinse 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 to reduce rapid intra-oral fluoride clearance (Chestnutt et al., 1998). Sodium fluoride mouthwash was used as a positive control in this study. It contained 225 ppm F, a commonly employed fluoride concentration in commercial mouth rinses. The tested NaF mouth rinse was not statistically significantly different compared to D/I water ($p= 0.9927$ for dentin and $p= 0.1946$ for enamel).

The explanation 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 recent review, the authors concluded that F rinse with elevated concentration (at least 450 ppm F) is essential in prophylaxis and management of dietary acid-mediated enamel erosion (Anan et al., 2010). Also, an in vitro study conducted by O'Toole et al., (2016) found that NaF mouth rinses with 225 ppm F effectively reduced enamel surface loss after the first cycle of the study. However, the result could have been promising after the fifth cycle of erosive challenge (O'Toole et al., 2016). However, another study showed erosion was reduced using a 225 ppm F rinse, but discrepancies in study design between this and the present study do not necessarily justify a comparison (Ullsfoss et al., 1994).

Lastly, the present study was conducted in vitro and did not consider the impact of soft tissues and oral mucosa, which could affect the intra-oral retention of active ingredients clinically. Fluoride and other actives, such as CHX, CPC, and EO, may be retained on the tongue, which has a large surface area that may increase retention and interaction of the active agents, 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 one hour after toothbrushing. However, it can be questioned whether patients would comply with these instructions. 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.

5. CONCLUSION

Within the limitations of the present study, we concluded that commonly used mouth rinses containing antimicrobial agents do not impair the erosion/abrasion protection afforded by conventional NaF and SnF2-containing dentifrices. Also, in the erosion-only model, the tested SnF2 dentifrice offered more excellent protection against enamel surface loss than the tested NaF dentifrice; the opposite result was found for dentin. Lastly, toothbrushing abrasion of previously eroded enamel and dentin significantly increased surface loss.

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Authors Contributions

Ebtehal Albeshir: Sample preparation, experiment, data collection, and writing.

Reem Albluwi, Ibtesam Almobarak, Abdulmohsen Alrabea: Data analysis, figures, tables, reviewing, and payment.

Norman B Cook, Anderson T Hara: Research idea and reviewing

George J Eckert: Statistical analysis

Frank Lippert: Writing, reviewing, and mentoring.

Informed consent

Not applicable.

Ethical approval

Not applicable.

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Conflict of interest

The authors declare that there is no conflict of interests.

Data and materials availability

All data sets collected during this study are available upon reasonable request from the corresponding author.

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