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Controlled Toothbrush Abrasion of Softened Human Enamel

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Key Words
Abrasion · Erosion · Force measurement · Pellicle-like layer · Soft/hard toothbrushes

Abstract
The aim of this in vitro study was to compare toothbrush abrasion of softened enamel after brushing with two (soft and hard) toothbrushes. One hundred and fifty-six human enamel specimens were indented with a Knoop diamond. Salivary pellicle was formed in vitro over a period of 3 h. Erosive lesions were produced by means of 1% citric acid. A force-measuring device allowed a controlled toothbrushing force of 1.5 N. The specimens were brushed either in toothpaste slurry or with toothpaste in artificial saliva for 15 s. Enamel loss was calculated from the change in indentation depth of the same indent before and after abrasion. Mean surface losses (95% CI) were recorded in ten treatment groups: (1) soft toothbrush only [28 (17–39) nm]; (2) hard toothbrush only [25 (16–34) nm]; (3) soft toothbrush in Sensodyne MultiCare slurry [46 (27–65) nm]; (4) hard toothbrush in Sensodyne MultiCare slurry [45 (24–66) nm]; (5) soft toothbrush in Colgate sensation white slurry [71 (55–87) nm]; (6) hard toothbrush in Colgate sensation white slurry [85 (60–110) nm]; (7) soft toothbrush with Sensodyne MultiCare [48 (39–57) nm]; (8) hard toothbrush with Sensodyne MultiCare [40 (29–51) nm]; (9) soft toothbrush with Colgate sensation white [51 (37–65) nm]; (10) hard toothbrush with Colgate sensation white [52 (36–68) nm]. Neither soft nor hard toothbrushes produced significantly different toothbrush abrasion of softened human enamel in this model ($p > 0.05$).

It has been shown that the characteristics of the brush head could influence the abrasivity of the toothpaste, insofar as soft filament toothbrushes produced more abrasion than hard brushes. This could be explained by the fact that soft brushes usually consist of filaments with smaller diameters, which allow better transportation of toothpaste over the tooth surface [Dyer et al., 2000]. However, pellicle formation and surface softening were not taken into account in these experiments, and measurements of abrasion of a standard substrate, acrylic, were made by profilometry.

The major factor in the wear of dental hard tissues is erosion, which can act in synergy with abrasive factors, including toothpaste [Addy and Hunter, 2003]. The abrasion resistance of softened dental hard tissues is lower than that of sound surfaces [Davis and Winter, 1980; Jaeggi and Lussi, 1999]. Eroded enamel is highly susceptible to physical forces [Attin et al., 2000], which normally have negligible effect on native enamel and only minimal effect on sound dentin [Hunter et al., 2002].

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The primary objective of the study was to compare toothbrush abrasion performed with soft and hard toothbrushes. The second objective was to assess the impact of toothpastes of different abrasivity on the abrasion of softened human enamel.

Materials and Methods

Preparation of Enamel Specimens

One hundred and fifty-six caries-free human molars with no cracks on the buccal surface as viewed under the stereomicroscope (Leica, Zoom 2000, USA; magnification ×25) were selected from a pool of extracted teeth. The teeth were rinsed and brushed thoroughly under running tap water. Subsequently, the crowns were separated from the roots by using a diamond abrasion wheel (Isomet, 11-1,180 Low Speed Saw, Buehler Ltd., USA). The teeth were then flattened on the lingual side to approximately 3 mm thickness using the high abrasive disk of a rotating polishing machine (Knuth-Rotor, Struers, Copenhagen, Denmark). Thereafter, each slab was embedded in resin (Paladur, Bad Homburg, Germany) in two planar parallel moulds [Lussi et al., 1993]. The thinner mould (200 μm thick) was then removed, and the thicker mould (7 mm thick) was polished on the Knuth-Rotor polishing machine with silicone carbide paper with a grain size of 18, 8, 5 μm under constant tap water cooling. The embedded enamel blocks were taken out of the moulds before being polished with 3-μm diamond abrasive under constant cooling for 90 s (Labo-Pol-6, DP-Mol Polishing, DP-Stick HQ, Struers). In this manner, specimens were produced with a flat ground enamel area having suffered from a surface substance loss of 200 μm at the most in the centre of the window. After each polishing step, the slabs were rinsed under running tap water and sonicated for 3 min in deionized water. All specimens were stored in a mineral solution (1.5 mM $CaCl_2$, 1.0 mM KH_2PO_4, 50 mM NaCl, pH 7.0) when not used for experiments [Zero et al., 1990]. Prior to the experimental procedures, the specimens were further polished with 1-μm diamond abrasive under constant cooling for 60 s (Labopol-6, DP-Mol Polishing, DP-Stick HQ, Struers).

Surface Microhardness Measurement

Surface microhardness (SMH) measurements were performed using a Knoop diamond under a load of 50 pounds (MNT-10, Anton Paar, Paar Physica, Graz, Austria). Indentations were made with their long axis parallel to the vertical borders of the ground enamel area at intervals of 25 μm. Thus, the indentations were positioned parallel to the coronoapical axis of the teeth. The lengths of the indentations were measured with an optical analysis system and transferred to a computer (Leica DMR Microscope, Leica Mikroskopie and Systeme GmbH, Wetzlar, Germany). The apparatus was recalibrated before each use. SMH was also determined for allocation of the samples to the groups prior to the experimental procedure.

Salivary Pellicle Formation

To simulate clinical conditions, pellicle formation was performed as follows. Paraffin-wax-stimulated saliva was collected between 8.00 and 8.30 a.m. into ice-chilled vials from one donor who was caries free, had no salivary gland dysfunction, and was not on any drug therapy. Whole saliva was centrifuged for 10 min at 4°C and 2,000 *g* [Nekrashevych et al., 2004]. For pellicle formation, the enamel specimens were immersed in the clarified saliva (6 ml per specimen) for 3 h at 37°C under constant agitation [Meurman and Frank, 1991; Schüpbach et al., 2001].

Color version available online

Fig. 1. Toothbrush design. The head of the toothbrush is changeable. Two strain gauges are attached on the upper and lower side of the metallic flap for measuring toothbrushing forces.

Surface Softening

Each sample was immersed in 20 ml 1% citric acid (pH 4) for 3 min under constant agitation at the temperature of 30°C to perform erosion in vitro.

Toothbrush Design and Force Measurement

A commercial toothbrush (Elmex Oeco-clic, GABA AG, Therwil, Switzerland) was modified in order to allow the measurement of the brushing force. As a force transducer a flat metallic flap was used, which was fixed to the toothbrush with a screw. On the upper and lower side of this flap, two commercial strain gauges were attached (Type EA-06-125BT, Micro Measurements, Romulus, Mich., USA), resulting in a half-bridge measuring circuit. Thus the elastic bending of the toothbrush together with the sensor allowed for measuring and quantifying the force applied to the bristles of the toothbrush (fig. 1). The half-bridge signal was amplified and measured with a full bridge setup according to a measurement setup of earlier force studies [Lussi and Buergin, 1987; Lussi et al., 1995] and recorded on a fast plotter (Rikadenki R-03, Rikadenki Kogyo Co., Ltd., Tokyo, Japan). The head of the toothbrush was changeable. Toothbrush heads with soft and hard bristles were used. The filament diameters for hard and soft brushes were 0.225 and 0.150 mm, respectively. The filament material of bristles was nylon and the bristle length varied between 8 and 10 mm.

Habitual toothbrushing force was assessed in previous experiments as follows: Four healthy volunteers brushed their teeth in their habitual way using the modified toothbrush and the average toothbrushing force (± SD) of 1.50 ± 0.05 N was obtained.

Applied forces were kept on this value while brushing the specimens. The force signal on the chart was monitored for this purpose. Each specimen was manually brushed for 15 s.

Then the following parameters were calculated: (1) the maximum toothbrushing force (N), calculated as the mean of the ten highest peaks of the force signal on the chart; (2) the average toothbrushing force (N), calculated from the integral of the force over time divided by the recorded effective toothbrushing time. Before each recording, careful calibration of the instrument was made with different forces.

Measurement of Toothbrush Abrasion

Detection of enamel abrasion was based on SMH measurements by calculating the depth of the indentations. The difference between the depth after erosion and the depth after abrasion of the same indentation provided a direct measure for the loss of substance by abrasion at this site [Jaeggi and Lussi, 1999]. The depths of the indentations (d) were calculated from their lengths (L) using the geometrical formula: $d = (L/2)\tan 3.75°$.

Treatment Media

Two toothpastes of different abrasivity were chosen for this study: Sensodyne MultiCare (RDA 30; 1,400 ppm F; silica abrasive) (GSK AG, Münchenbuchsee, Switzerland) and Colgate Sensation White (RDA 120; 1,450 ppm F; silica abrasive) (Colgate-Palmolive AG, Thalwil, Switzerland).

The control specimens were immersed in artificial saliva during brushing, thus simulating the conditions during toothbrushing without toothpaste.

The test specimens in groups 3–6 were brushed and immersed in toothpaste slurry. The toothpaste slurry was prepared with a fluoridated dentifrice and artificial saliva in a weight ratio 1:3 [Lagerweij et al., 2006]. It was freshly mixed until homogeneity was achieved before each experimental run. This manner of application was chosen to simulate toothpaste dilution with saliva and formation of slurry during toothbrushing.

Groups 7–10 were brushed and immersed in artificial saliva. The toothpaste was applied directly with the toothbrush. This manner of application was chosen to simulate the beginning of toothbrushing, when the toothpaste does not take the form of slurry and is not well mixed with saliva yet.

Study Design

Six (pre-experimental) indentations were made in the centre of the enamel window of each prepared specimen and the average SMH was calculated, making the tooth as the statistical unit. Based on these data, the specimens were assigned to twelve vials according to the distribution of all specimens. Each of the twelve vials alternatingly contained twelve or fourteen specimens, resulting in six vials of the same amount of teeth each. Then, one specimen from each of the twelve vials was randomly selected and assigned to a test group. For the control groups, the procedure was carried out twice, plus six more specimens were selected from every second vial. The treatment groups were as follows: (1) soft toothbrush only (control 1, n = 30); (2) hard toothbrush only (control 2, n = 30); (3) soft toothbrush in Sensodyne MultiCare slurry (n = 12); (4) hard toothbrush in Sensodyne MultiCare slurry (n = 12); (5) soft toothbrush in Colgate Sensation White slurry (n = 12); (6) hard toothbrush in Colgate Sensation White slurry (n = 12); (7) soft toothbrush with Sensodyne MultiCare (n = 12); (8) hard toothbrush with Sensodyne MultiCare (n = 12); (9) soft toothbrush with Colgate Sensation White (n = 12); (10) hard toothbrush with Colgate Sensation White (n = 12). SMH measurements (6 indentations) were conducted on enamel specimens before and after pellicle formation and after erosive challenge. The array was such that the corresponding indentations were neighboring within a distance of 25 μm.

After toothbrush abrasion, the indentations after softening were located and surface loss was calculated. On each occasion, after immersion in saliva or citric acid, each specimen was rinsed with deionized distilled water and gently dried with air.

Statistics

The change of surface hardness of the specimens after pellicle formation and after erosion was compared by two paired t tests (Bonferroni corrected). The relationship between abrasion (dependent variable) and type of toothpaste, toothbrush, toothbrushing force and presence of slurry (independent variables) under study was investigated by fitting a linear model. All computations were made using SAS Enterprise Guide 4.1 by SAS Institute Inc. The significance level for all statistical tests was set at 0.05.

Statistical Evaluation of Measurement Error

In order to report the measurement error, the British Standards Institution [1979] recommended the repeatability coefficient – the maximum difference likely to occur between two successive measurements. The best way to examine repeatability is to take repeated measurements on a series of indentations [Bland and Altman, 1986]. Since the same method is used for the repeated measurements, the mean difference should be zero. The Bland and Altman plot was used to assess the repeatability of the method by comparing repeated measurements. The repeatability coefficient was read from the Bland and Altman plot (MedCalc for Windows, version 9.3.0.0, Mariakerke, Belgium).

Results

The mean baseline SMH of the 10 groups ranged from 343.2 Knoop hardness number (KHN; group 6) to 348.4 KHN (group 7). After immersion in saliva, the mean SMH ranged from 363.7 KHN (group 1) to 369.0 KHN (group 10). After softening, the mean SMH ranged from 313.1 KHN (group 2) to 325.9 KHN (group 8). The changes in SMH after immersion in saliva and softening were statistically significantly different from the baseline ($p < 0.001$). Table 1 shows the mean surface losses of the different treatment groups as well as the lower and upper bounds of the 95% confidence interval. Substance loss after toothbrush abrasion was greatest for group 6 (85 nm). The smallest abrasion was found in group 2 (25 nm). The maximal toothbrushing forces ranged from 1.78 N (group 5) to 1.92 N (group 6). The mean toothbrushing forces ranged from 1.42 N (group 9) to 1.55 N (group 6).

Table 1. Mean loss of surface substance and lower and upper bounds of the 95% confidence interval by toothbrush abrasion in the experimental groups and RDA values of the toothpastes

Groups		RDA	Mean surface loss, nm
1	Soft only	–	28 (17–39)
2	Hard only	–	25 (16–34)
3	Soft in Sensodyne/slurry	30	46 (27–65)
4	Hard in Sensodyne/slurry	30	45 (24–66)
5	Soft in Colgate/slurry	120	71 (55–87)
6	Hard in Colgate/slurry	120	85 (60–110)
7	Soft with Sensodyne	30	48 (39–57)
8	Hard with Sensodyne	30	40 (29–51)
9	Soft with Colgate	120	51 (37–65)
10	Hard with Colgate	120	52 (36–68)

After backward selection, only type of toothpaste, presence of slurry and the interaction between the two remained in the linear model. Toothbrush and toothbrushing force had no significant influence on abrasion. Sensodyne MultiCare produced significantly less abrasion than Colgate sensation white ($p = 0.0128$) in this model. Presence of slurry significantly increased the abrasion ($p = 0.0004$). Difference in the ability to abrade between two toothpastes significantly increased in the presence of slurry ($p = 0.0202$).

Analyses of measurement error revealed that two measurements on the same indentation did not differ from each other by more than 12 nm (0.8%) before softening and 20 nm (1.2%) after softening, respectively.

Discussion

A wide range of methods has been developed to measure mineral loss of erosively altered tooth substance, such as surface hardness measurements, profilometry, iodide permeability, microradiography, atom force microscopy. The main advantage of microhardness measurements is the long experience with the system [Attin, 2006]. In the present study, the measurement error of indentations made on softened enamel was slightly higher than for sound enamel, 1.2 and 0.8%, respectively. These values are lower than the error determined by Collys et al. [1993]: 3.25 and 2.53%, respectively. A modification of this method allows the amount of abrasion of softened enamel to be quantified [Jaeggi and Lussi, 1999].

In order to mirror conditions closer to the in vivo situation, human fresh clarified saliva was used to form a pellicle-like layer. In an in vitro study significant protection against erosion was found after the exposure of enamel to saliva for ≥1 h [Wetton et al., 2006]. However, Zahradnik et al. [1976] showed that pellicle developed during a period of 1 day does not protect against demineralization in a caries model. Further research is needed to evaluate protective properties of pellicle against erosion.

In this study, citric acid was chosen because of its widespread use as an additive to foodstuffs with erosive potential in order to minimize uncontrollable effects of other ingredients. To produce initial erosive lesions, a short immersion time was chosen. The pre-experimental allocation resulted in a homogenous hardness distribution. Thus, it was possible to create initial erosive lesions without significant differences of mean SMH between the samples.

Two toothpastes with different abrasivity were used in the present study. The application form of toothpaste was chosen to simulate the natural conditions when after a few seconds the applied toothpaste will be diluted with saliva and form slurry. The more abrasive the toothpaste the greater was the surface loss. Interestingly, the surface loss in groups treated with toothpaste applied directly was smaller than in groups where the same toothpaste was applied as slurry. It is probable in the case of well-mixed slurry that abrasive particles, suspended in artificial saliva, may be trapped at the moment of contact between the filament tip and the surface. They are propagated into the softened layer and cause particles of enamel to break away. In the case of toothpaste applied directly, the toothpaste is not well mixed with artificial saliva and the larger particles are trapped between filaments or contact the tooth only on the side of a bent filament, thus producing less abrasion.

The results of the present study showed that in this model, soft and hard toothbrushes present no significant differences in toothbrush abrasion of softened human enamel. The more abrasive the toothpaste, the greater was the surface loss. Obviously, more research is needed to improve the prevention measures for patients suffering from dental erosion.

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References

Addy M, Hunter ML: Can tooth brushing damage your health? Effects on oral and dental tissues. Int Dent J 2003;53(suppl 3):177–186.

Attin T: Methods for assessment of dental erosion; in Lussi A (ed): Dental Erosion. Monogr Oral Sci. Basel, Karger, 2006, vol 20, pp 152–172.

Attin T, Buchalla W, Gollner M, Hellwig E: Use of variable remineralization periods to improve the abrasion resistance of previously eroded enamel. Caries Res 2000;34:48–52.

Bland JM, Altman DG: Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;i:307–310.

British Standards Institution: Precision of test methods. Guide for the determination and reproducibility for a standard test method (BS 5497, part 1). London, British Standards Institution, 1979.

Collys K, Cleymaet R, Coomans D, Michotte Y, Slop D: Rehardening of surface softened and surface etched enamel in vitro and by intraoral exposure. Caries Res 1993;27:15–20.

Davis WB, Winter PJ: The effect of abrasion on enamel and dentine and exposure to dietary acid. Br Dent J 1980;148:253–256.

Dyer D, Addy M, Newcombe RG: Studies in vitro of abrasion by different manual toothbrush heads and a standard toothpaste. J Clin Periodontol 2000;27:99–103.

Hunter ML, Addy M, Pickles MJ, Joiner A: The role of toothpastes and toothbrushes in the aetiology of tooth wear. Int Dent J 2002;52:399–405.

Jaeggi T, Lussi A: Toothbrush abrasion of erosively altered enamel after intraoral exposure to saliva: an in situ study. Caries Res 1999;33:455–461.

Lagerweij MD, Buchalla W, Kohnke S, Becker K, Lennon AM, Attin T: Prevention of erosion and abrasion by a high fluoride concentration gel applied at high frequencies. Caries Res 2006;40:148–153.

Lussi A, Brunner M, Portmann P, Buergin W: Condensation pressure during amalgam placement in patiens. Eur J Oral Sci 1995;103:388–393.

Lussi A, Buergin W: A New method to measure the condensation pressure of amalgam under in vivo conditions. J Dent Res 1987;66:737–739.

Lussi A, Jaeggi T, Schaerer S: The influence of different factors on in vitro enamel erosion. Caries Res 1993;27:387–393.

Meurman JN, Frank RM: Scanning electron microscopic study of the effect of salivary pellicle on enamel erosion. Caries Res 1991;25:81–87.

Nekrashevych Y, Hannig M, Stösser L: Assessment of enamel erosion and protective effect of salivary pellicle by surface roughness analysis and scanning electron microscopy. Oral Health Prev Dent 2004;2:5–11.

Schüpbach P, Oppenheim FG, Lendenmann U, Lamkin MS, Yao Y, Guggenheim B: Electromicroscopic demonstration of proline-rich proteins, statherin, and histatins in acquired enamel pellicles in vitro. Eur J Oral Sci 2001;109:60–68.

Wetton S, Hughes J, West N, Addy M: Exposure time of enamel and dentine to saliva for protection against erosion: a study in vitro. Caries Res 2006;40:213–217.

Zahradnik RT, Moreno EE, Burke EJ: Effect of salivary pellicle on enamel subsurface demineralization in vitro. J Dent Res 1976;55:664–670.

Zero DT, Rahbek I, Fu J, Proskin HM, Featherstone JDB: Comparison of the iodide permeability test, the surface microhardness test and mineral dissolution of bovine enamel following acid challenge. Caries Res 1990;24:181–188.