Protocol for measurement of enamel loss from brushing with an anti-erosive toothpaste after an acidic episode

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Tooth erosion from an acidic insult may be exacerbated by toothbrushing. The purposes of this study were to develop an in vitro methodology to measure enamel loss after brushing immediately following an acidic episode and to investigate the effect of brushing with an anti-erosive toothpaste. The null hypotheses tested were that tooth erosion after brushing with the toothpaste would not be different from brushing with water and that a 1-hour delay before brushing would not reduce tooth erosion. Forty bovine enamel slabs were embedded, polished, and subjected to baseline profilometry. Specimens were bathed in hydrochloric acid for 10 minutes to simulate stomach acid exposure before post-acid profilometry. Toothbrushing was then simulated with a cross-brushing machine and followed by postbrushing profilometry. Group 1 was brushed with water; group 2 was brushed with a 50:50 toothpaste-water slurry; and groups 3 and 4 were immersed in artificial saliva for 1 hour before brushing with water or the toothpaste slurry, respectively. The depth of enamel loss was analyzed and compared using 1-way analysis of variance and post hoc testing ($\alpha = 0.05$). Greater enamel loss was measured in groups brushed with toothpaste than in groups brushed with water. One-hour immersion in artificial saliva significantly reduced enamel loss when teeth were brushed with water (group 3; $P < 0.05$) but not with toothpaste (group 4). This study established a protocol for measuring enamel loss resulting from erosion followed by toothbrush abrasion. The results confirmed the abrasive action of toothpaste on acid-softened enamel.

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Dental erosion remains a significant dental health problem for many patients who experience recurrent acidic episodes. Exposure to intrinsic acids, such as those found in gastric refluxate, softens tooth enamel and causes tooth surface loss.[1] Erosive tooth wear is found in all age groups.[2] Recurrent acidic exposure can be a manifestation of systemic conditions, such as bulimia, acid reflux, pregnancy morning sickness, or frequent consumption of highly acidic foods and drinks.[1,3,4] Dental erosion can result in extensive tooth damage, resulting in costly dental treatment to restore the teeth to their optimal form and function. Erosive wear is multifactorial, as biological, behavioral, and chemical factors can interact to further destroy or, in some cases, protect the tooth structure.[1]

Dentists are in a unique position to detect the early signs of dental erosion in their patients and thus can help prevent or slow tooth destruction. A comprehensive knowledge of the risk factors involved—including the frequency of acidic exposure in patients suffering from systemic conditions—is imperative for developing customized home care regimens for these patients. Appropriate home care for patients who suffer from recurrent acidic episodes can be instrumental in the prevention and treatment of acid erosion.

Many patients are under the erroneous assumption that meticulous and overzealous brushing after the consumption of acidic foods and drinks or the regurgitation of stomach acids is an effective means to protect tooth structures from acid erosion.[5,6] However, enamel softened by acid exposure is inherently more susceptible to toothbrush abrasion.[7,8] Brushing immediately after an acidic episode can intensify the tooth wear process significantly, whereas waiting to brush for 1 hour after acid exposure can decrease surface loss.[9]

In an in vitro study, Dzakovich & Oslak found more tooth wear after the use of common commercial toothpastes than after brushing with water alone.[10] The abrasivity of toothpastes can be measured using the relative dentin abrasivity (RDA) method.[11] Although toothpastes with high RDA values (indicating high abrasivity) reportedly often have higher cleaning potential, the relationship is not always straightforward.[12] Conventional fluoride toothpastes have been shown to reduce erosive tooth loss but to have limited efficacy if the erosion is followed by a brief toothbrushing session.[13]

In a normal, healthy population, toothpastes containing sodium fluoride, stannous fluoride, or other cleaning ingredients are effective in plaque removal and caries prevention. However, in patients suffering from systemic recurrent acidic

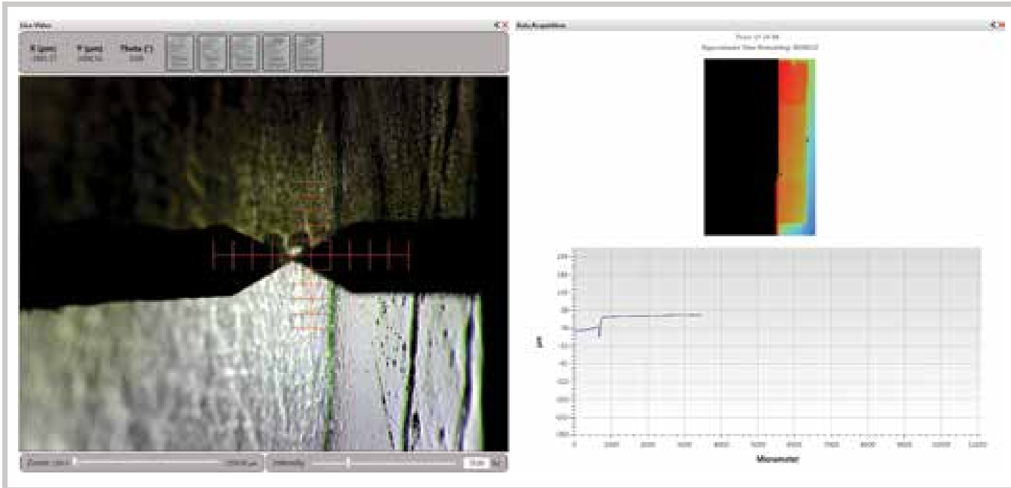

Fig 1. Baseline profilometry of a tooth specimen.

Fig 2. Polytetrafluoroethylene tape protecting both edges of the tooth specimen from acid erosion and serving as a reference area for analysis of surface loss.

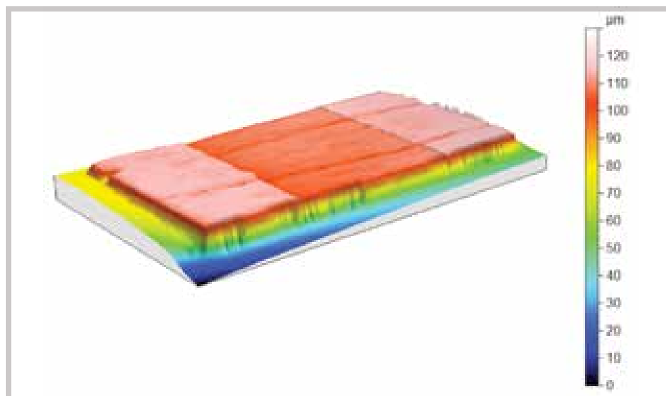

Fig 3. Surface profilometry of an enamel specimen after acid erosion. The height of the enamel surface relative to the base of the specimen is shown by the color scale. The shoulder areas are higher than the middle portion because the shoulders were protected from acid erosion.

episodes with resultant erosive tooth wear, the same toothpastes can create further tooth destruction. A clinical case report verified increased tooth surface destruction in a bulimic patient who brushed immediately after vomiting.[14]

Certain toothpastes are marketed as specifically formulated to help protect against acid erosion, including Sensodyne ProNamel (GlaxoSmithKline), which has an RDA value of 35, among the lowest of any toothpaste on the market.[12,15] Unfortunately, there are few in vitro or clinical studies determining the effectiveness of these products. The present study aimed to develop an in vitro methodology to capture the very small enamel loss from a single episode of acid erosion followed by toothbrushing. Once established, the methodology was used to test 2 hypotheses. The first null hypothesis was that tooth surface destruction after an acidic episode followed by brushing with a toothpaste marketed as being helpful against acid erosion would not be different from enamel loss after brushing with water. The second null hypothesis was that 1-hour immersion in saliva before brushing would not reduce tooth erosion.

Materials and methods

Specimen preparation

Forty bovine teeth were cut into 5 × 10-mm enamel slabs and embedded in acrylic resin. The specimens were sequentially polished with 240-, 400-, and 600-grit silicon carbide paper followed by 0.5- and 1.0-µm alumina suspensions.

Surface profilometry

To obtain baseline profilometry measurements, each enamel specimen was placed on a wafer fixture with imprinted cross-sectional markings in a profilometer (DektakXT, Bruker) (Fig 1). The markings were used to allow precise repositioning of the specimen at the different phases. The specimen was fixed with double-sided tape to the wafer base. The wafer fixture was then coupled to an X-Y auto stage with a single-arch bridge to reduce sensitivity to environmental conditions. The measurement parameters were set at 524-µm range, 4.995-µm resolution, 4-mg stylus force, and 40 traces; the total scanning time was approximately 15 minutes.

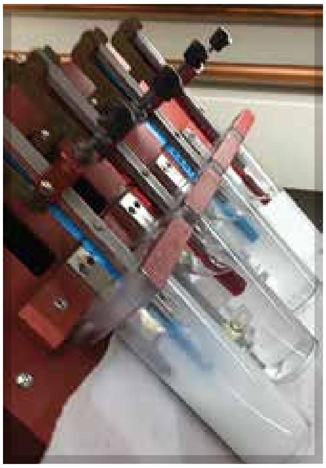

Fig 4. Simulated toothbrushing with a cross-brushing machine.

Table. Mean (SD) depth of enamel loss (in mm) after acid erosion and toothbrushing.

	Brushing immediately		Brushing after 1 hour	
Measurement	Group 1	Group 2	Group 3	Group 4
Post-acid	3.61 (1.87)[a]	2.83 (1.49)[a]	4.76 (1.64)[a]	3.97 (1.92)[a]
Postbrushing	5.66 (2.05)[a]	5.55 (1.57)[a]	6.54 (1.52)[a]	6.96 (2.14)[a]
Difference*	2.05 (0.55)[ab]	2.72 (1.48)[ab]	1.78 (1.10)[a]	2.99 (0.50)[b]

Groups: 1, brush with water alone immediately after acidic episode; 2, brush with toothpaste immediately after acidic episode; 3, brush with water alone after 1-hour storage in artificial saliva after acidic episode; 4, brush with toothpaste after 1-hour storage in artificial saliva after acidic episode.

*Difference between postbrushing and post-acid values.

Mean values with the same superscript lowercase letter (in rows) were not significantly different (ANOVA and post hoc test; significance level 0.05).

Hydrochloric acid erosion

Both edges of the enamel slabs (known as *shoulders*) were covered with polytetrafluoroethylene tape to protect them from acid erosion (Fig 2). This design allowed the shoulder areas to be used as reference in the analysis of enamel loss. Specimens were immersed in 10 mM of hydrochloric acid (pH 1.5) for 10 minutes before they were subjected to post-acid profilometry. An example of surface profilometry of an enamel specimen after acid erosion is shown in Fig 3.

Toothbrushing simulation

Toothbrushing was carried out in a V8 cross-brushing machine (Sabri Dental Enterprises) using toothbrushes that met American Dental Association standards of soft-bristle brushes (Fig 4). The brushing was done at 1.5 Hz with 350 g of force for 1 minute. Enamel specimens were divided into 4 groups (n = 10). Group 1 was brushed with deionized water. Group 2 was brushed with a 50:50 (by weight) Sensodyne ProNamel toothpaste and deionized water slurry. Specimens in groups 3 and 4 were immersed for 1 hour in artificial saliva before brushing with deionized water (group 3) or the Sensodyne ProNamel–water slurry (group 4). The artificial saliva (pH 7.0) consisted of 1.5 mM of calcium chloride, 0.9 mM of monopotassium phosphate, 20 mM of 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), and 130 mM of potassium chloride in deionized water. After brushing, the enamel specimens were subjected to postbrushing profilometry.

Surface loss and statistical analysis

The data obtained for surface profilometry were uploaded to TalyMap Platinum 6.2 Profilometry analysis software (Taylor Hobson). Depths of enamel loss were calculated for each specimen, first after the acid treatment and then after the brushing simulation, with the following formula: Depth of enamel loss = *ED* (shoulders) – *ED* (post-acid or postbrushing), where *ED* is the averaged depth over a selected area.

Comparisons among the tested groups were done using analysis of variance (ANOVA) followed by a Student-Newman-Keuls post hoc test at a significance level of 0.05.

Results

The depths of enamel loss are shown in the Table. The hydrochloric acid immersion used in this study to simulate exposure to stomach acid caused enamel losses in the range of 3-5 mm deep. There were no statistically significant differences in post-acid enamel loss among the groups.

Brushing after the acidic episode resulted in further loss of enamel. Differences in the surface loss between the post-acid and postbrushing stages were higher when brushing with the toothpaste than when brushing with deionized water, but the differences were not significant (ANOVA and post hoc test; significance level 0.05). One-hour immersion in artificial saliva significantly reduced enamel loss when specimens were brushed with water (group 3; significance level 0.05). However, the depths of enamel loss when specimens were brushed immediately or after 1-hour immersion in artificial saliva were not significantly different when the toothpaste slurry was used (groups 2 and 4). The differences between postbrushing and post-acid measurements were significantly greater in group 4 than in group 3 (significance level 0.05).

Discussion

Conventional toothpastes containing fluoride have been important for basic caries prevention, and they may prevent erosive demineralization from daily exposure to food and drinks with high acidic content.[13,16,17] Saliva plays an important role in remineralizing at early stages of tooth erosion, which is characterized by a softened surface on the eroded lesion.[18] However, patients who suffer from recurrent acidic episodes occasionally develop extensive tooth surface loss despite the fact that they regularly brush their teeth with fluoride-containing toothpastes. Enamel and dentin that are softened by acid erosion are vulnerable to mechanical abrasion, even from tongue movement.[19] In addition, many patients with systemic conditions such as acid reflux or bulimia also have low salivary flow, which minimizes the neutralization and remineralization of the tooth surface.[20,21] To reduce the abrasive effects of these factors, patients are instructed to refrain from toothbrushing for at least 1 hour after an erosive attack.[9,22]

Fig 5. Severe tooth surface loss resulting from acid erosion and toothbrush abrasion in a patient with gastroesophageal reflux disease. The cervical lesion on the mandibular left canine is associated with the use of chewing tobacco.

Fig 6. Severe tooth surface loss resulting from acid erosion and toothbrush abrasion in a patient with gastroesophageal reflux disease. Deep grooves from toothbrush abrasion surround the cervical composite restorations on the mandibular left canine and premolars.

Several commercial toothpastes that are designed specifically for anti-erosive action have been introduced recently. Certain ingredients in toothpaste (such as fluoride) can promote remineralization and thus rehardening of an erosive lesion.[23] However, toothpastes are used for brushing teeth, and the brushing itself is an abrasive action. This issue was highlighted in a study where conventional fluoride toothpastes reduced erosive tissue loss but had limited efficacy for preventing brushing abrasion.[13] Toothpaste formulations with anti-erosion claims were reported to be not better, or even less effective, than conventional sodium fluoride toothpaste.[13]

In the present study, an in vitro erosion/abrasion model was established by creating eroded enamel lesions approximately 3-5 mm deep. This was accomplished by exposing the tooth enamel to hydrochloric acid, which mimics stomach acid regurgitation. Each acidic episode was followed by a 1-minute brushing period, which caused further enamel loss. The results of this study showed that the depth of enamel loss resulting from brushing with toothpaste or brushing with water was not significantly different, and therefore the first null hypothesis was not rejected. In other words, any anti-erosive effect resulting from the active ingredient of the toothpaste was cancelled out by the abrasivity of the toothpaste.

The second null hypothesis was that 1-hour immersion in saliva between the acid erosion and brushing procedures would not reduce tooth surface loss. A previous study reported that 1-hour immersion in artificial saliva significantly increased the surface hardness of tooth enamel softened by acid erosion, although the hardness was not restored to its original value.[24] However, in another study, up to 4 hours' storage in human saliva did not increase surface hardness or decrease tooth wear of the enamel after an erosive attack and subsequent brushing abrasion using fluoride toothpaste.[25] The results from the current study concur with the latter study when the brushing included use of the toothpaste-water slurry. Without the abrasivity of the toothpaste, however, the tooth surface loss was significantly reduced after 1-hour immersion in artificial saliva and brushing with water alone. In other words, the 1-hour delay was not sufficient to remineralize the softened enamel enough to withstand the abrasivity of the toothpaste. Therefore, the second null hypothesis was partially rejected.

The present results support those of Lussi et al, who found that exposure to natural saliva for any clinically relevant period of time was not sufficiently effective to decrease tooth surface loss.[25] As a result, Lussi et al proposed that the recommendation to delay toothbrushing after an erosive attack be reconsidered.[25] Clearly, further study is needed to find the best approach for patients who suffer from recurrent acidic attacks. According to the manufacturer, Sensodyne ProNamel has a relatively low abrasivity index compared to most commercially available toothpastes.[15] Therefore, the amount of enamel loss that occurs with brushing may be different when other toothpastes are used. This aspect is currently under investigation in a follow-up study by the authors of the present research.

The clinical relevance of the erosion/abrasion model can be illustrated by 2 case descriptions. A 29-year-old man with extensive tooth erosion from gastroesophageal reflux disease (GERD) had acid regurgitation for more than 10 years. Generalized loss of tooth enamel on the anterior teeth was noticeable (Fig 5). In addition, crater-shaped, noncarious cervical lesions were observed on the mandibular left canine and second premolar. The cervical lesions were a result of smokeless ("chewing") tobacco. A form of smokeless tobacco known as *snuff* is popular in the United States. Snuff is finely ground tobacco, usually placed between the bottom lip and the gingiva.[26] Smokeless tobacco typically contains abrasive substances that cause abrasion on teeth already vulnerable as a result of acid erosion.

In another case of abrasive tooth wear on acid-eroded teeth, a 49-year-old man had extensive tooth erosion and history of GERD for more than 30 years. The patient reported almost nightly acid regurgitation. He practiced vigorous toothbrushing to eliminate the bitter taste of the refluxate. He had numerous cervical lesions, some of which had been restored with composite resin (Fig 6). The effects of toothbrush abrasion were apparent as deep grooves surrounding composite restorations on the mandibular canines and premolars.

Proper oral care behavior is critical in maintaining oral health. Toothbrushing after an acid attack may do more harm to the acid-softened teeth via mechanical debridement. A case report by Harrison et al is illustrative.[14] They described 2 bulimic women in their 30s who had undergone purging episodes approximately 3 times a day for 6 and 13 years, respectively. The patient with 13 years of purging had relatively minor enamel erosion, whereas the patient with 6 years of purging had extensive erosion. The latter patient had completely lost her lingual enamel into dentin pulp chambers beneath the dentinal surface. The authors observed that the discrepancy in tooth destruction might have been the result of the different toothbrushing habits of the patients. The patient with less severe erosion did not brush immediately after purging episodes, whereas the patient with more extensive destruction did.[14] For ethical reasons, this observation cannot be tested in a clinical study, but the results of the current study offer support for the authors' explanation; the in vitro method developed for the current study can help to investigate and propose treatment and prevention strategies.

General dentists play an important role in the diagnosis and management of tooth erosion. Recognizing the contributing factors enables practitioners to advise their patients. This study showed that brushing with toothpaste resulted in further loss of acid-softened enamel due to the dentifrices' abrasive components, even in a toothpaste designed to be anti-erosive. A 1-hour delay before brushing only helped to reduce enamel surface loss if the brushing was done without toothpaste. However, it is important to acknowledge patients' need to clean their teeth after an acidic episode. The dental profession must develop other innovative options that avoid abrasivity and promote enamel rehardening for patients for whom toothbrushing, with or without toothpaste, is not a viable option.

Conclusion

This study established an in vitro protocol for measuring enamel loss after an episode of acid erosion followed by 1 minute of brushing. Findings confirmed the abrasive action of toothpaste on acid-softened enamel, despite the anti-erosive properties of the dentifrice. A delay of 1 hour was not sufficient to allow enough remineralization to reduce tooth surface loss resulting from abrasion when enamel was brushed with toothpaste following acid erosion.

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Disclosure

Drs Dehghan and Tantbirojn are inventors on a pending patent application, "Methods and composition for preventing and treating tooth erosion" (WO2014134463 A1; February 28, 2014). Other coauthors have no financial, economic, commercial, or professional interests related to topics presented in this article.

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