

# An in situ Investigation into the Abrasion of Eroded Dental Hard Tissues by a Whitening Dentifrice

C.P. Turssi<sup>a</sup> J.J. Faraoni<sup>b</sup> A.L. Rodrigues Jr.<sup>c</sup> M.C. Serra<sup>b</sup>

<sup>a</sup>Department of Restorative Dentistry, School of Dentistry of Piracicaba, State University of Campinas, <sup>b</sup>Department of Restorative Dentistry, School of Dentistry of Ribeirão Preto, and <sup>c</sup>Department of Social Medicine, Clinical Hospital of School of Medicine of Ribeirão Preto, University of São Paulo, Brazil

## Key Words

Abrasion · Dental wear · Erosion · Toothbrushing · Whitening dentifrice

## Abstract

This crossover study aimed to investigate abrasion of previously eroded hard dental tissues by a whitening dentifrice compared to a regular dentifrice. After a 3-day lead-in period, 14 volunteers were randomly assigned to use one of the toothpastes while wearing a removable appliance, containing 3 enamel and 3 root dentine slabs on each side. On the first day salivary pellicle was allowed to form. Twice daily for the following 3 days, one side of each appliance was immersed in an acidic carbonated drink *ex vivo* while the other side remained unexposed. Specimens were then brushed with the allocated dentifrice. After a 3-day washout period, new sets of enamel and dentine slabs were mounted in the appliances and the participants commenced period 2 using the alternative toothpaste. Acid-treated specimens always showed more wear than untreated specimens. The whitening dentifrice did not significantly increase the wear of softened enamel compared with the regular dentifrice. Brushing with the whitening toothpaste led to sig-

nificantly greater wear of sound enamel and of both eroded and sound dentine than the regular dentifrice. The results suggest that whitening dentifrices may not increase the wear of acid-softened enamel but may have a more deleterious effect on dentine than regular toothpastes.

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Various strategies have been described to reduce erosive wear. One of these is to minimize abrasive influences such as provided by toothbrushing [Imfeld, 1996], as this can act in synergy with erosion [Davis and Winter, 1980; Hunter et al., 2002; Hooper et al., 2003].

Although a multitude of factors seem to be involved in the process of abrasion resulting from toothbrushing [Imfeld, 1996], it has been recommended that patients suffering from erosion should use only low-abrasive toothpastes [Imfeld, 1996; Gandara and Truelove, 1999] and there appears to be some evidence relating the type of toothpaste to erosion exacerbation [Al-Dlaigan et al., 2002; Mathew et al., 2002]. In this regard, it can be asked whether whitening toothpastes are harmful to enamel and dentine after the consumption of acidic drinks. There is some evidence that such dentifrices can be associated with wear

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Mônica Campos Serra  
Faculdade de Odontologia de Ribeirão Preto, USP  
Av. do Café, s/n, Monte Alegre, CEP 14040-904  
Ribeirão Preto, SP (Brazil)  
Tel. +55 16 602 4075, Fax +55 16 633 0999, E-Mail [mcserra@forp.usp.br](mailto:mcserra@forp.usp.br)

because of their high abrasivity [Mathew et al., 2002]. However, investigations focusing on the role of whitening toothpastes on previously eroded hard dental tissues are lacking. This study was therefore designed to determine whether a whitening toothpaste would increase the abrasion of enamel and root dentine previously exposed to acidic challenges compared to a conventional toothpaste, using an in situ model.

## Materials and Methods

### Volunteers

Fourteen volunteers (11 females and 3 males, aged 20–28 years) who fulfilled the inclusion criteria (mean stimulated saliva flow rate  $\geq 0.7$  ml/min, willing to follow the research schedule) without violating the exclusion criteria (use of any form of medication likely to interfere with salivary secretion, use of fixed or removable orthodontic appliances, pregnancy or breastfeeding, general/systemic illness) were enrolled. The experimental procedures used were performed with the informed consent of the subjects, following protocols reviewed and approved by the Ethics Committee of the Faculty of Dentistry of Ribeirão Preto, USP.

### Experimental Design

This study was a two-period crossover design. Each phase lasted for 4 days and a washout period of 3 days was allowed between each phase. The factors under evaluation were substrate condition (eroded and sound) and dentifrice (Aquafresh Whitening and Aquafresh Fluoride Protection, GlaxoSmithKline, Pittsburgh, Pa., USA). The whitening dentifrice contained NaF and the regular dentifrice sodium monofluorophosphate, both at 0.15%. The abrasive in the whitening paste was hydrated silica, while that in the regular paste it was a mixture of silica and calcium carbonate. However, we have been unable to obtain quantitative information on abrasivity (RDA or REA values).

Volunteers were randomly given either the regular or whitening dentifrice in the first period and then crossed over to the other toothpaste. In each phase, half of the volunteers' slabs were exposed to the erosive drink, creating four experimental groups for both enamel and root dentine: (1) eroded substrates brushed with whitening dentifrice; (2) eroded substrates brushed with regular dentifrice; (3) sound substrates brushed with whitening dentifrice, and (4) sound substrates brushed with regular dentifrice. Each group comprised 42 enamel or dentine slabs, or 14 experimental units, in triplicate, randomly assigned to the 14 volunteers, which were considered as statistical blocks. The response variable was the wear depth measured profilometrically ( $\mu\text{m}$ ).

### Preparation of Enamel and Root Dentine Slabs

Sixty freshly extracted bovine incisors were scraped of any remaining soft tissues, polished with pumice slurry, and disinfected by soaking in 10% formalin-buffered solution (pH 7.0) for 7 days [Dominici et al., 2001]. Each tooth was cut at the cemento-enamel junction, using a low-speed water-cooled diamond saw (Minitom, Struers A/S, Rodovre, Denmark). Next, two rectangular enamel and dentine slabs ( $3 \times 3 \times 2$  mm) were obtained from the coronal and root portions of the tooth, respectively. Sectioned pieces were mini-

mally ground and polished on a water-cooled mechanical grinder (Struers A/S) with 400-, 600- and 1,200-grit  $\text{Al}_2\text{O}_3$  papers to produce a flat surface. Final polishing was performed with 3- $\mu\text{m}$  diamond abrasive paste (Arotec S.A., Cotia, São Paulo, Brazil) on cloths. The slabs were cleansed ultrasonically in distilled water for 10 min, inspected for surface defects with a magnifying lens and rejected if pitted or cracked. Sections were stored at 37°C in 100% relative humidity.

### Selection of Enamel and Root Dentine Slabs

Using a Shimadzu microhardness tester, three Knoop indentations spaced 500  $\mu\text{m}$  apart were made 500  $\mu\text{m}$  from the edge of each slab. The KHN was measured under a 25-gram load for enamel and a 10-gram for root dentine for 5 s. The average value of three readings was used as the outcome for each slab. Based on the averages for both the 120 enamel and 120 dentine sections, 84 slabs of each dental hard tissue were selected so that among slabs of the same substrate the mean values did not vary by more than 10% from each other.

### Appliance Preparation and Mounting of the Slabs

Each volunteer had an impression of his/her maxillary arch recorded in alginate using a stock tray. This was poured in dental stone and an upper acrylic removable appliance was constructed. Appliances had six retention slots on either side of the midline, which accommodated three aligned enamel and three aligned dentine slabs facing the oral cavity. Dental sections were attached to the appliances and coated with carving wax, leaving an exposed strip, approximately 1.0 mm wide. The wax-covered areas provided reference surfaces for measuring wear.

### Intraoral Phases

During a 3-day lead-in period, the volunteers were instructed to use only the toothpaste (Colgate Cavity Protection Gel, Colgate-Palmolive, Divisão da Kolynos do Brasil Ltda., Osasco, São Paulo, Brazil) and toothbrush (Oral-B Indicator 40, Gillette do Brasil Ltda., Manaus, Amazonas, Brazil) supplied. After this lead-in phase, the palatal device was worn for 4 consecutive days. The volunteers started wearing the appliance 1 day prior to the beginning of the erosive/abrasive challenges, to allow formation of salivary pellicle. Twice daily for the following 3 days, the appliance was removed from the mouth and one side (right or left, as determined by randomization) was immersed in 25 ml of fresh Sprite Light (Companhia de Bebidas Ipiranga, Ribeirão Preto, São Paulo, Brazil) for 90 s at room temperature *ex vivo*, under supervision. The contralateral side remained unexposed to the acidic beverage. Afterwards, all specimens were brushed with a soft end-tufted toothbrush (Bitufo, Montagem e Comércio de Escovas Ltda., Jundiá, São Paulo, Brazil) using the allocated toothpaste for that period. A slurry (1:3, w/w of the allocated dentifrice/distilled water) was dropped on each slab and each row (containing either enamel or dentine slabs) was subjected to a linear toothbrushing abrasion movement of 40 strokes (back and forth). Then, specimens were washed thoroughly with running tap water before reinserting the appliance in the mouth. The subjects were blind as to which dentifrice they were using.

On completion of phase 1, the specimens were removed from the appliances and volunteers used the Colgate toothpaste, without wearing palatal devices for a 3-day washout period. Appliances were refilled with a new set of enamel and dentine slabs and the participants commenced period 2 using the alternative toothpaste.

During each phase, participants were instructed to use only the coded toothpaste and soft-bristled toothbrush supplied and to refrain from using any fluoridated products or mouthrinses. Volunteers wore their appliances continuously except during eating, drinking, or carrying out oral hygiene procedures. During these periods, the palatal devices were protected from dehydration by being placed in a plastic box with moist paper lining.

#### Measurement of Wear

At the end of each experimental period, the specimens were taken from the appliances. Wax was carefully removed with a plastic spatula and specimens were cleansed ultrasonically for 10 min. The wear depth, on both enamel and dentine, was measured using profilometry (Surfcorder SE-1700, Kosaka Laboratory Ltd.). The diamond stylus of the profilometer had a tip radius of 2  $\mu\text{m}$  and recordings were performed at a head velocity of 0.1 mm/s and a load of 0.7 mN. Five measurements were taken across the previously exposed area, using the covered area as a reference, on each triplicate. The average of these five readings was considered as the representative value for each triplicate.

#### Statistical Analysis

The average wear of triplicates was used as the outcome value for each experimental group ( $n = 14$ ). To avoid bias in the estimation of the treatment effect, both period and carryover effects were analyzed by using matched-pairs Student's  $t$  test ( $\alpha = 0.05$ ) [Senn, 1993].

After crossover design assumptions had been verified, the homogeneity of variances and normal distribution of errors were tested by Hartley's test and Shapiro-Wilks' test, respectively. The outcomes from enamel and root dentine data were statistically evaluated using two-way analysis of variance (ANOVA). If interaction between factors or main effects was significant, the differences between groups were analyzed using Tukey's test. The significance level was set at 5%. SAS 6.11 software (SAS Institute Inc., Cary, USA) was used to perform the statistical analyses.

## Results

Hartley's test and Shapiro-Wilks' test, respectively, showed that the data set had homogeneity of variance and was normally distributed. As verified by Student's  $t$  tests, the effects of period and carryover were not statistically significant (for enamel:  $p = 0.178$  and  $p = 0.127$ , respectively; for dentine:  $p = 0.535$  and  $p = 0.584$ ), which fulfilled the entire assumptions of the mathematical model.

The wear depth data, expressed in micrometers, are summarized in table 1. For enamel, ANOVA revealed a statistically significant interaction between substrate condition and dentifrice ( $p < 0.001$ ). For dentine, interaction was not significant ( $p = 0.206$ ), but both main factors under study (substrate condition and dentifrice) were significant by ANOVA ( $p < 0.001$ ).

Irrespective of the dental substrate, enamel or root dentine, higher wear was found for the specimens pre-

**Table 1.** Means of wear depth ( $\mu\text{m}$ ) of eroded and sound dental substrates according to the dentifrice used

Dentifrice	Enamel		Dentine	
	eroded	sound	eroded	sound
Whitening	2.91 (0.66) <sup>b</sup>	2.14 (0.39) <sup>a</sup>	4.36 (0.83) <sup>b</sup>	2.69 (0.41) <sup>a</sup>
Regular	2.56 (0.53) <sup>b</sup>	1.31 (0.19) <sup>a</sup>	3.12 (0.52) <sup>b</sup>	1.85 (0.24) <sup>a</sup>

Standard deviations in parentheses.

Means connected by vertical lines are not statistically different.

Statistical differences between eroded and sound substrates, within either enamel or dentine columns, are expressed by different letters.

viously exposed to the erosive challenge. For eroded enamel, no significant difference was observed between specimens brushed with either the whitening dentifrice or the regular counterpart. For sound enamel and dentine (both erosively altered and sound), brushing with the whitening toothpaste led to a significantly greater wear compared to the specimens brushed with the regular dentifrice.

## Discussion

In this study, ten percent formalin solution, which has been described as sufficient to kill all bacteria and viruses on tooth surfaces and within the dentinal tubules, was used to avoid the risk of cross-infection [Tate and White, 1991]. Moreover, this storage medium limits changes in dentine permeability over time [Jameson et al., 1994], which would be important for acidic diffusion control.

This intraoral model provided conditions for acquired salivary pellicle formation, which resists erosion to some extent [Hannig and Balz, 1999]. As previous investigations had indicated that in vivo formed pellicle was thinned down or even partially removed by erosive episodes [Hannig and Balz, 1999] and by brushing with toothpaste [Hannig, 2002], the slabs were kept in the mouth for at least 4 h to reestablish the pellicle.

In situ models represent an intermediate stage between in vitro studies and clinical trials [Sonju Clasen and Øgaard, 1999] and are particularly useful in erosion investigations [Millward et al., 1997; Rugg-Gunn et al., 1998; Hall et al., 1999; Hannig and Balz, 1999; Hunter et al., 2000, 2003] and abrasion tests [Kuroiwa et al., 1993;

Addy et al., 2002]. Results should be considered, however, only as indicators of possible trends. As the erosive episodes were performed extraorally in the current investigation, they were not counterbalanced by oral clearance – as a result of the stimulation of increased salivary flow – and by the buffering capacity of saliva [Millward et al., 1997]. Also, in between acidic exposure and toothbrushing abrasion, the calcium and phosphate contained in saliva did not produce any remineralization [Meurman and ten Cate, 1996; Hall et al., 1999]. Although the abrasion resistance of the softened enamel could be diminished by postponing the toothbrushing procedure after erosive events [Jaeggi and Lussi, 1999; Attin et al., 2001b], this benefit would not be accomplished by dentine [Hara et al., 2003]. Within this context and with the intention of maximizing abrasion, toothbrushing was performed immediately after each acidic challenge, even though this is unlikely in a normal lifestyle.

In contrast to other *in situ* studies [Hunter et al., 2000; Addy et al., 2002], statistically significant intersubject variation was not observed, possibly due to the effort to standardize specimens and experimental procedures. Enamel and dentine slabs were selected according to microhardness since initial microhardness is correlated with the susceptibility of eroded enamel to toothbrushing abrasion [Attin et al., 1997]. Indeed, exposure to soft drinks and toothbrushing procedures were conducted *ex vivo* under supervision to ensure that all participants strictly followed the test protocol.

The use of an end-tufted toothbrush was preferred in order to guarantee contact between slab and brush and to prevent brushing of adjacent slabs. At each erosion/abrasion session, triplicates were subjected to 40 brushing strokes each, based on a previous study [Attin et al., 1998]. Moreover, the 1:3 dilution of toothpastes is similar to that achieved during intraoral use [Duke and Forward, 1982].

The results confirm previous studies showing that dental hard tissues exposed to acids and then to brushing wore more than sound substrates [Davis and Winter, 1980; Attin et al., 1998, 2001a], because samples of hard tissues exposed to acid are partly demineralized and thereby softened [Amaechi et al., 2003].

The data showed that for eroded enamel there was no difference between the regular and whitening toothpastes. Conversely, for sound enamel there was a significant difference between dentifrices. An explanation could be that the softened surface layer is easily removed even by the less abrasive toothpaste, whereas toothpaste abrasivity would be relevant only for sound tissue.

Both acid-challenged and sound dentine showed higher wear depth after brushing with the whitening dentifrice. Therefore, this product posed no extra danger to abrasion of eroded enamel, but was deleterious to previously softened root dentine. These findings may be due to the chemical and structural differences between dentine and enamel. Dentine is more soluble and more porous than enamel [Meurman and ten Cate, 1996] and is less resistant to acid exposure [Hunter et al., 2000].

Although it has been speculated that *in vitro* collagen matrix serves to maintain the integrity of the softened zone of dentine, it is likely that such protection is cancelled under *in situ* conditions due to physical influences [Vanuspong et al., 2002]. In this respect, as presumably the abrasivity of the whitening dentifrice is higher, dentine would be more deleteriously affected by this product as compared to the regular counterpart.

In conclusion, acid exposure increased wear of both enamel and dentine. The whitening dentifrice did not cause enamel to be more abraded after acidic exposure but had a more deleterious effect on sound enamel and dentine (both eroded and sound) than the regular toothpaste. Although this experimental system appears to be well controlled and sensitive, the relevance of the results to the clinical situation is not established and caution must be exercised in extrapolating these findings.

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