Acta Odontologica Scandinavica, 2008; 66: 231–235

ORIGINAL ARTICLE

Impact of toothpaste slurry abrasivity and toothbrush filament stiffness on abrasion of eroded enamel – an *in vitro* study

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Abstract

Objective. Toothbrush abrasion is significant in the development of tooth wear, particularly when combined with erosion. This *in vitro* study aimed to evaluate the impact of toothpaste slurry abrasivity and toothbrush filament stiffness on abrasion of eroded enamel. ***Material and methods.*** Eroded enamel samples (hydrochloric acid, pH: 2.6, 15 s) were brushed with 40 strokes in an automatic brushing machine using manual toothbrushes with different filament stiffness (filament diameter: 0.15, 0.20, or 0.25 mm). A paste-free control slurry (relative enamel abrasion (REA) value 2) and toothpaste slurries with different abrasivity (REA values 6 or 9) were used for brushing. Erosion and abrasion were followed by storing the enamel samples in artificial saliva for 3 h. After each 4th cycle, the samples were stored in artificial saliva for 15 h. After 60 cycles, enamel loss was measured by profilometry and statistically analyzed by two-way and one-way ANOVA and Bonferroni/Dunn post-hoc tests. ***Results.*** Loss of enamel (mean, μm) was influenced mainly by the abrasivity of the slurry and increased along with REA value (REA 2: 0.0–0.2, REA 6: 2.1–3.3, REA 9: 2.9–3.7). Abrasion of eroded enamel was also affected by filament stiffness of the toothbrush, but only groups brushed with toothpaste slurry of REA 6 showed any significant difference between the different toothbrushes. Thereby, toothbrushes with 0.2 mm filament diameter caused higher enamel loss than 0.15 and 0.25 mm filaments. ***Conclusions.*** Toothbrush abrasion of eroded enamel is influenced mainly by the abrasivity of the toothpaste slurry, but is also modified by toothbrush filament stiffness.

Key Words: *Abrasion, enamel, erosion, toothbrush, toothpaste*

Introduction

Abrasive wear of sound dental hard tissues is significantly related to the abrasivity of the toothpaste [1]. Additionally, the toothbrush as a delivery vehicle might modulate the abrasivity of the toothpaste [2], depending on the characteristics of the toothbrush, such as type, filament stiffness, and filament end-rounding.

It has been shown in previous studies that powered and manual toothbrushes, as well as manual toothbrushes applied with different brushing loads, vary in their ability to remove the fragile surface of demineralized enamel [3,4]. On the basis of the observation that toothbrush hardness influences the amount of abrasion of sound dentine [5], it is assumed that hard and soft brushes also differ in their potential to remove the demineralized layer of eroded dental hard tissues [6]. Toothbrush stiffness is affected by the bristle modulus of elasticity, bristle and tuft diameter, number of tufts, the number of bristles per unit area packed into a tuft hole, and the trim length of bristles [7]. Filament stiffness is suggested to be dependent on the diameter and length of the filament and its modulus of elasticity [8]. Assuming that the lengths of different filaments and their moduli of elasticity are constant, their stiffness will be affected only by the filament diameter.

Acid-softened enamel is highly susceptible to mechanical abrasion, such that even brushing without toothpaste or friction from the tongue can

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(Received 21 March 2008; accepted 12 May 2008)
ISSN 0001-6357 print/ISSN 1502-3850 online
DOI: 10.1080/00016350802195041

remove the etched softened surface layer [9]. Analogously, the application of toothpastes with different abrasivities might also remove different amounts of the demineralized surface layer. Kielbassa et al. [10] found that brushing abrasion of artificial caries-like enamel lesions increased with increasing abrasivity of the toothpaste. Determination of toothbrush abrasivity is based on a radiotracer method that provides relative enamel and dentine abrasivity (REA and RDA, respectively) values compared to a reference abrasive (ISO). However, RDA values of toothpastes are not consistently correlated to their relative enamel abrasion (REA) [11,12]. Indeed, Hooper et al. [13] found no correlation between abrasion of eroded enamel and toothpaste abrasivity. This could lead to the hypothesis that resistance of the outermost very fragile eroded enamel structure to toothbrushing wear is not dependent on the abrasivity of the toothpaste used.

However, it has to be noted that in the majority of experiments evaluating the abrasion susceptibility of eroded dental hard tissues a high number of strokes was applied, i.e. exceeding the number of strokes usually applied during tooth-cleaning. The outermost fragile enamel layer might thereby be removed with just a few brushing strokes, irrespective of differences in terms of toothbrush stiffness or toothpaste abrasivity, leading to the exposure of deeper layers. It might be assumed that these deeper, less demineralized, enamel layers behave differently compared to the highly fragile surface layer.

Despite the above-mentioned considerations, patients suffering from erosive tooth wear are often given the recommendation to use toothbrushes with soft filaments and toothpastes with low abrasivity in order to reduce the risk of damaging the eroded enamel surface [6,14]. This is done, although the impact of tooth-brushing on the very fragile outermost surface layer is not yet elucidated. Thus, the present *in vitro* study aimed to analyze the impact of toothpaste slurries with different REA values applied with toothbrushes of different hardness on abrasion of acid-softened enamel using a low number of brushing strokes applied after an erosive challenge.

The null hypothesis tested was that the amount of enamel loss after erosion and brushing abrasion is not dependent on the abrasivity of the toothpaste slurry and the filament diameter of the toothbrush.

Material and methods

Preparation of enamel specimens

Enamel samples (3 mm in diameter) were obtained from the labial surfaces of 72 freshly extracted, non-damaged bovine incisors, which are stored in 0.9% NaCl solution until use. The samples were embedded in molds of a ceramic disk (Degussit, Friatec/Degussa, Düsseldorf, Germany) and fixed with composite material (Tetric flow; Ivoclar Vivadent, Schaan, Liechtenstein). The enamel surfaces were ground flat and polished with water-cooled carborundum disks (1200, 2400 and 4000 grit, Water Proof Silicon Carbide Paper; Stuers, Erkrat, Germany), thereby removing approximately 100–150 μm of the outermost enamel layer, as verified with a micrometer (Digimatic, Mitutoyo, Tokyo, Japan). The samples were randomly assigned to 9 groups ($n = 8$ each) and applied to resin containers (Eracetal, Angst+Pfister, Zürich, Switzerland), which allowed for exact reposition in the wells of the brushing machine and profilometer.

Prior to the experiment, baseline surface profiles were obtained from all specimens to serve as reference surfaces for calculating enamel loss after the experiment. A preliminary test showed no signs of abrasion on the ceramic mountings of the enamel samples by brushing. This was also shown in a previous study by Attin et al. [15].

Preparation of toothpaste slurries

Three experimental slurries with low (REA: 2), medium (REA: 6), and high (REA: 9) abrasivity were prepared. The baseline formulation of the slurries consisted of a mixture of saliva substitute (79.2%) formulated by Göhring et al. [16], i.e. 85% glycerine (10%), 1.62% sodium bicarbonate (10.3%), and carboxymethylcellulose (CMC, 0.5%), and exhibited the REA value 2. For preparation of the slurry with REA 6, 100 g of the above baseline formulation was supplemented and 20 g calcium pyrophosphate (Fluka, Buchs, Switzerland; Lot 88HO466). The slurry had a mean particle diameter of 9.5 μm. Particle size was measured by Ivoclar Vivadent (Schaan, Liechtenstein) using a laser particle-size analyzer (CILAS 1064, liquid mode; CILAS, Madison, Wisc., USA). For preparation of the slurry with REA 9, 7.4 g calcium pyrophosphate (Fluka, Buchs, Switzerland; Lot 88HO466) and 12.6 g calcium pyrophosphate (Budenheim KG, Budenheim, Germany; Lot C 54-80) were added to 100 g of the baseline formulation. This slurry had a mean particle diameter of 9.1 μm. All slurries were supplemented with 20 μg silicone antifoam (Fluka, Buchs, Switzerland) to avoid slurry loss due to foaming during the brushing procedure. REA values were determined by the radiotracer method using American Dental Association specifications (Oral Health Research Institute, Indiana University) as described previously [17]. REA scores of the test slurries were related to a standard abrasive, calcium pyrophosphate, which is by definition given a REA of 10.

Toothbrushing abrasion

Experimental toothbrushes (Paro M 43; Esro AG, Thalwil, Switzerland) with a flat trim and nylon filament diameters of 0.15, 0.20, or 0.25 mm were used for abrasion of the enamel samples. Additional information about the toothbrushes is given in Table I. Brushing was done in an automatic brushing machine, as in previous studies [18,19]. The toothbrushes were fixed in the brushing machine so that the long axes were at an angle of 12° to the direction of brushing. This was done to avoid the development of bristle tracks on the enamel surface. The heads of the toothbrushes were aligned parallel to the surfaces of the samples.

To each well, 1 mL of the respective toothpaste slurry was added. Enamel specimens were brushed with 40 strokes in each cycle at a load of 250 g. Toothpaste slurries were replaced after each cycle, while the same toothbrush head was used for one sample throughout the study.

Experimental setup

The samples were subjected to 60 cycles each consisting of 15 s erosion, toothbrushing abrasion, and storage in artificial saliva. Enamel surfaces were eroded by unstirred storage in 1 ml hydrochloric acid (pH: 2.6, 0.0025 mol/l; Merck, Zug, Switzerland) for 15 s and then rinsed for 10 s in distilled water. Thereafter, the samples were brushed with 40 strokes in an automatic brushing machine with a load of 250 g using manual toothbrushes with different filament diameter (groups 1, 4, and 7: 0.15 mm, groups 2, 5, and 8: 0.20 mm, groups 3, 6, and 9: 0.25 mm) and toothpaste slurries with different abrasivity (groups 1–3: REA 2, groups 4–6: REA 6, and groups 7–9: REA: 9). After erosion and abrasion treatment, the enamel samples were stored in artificial saliva [20] for 3 h. After 4 cycles each, the samples were stored in artificial saliva for 15 h. After 60 cycles of erosion and toothbrushing abrasion, enamel loss was measured by profilometry.

Profilometry

Enamel loss was determined quantitatively using a profilometer (Perthometer S2; Mahr, Göttingen, Germany) with a diamond stylus moving across the brushed surface and the references area perpendicular to the direction of the toothbrushing movement. Prior to the experiment, five baseline surface profiles had been obtained from all specimens as references for calculating enamel loss. After the experiment, profilometric analysis was again performed, and the average enamel loss relative to the baseline surface profiles was calculated with the respective software (Mahr Perthometer Concept 7.0; Mahr, Göttingen, Germany). Since the enamel samples could be repositioned exactly in the wells of the profilometer, matching of the respective baseline and final reading was possible.

Table I. Manufacturer's information about the experimental toothbrushes.

Parameter	Paro M43 soft	Paro M43 medium	Paro M43 hard
Filament diameter (mm)	0.15	0.20	0.25
Bristle length (mm)	11	11	11
Tufts (no.)	43	43	43
Bristles/tuft (no.)	60	36	20
Bristles/toothbrush (no.)	2580	1548	860

Statistical analysis

The average enamel loss of five matched profiles was calculated for each sample and used for statistical analysis. The descriptive statistics (mean $\pm$ standard deviation) of enamel loss in each group were computed. The data were statistically analyzed by two-way ANOVA, considering both REA value (factor with 3 levels) and filament diameter (factor with 3 levels) as independent variables. To allow for the interaction (REA*filament diameter) between both factors, the one-way ANOVA and Bonferroni/Dunn post-hoc tests with respect to the interaction factor with 9 levels were computed. The level of significance was set at $p < 0.05$.

Results

Mean (SD) enamel loss (μm) by brushing of the eroded enamel specimens in the different groups is presented in Figure 1.

The two-way ANOVA found that both the REA value of the experimental toothpaste slurries ($p < 0.0001$) and the filament stiffness of the toothbrushes ($p = 0.0001$) influenced enamel loss significantly. Moreover, a significant interaction between both factors was found ($p = 0.0315$). Enamel loss was mainly influenced by the abrasivity of the toothpaste slurry and increased with higher abrasivity of the slurries. Enamel loss in groups 1–3 (REA 2) was about 2.7 μm lower ($p < 0.0001$) compared to that in groups 4–6 (REA 6) and groups 7–9 (REA 9) (Figure 1). Moreover, groups brushed with toothpaste slurry of REA 6 (groups 4–6) had less enamel loss (about 0.5 μm) compared to groups brushed with REA 9 (groups 7–9), but only between groups 6 and 9 were any significant differences found.

Enamel loss was also affected by the filament diameter, but to a lesser degree compared to the abrasivity of the toothpaste slurry. Statistical analysis revealed significant differences between the different toothbrushes only for groups brushed with toothpaste slurry of REA 6. Thereby, toothbrushes with 0.2 mm filament diameter caused about 1 μm greater enamel loss ($p < 0.0012$) than brushes with

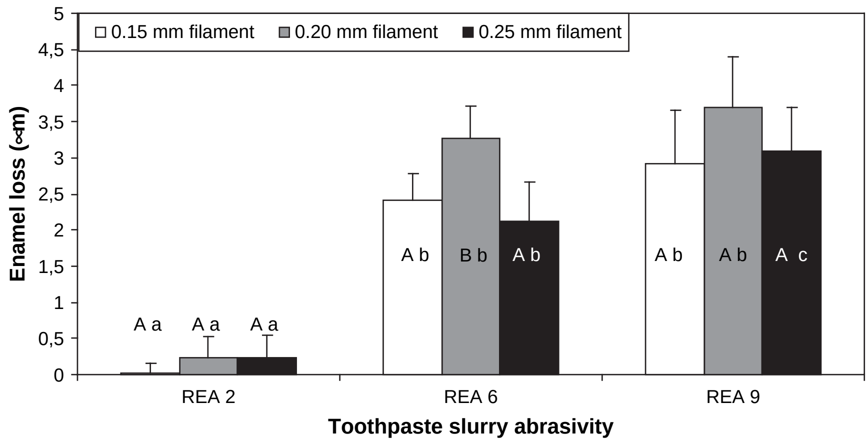

Figure 1. Mean (SD) enamel loss in groups brushed with toothpaste slurries of REA 2 (groups 1–3), REA 6 (groups 4–6), and REA 9 (groups 7–9). The slurries were applied using toothbrushes with 0.15, 0.2, or 0.25 filament diameter. Within the same REA value, groups marked with the same capital letter are not significantly different. Within groups brushed with the same toothbrush, groups marked with the same small letter are not significantly different.

0.15 or 0.25 mm filament diameter. Between-group comparisons yielded no significant differences within groups brushed with REA 2 (groups 1, 2, and 3) and REA 9 (groups 7, 8, and 9).

Discussion

The present study simulated a worst-case scenario in analyzing and evaluating the impact of toothpaste slurry abrasivity and toothbrush filament stiffness on abrasion of eroded enamel. In each cycle, enamel samples were eroded for 15 s to simulate brief erosion during a bulimic acid attack [21]. Toothbrushing abrasion was performed at 250 g brushing load, which is in the range of brushing pressure found clinically [22,23]. Assuming a maximum contact time of 10 s per single tooth during twice-daily toothbrushing [24] and a frequency of 4.5 strokes/s [25], the application of 40 brushing strokes per experimental cycle corresponds to the amount of brushing strokes applied daily under clinical conditions. To focus on the abrasion potential of the slurry only, the toothpaste slurries were prepared without fluoride. For the same reason, the experiments were carried out without storage in saliva in between erosion and abrasion to avoid rehardening of the acid-softened layer. The toothpastes were applied as slurries and not as undiluted toothpastes, because toothpaste is considerably diluted by saliva in the clinical situation. Only one kind of abrasive (calcium pyrophosphate) was used for preparation of the toothbrushing slurries of REA 6 and 9 to avoid the results being affected by interactions of different toothpaste abrasives with the eroded enamel surface. However, in preliminary tests it was shown that the respective REA values could only be adjusted by using one or a combination of both brands. For preparation of the control slurry (REA 2), which is free from abrasive particles, only glycerine and CMC were added to the saliva substitute, as these are used as wetting agents and thickeners in toothpastes. For standardization, antifoam was also added to the control slurry.

As for sound enamel, abrasion of acid-softened enamel was mainly influenced by the abrasivity of the toothpaste slurry and less by the filament stiffness. In the study by Dyer et al. [26], toothbrushes with filament diameters of 0.006 inches (0.15 mm), 0.007 inches (0.18 mm), and 0.008 inches (0.2 mm) were applied on acrylic resin using a slurry containing fluoridated toothpaste and water. Acrylic abrasion was highest for the soft toothbrush (0.006 inches filament diameter) followed by the medium and hard toothbrush. This was explained by the greater flexion of the soft bristles leading to increased duration and area of bristle contact with the brushed surface, and thus to an increased quantity of toothpaste moving over the surface. It is assumed from the results of the present study that toothbrush filament stiffness is of secondary importance for abrasion of eroded enamel. Only with toothpaste slurry "REA 6" did the toothbrush with 0.2 mm filament diameter cause higher enamel loss than the other toothbrushes. This observation might be explained by a better capacity of the toothbrush with 0.2 mm filament diameter to retain the abrasives of the slurry "REA 6" on the enamel surface compared to the toothbrushes with 0.15 or 0.25 mm filament diameter.

As indicated by the results of Kielbassa et al. [27], abrasivity (REA value) of the toothpaste slurry is a major factor in toothbrushing abrasion of eroded enamel. The different amounts of abrasives of the toothpaste slurries probably vary in their ability to remove different parts of the eroded enamel layer. Thereby, the less abrasive toothpaste slurry might only abrade the outermost, fragile crystals of the etched layer, while the inner, less eroded, region might be removed – at least partially – by the application of toothpaste slurries with higher abrasivity.

The extrapolation from *in vitro* data to the clinical situation should be done with care, not least because eroded bovine enamel offers less resistance to brushing abrasion than human enamel [28]. However, the findings of this study suggest that abrasion of acid-softened enamel is influenced mainly by the REA value of the toothpaste, while toothbrush hardness is of less relevance. Thus, patients with erosive enamel lesions should be advised to use toothpastes with low REA values to minimize enamel loss. In this context, it is worth mentioning that cleaning efficacy of toothpaste is dependent less on REA value and more on the kind of abrasive used [12]. Therefore, the application of toothpastes with low REA value might not inevitably be at the risk of reduced cleaning efficacy but of less abrasion of eroded enamel than toothpastes with high REA values.

The working hypothesis that enamel loss after erosion and toothbrushing is not affected by the abrasivity of the toothpaste slurry and the filament diameter of the toothbrush was rejected.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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