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Effect of Salivary Stimulation on Erosion of Human and Bovine Enamel Subjected or Not to Subsequent Abrasion: An in situ/ex vivo Study

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Key Words
Toothbrush abrasion · Erosion · Dental wear · Demineralization · Enamel · Soft drinks · Stimulated saliva

Abstract
This in situ/ex vivo study evaluated whether saliva stimulated by chewing gum could prevent or reduce the wear and the percent change in microhardness (%SMH) of bovine and human enamel submitted to erosion followed by brushing abrasion immediately or after 1 h. During 2 experimental 7-day crossover phases, 9 previously selected volunteers wore intraoral palatal devices, with 12 enamel specimens (6 human and 6 bovine). In the first phase, the volunteers immersed the device for 5 min in 150 ml of cola drink, 4 times per day (at 8, 12, 16 and 20 h). Immediately after the immersions, no treatment was performed in 4 specimens, 4 other specimens were immediately brushed (0 min) using a fluoride dentifrice, and the device was replaced into the mouth. After 60 min, the remaining 4 specimens were brushed. In the second phase, the procedures were repeated, but after the immersions, the volunteers stimulated the salivary flow rate by chewing a sugar-free gum for 30 min. Changes in wear and %SMH were measured. ANOVA and Tukey's test showed statistical differences ($p < 0.05$) for the following comparisons. The chewing gum promoted less wear and %SMH. A decreasing %SMH and an increasing enamel wear were observed in the following conditions: erosion only, 60 min and 0 min. The human enamel presented greater %SMH and less wear compared to bovine enamel. The data suggest that the salivary stimulation after an erosive or erosive/abrasive attack can reduce the dental wear and the %SMH.

The incidence of dental caries has declined in developed countries [Peterson and Bratthall, 1996], but this has been followed by the increase in other dental lesions, such as pathological tooth wear [Attin et al., 1998]. In clinical situations, tooth wear can involve attrition, abrasion and erosion simultaneously. Evidence of erosion being a major factor in tooth wear has been well documented [Smith and Knight, 1984; Moss, 1998].

Erosion is defined as the loss of dental hard tissue resulting primarily from nonbacterial chemical attack, usually involving acidic substances (foods and soft drinks) [ten Cate and Imfeld, 1996; Imfeld, 1996; Lussi et al.,

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2004]. The acidic attack leads to loss and softening of the enamel surface and decreases the wear resistance of the enamel surface, thus rendering it more susceptible to the effects of mechanical abrasion, such as tooth-brushing [Davis and Winter, 1980; Attin et al., 2001]. Because these erosive and abrasive processes are frequently observed, efforts have been made to elucidate how erosive/abrasive lesions can be prevented.

Saliva seems to play an important role in minimizing enamel wear in erosive/abrasive attack [Amaechi and Higham, 2001]. The buffering capacity, calcium and phosphate contents of saliva and the acquired pellicle may counteract the erosive attacks, by reducing enamel loss and softening, enhancing its rehardening and minimizing the surface wear by subsequent tooth-brushing procedures [Hall et al., 1999; Jaeggi and Lussi, 1999; Attin et al., 2001; Hara et al., 2003]. However, Attin et al. [2001], in a previous study, showed that even after 1 h, abrasion of the previously eroded samples was significantly higher as compared to uneroded controls or eroded unbrushed samples. There is an evident relationship between a reduced salivary flow rate and the ability to clear dietary acids from the mouth [Lussi et al., 2004; Sánchez and Preliasco, 2003]. In addition, the bicarbonate in saliva is positively correlated with salivary flow rate, which means that saliva produced at a high flow rate has a higher pH and a higher buffering capacity [Moss, 1998].

Nevertheless, salivary parameters are not replicated in in vitro studies. This in situ/ex vivo study was therefore designed to evaluate whether the salivary flow rate stimulated by chewing gum could avoid or reduce the wear and the superficial microhardness change of enamel submitted to erosion followed by tooth-brushing abrasion. In addition, the behavior of bovine and human dental enamel substrates in in situ/ex vivo erosive/abrasive lesions was compared quantitatively.

Material and Methods

Ethical Aspects

This study was approved by the IRB of the Faculty of Dentistry of Bauru, University of São Paulo. Nine adult volunteers (4 males and 5 females) with an average age of 26.2 years (range 22–29 years) and a normal stimulated saliva flow rate (>1 ml/min) took part in this study after signing an informed, written consent.

Experimental Design

This in situ/ex vivo study involved a randomized complete block design, performed in two crossover phases of 7 days. The factors under evaluation were: type of enamel in two levels, human and bovine; salivary stimulation in two levels, stimulated by chewing gum and not stimulated; time elapsed between erosive and abrasive procedures in three levels, 0 min, 1 h and erosive challenge only. Nine volunteers wore acrylic palatal appliances each containing 6 dental enamel slabs of each substrate (6 human and 6 bovine). A new appliance was constructed for the volunteers in each phase. The groups under study in phase 1 (A, B, C), without salivary stimulation (no chewing gum) and in phase 2 (D, E, F), with salivary stimulation by chewing gum after the erosive challenge were: groups A/D, erosive challenge only; groups B/E, abrasive procedures 0 min after erosive challenge; groups C/F, abrasive procedures 1 h after erosive challenge. The response variables were depth of enamel surface wear and percent superficial microhardness change (%SMH).

Preparation of the Enamel Specimens

Enamel slabs (4 × 4 mm) were obtained from recently extracted, caries-free, unerupted human third permanent molars and bovine incisor teeth, which were stored and sterilized in 2% formaldehyde solution, pH 7.0, for 30 days at room temperature. For the human enamel, all tooth surfaces were used for preparation of the specimens, while for the bovine enamel only the buccal surfaces were used. The enamel surfaces of the slabs were ground flat with water-cooled carborundum disks (320, 600 and 1,200 grades of Al_2O_3 papers; Buehler, Lake Bluff, Ill., USA) and polished with diamond spray (1 μm; Buehler). A surface Knoop microhardness test was performed (5 indentations in different regions of the slab, 25 g, 5 s, HMV-2000; Shimadzu Corp., Tokyo, Japan) to select 120 human (KHN 320-358) and 120 bovine (KHN 321-344) enamel slabs.

Palatal Device Preparation

Custom-made acrylic palatal devices were made with 6 sites (6 × 6 × 3 mm) recessed into the polished surface of each appliance. Two enamel slabs (1 human and 1 bovine) were randomly assigned to each of the 6 sites and fixed with wax. The position of each group in the device was randomly determined for each volunteer. In order to maintain reference surfaces for lesion depth determination, two layers of nail varnish were applied on half of the specimen surfaces.

Intraoral Phase

A 1-week lead-in period was used. During this period and throughout the experimental phase, the volunteers brushed their teeth with fluoride dentifrice (1,100 ppm F as NaF, pH 6.8; Crest, USA). The palatal device was worn for two phases of 7 consecutive days with an interval of 2 days between them. One day before the experimental phases, the device was worn and specimens were not subjected to erosive/abrasive processes, to allow the formation of a salivary pellicle [Hara et al., 2003]. During the following 7 days, erosive/abrasive challenges were carried out extraorally 4 times/day (at 8, 12, 16 and 20 h).

In each challenge, the device was immersed in a cup containing 150 ml of a freshly opened bottle of a cola soft drink (Coca-Cola®; Companhia Fluminense de Refrigerantes, Porto Real, Rio de Janeiro, Brazil) for 5 min. Subsequently, the device was removed and specimens of groups B and E were immediately brushed by the volunteers. The brushing procedure consisted of 30 brushing strokes, made by each volunteer with a soft end-rounded toothbrush (Bitufo; Sanifil, Jundiai, São Paulo, Brazil) with a small portion of the described dentifrice (approx. 0.3 g). Volunteers were trained and in-

structed to carefully perform this procedure, avoiding contact of the toothbrush and dentifrice with the remaining specimens. Groups A and D were submitted only to the erosive process. The brushed specimens were washed under running tap water and the volunteers were instructed to take one sip of the beverage, before reinserting the device into the mouth. After these procedures, in phase 1, the palatal device was kept under unstimulated salivary flow rate, while in phase 2, it was kept under stimulated salivary flow rate [Dawes and Macpherson, 1992; Polland et al., 2003]. For this purpose the volunteers chewed a tablet of sugar-free chewing gum (Trident, Cadbury Adams Indústria e Comércio, Bauru, São Paulo, Brazil) for 30 min. After the intraoral device had been worn for 1 h in different situations of salivary conditions, in phases 1 and 2, respectively, groups C and F were brushed as described above.

The volunteers were instructed to avoid acidic foods and to wear the intraoral devices throughout the intraoral phase of the study. The devices should be removed only for the above-described procedures, during meals and for oral hygiene purposes. Plaque control on the specimens was achieved by dipping the intraoral device in 0.2% chlorhexidine gluconate mouthrinse for 5 min at the end of each day of the study [West et al., 1999].

Microhardness Analysis

By the end of the 7th day, the volunteers stopped wearing the palatal devices. The nail varnish over the reference surfaces was carefully removed and the slabs were removed from the device. After that, surface microhardness of the enamel slabs was measured again using a microhardness tester (Shimadzu HMV-2000, Shimadzu Corp., Japan) with a Knoop diamond under a 25-gram load for 5 s. Ten indentations were made on each specimen, 5 on the previously protected enamel surface and 5 on the experimental areas. The %SMH was calculated as a percentage of the initial hardness.

Wear Analysis

The enamel wear was determined in relation to the reference surfaces, using a profilometer (Hommel Tester T 1000, Hommelwerke VS, Schwenningen, Germany). Five readings were performed on each slab. These profilometric traces were taken from the reference surface, across the exposed surface. The average wear depth of an experimental unit was computed by using the 10 readings: 2 slabs × 5 readings.

Statistical Analysis

The assumptions of equality of variances and normal distribution of errors were checked for the tested response variables. Since the assumptions were satisfied, 3-way analysis of variance (ANOVA with $\alpha = 0.05$) was performed. No significant interactions among the factors were observed. Comparisons among erosion and different times of abrasion (0 and 60 min) were made by Tukey's test. The analyses were performed with the SAS system 6.11 software (SAS Institute, Cary, N.C., USA).

Results

ANOVA revealed a significant effect of the factors enamel, salivary stimulation and time, for both variables (microhardness and wear). No significant interactions among the factors were observed. Table 1 shows the %SMH and the wear of enamel surface of the factors under study. Tukey's test showed that erosion alone promoted a significantly higher %SMH and a significantly lower wear than erosion subjected to abrasion by toothbrushing after 1 h. This condition also promoted a significantly higher %SMH and a significantly lower wear than erosion subjected to abrasion by immediate toothbrushing. Salivary stimulation resulted in a significantly lower %SMH and wear. The bovine enamel showed significantly less %SMH and higher wear as compared to human enamel.

Table 1. ANOVA for the factor salivary stimulation by chewing gum and dental substrate

Factors		Variables	
		%SMH	Wear
Salivary stimulation	not stimulated	-67.9 ± 19.8^{a}	13.5 ± 5.6^{a}
	stimulated	-61.4 ± 22.5^{b}	11.8 ± 5.9^{b}
Enamel	human	-70.8 ± 17.1^{a}	11.6 ± 5.9^{a}
	bovine	-58.5 ± 23.5^{b}	13.6 ± 5.7^{b}
Time	Erosion only	-85.1 ± 10.5^{a}	6.4 ± 3.0^{a}
	60 min	-58.9 ± 16.8^{b}	14.2 ± 4.0^{b}
	0 min	-49.9 ± 17.7^{c}	17.3 ± 3.9^{c}

Tukey's test for time elapsed between erosive and abrasive procedures. Means followed by different superscript letters are significantly different ($p < 0.05$). No significant interaction was found among the 3 factors evaluated.

Figure 1 illustrates %SMH and enamel wear behaviors for the groups with and without saliva stimulation. The %SMH and enamel wear show an inversely proportional relationship ($r = 0.99$; $p < 0.05$).

Discussion

Usually after consumption of beverages and foods, people brush their teeth to prevent caries development, thus exerting mechanical forces on enamel surface [Al-Dlaigan et al., 2002; Addy and Hunter, 2003]. Acidic components of foods may lead to erosion, thus making enamel more susceptible to wear by tooth-brushing [Davis and Winter, 1980; Jaeggi and Lussi, 1999; Attin et al., 2001]. Several authors have postulated that tooth-brushing after an erosive attack should be delayed to allow the saliva to exert its natural remineralizing action on the

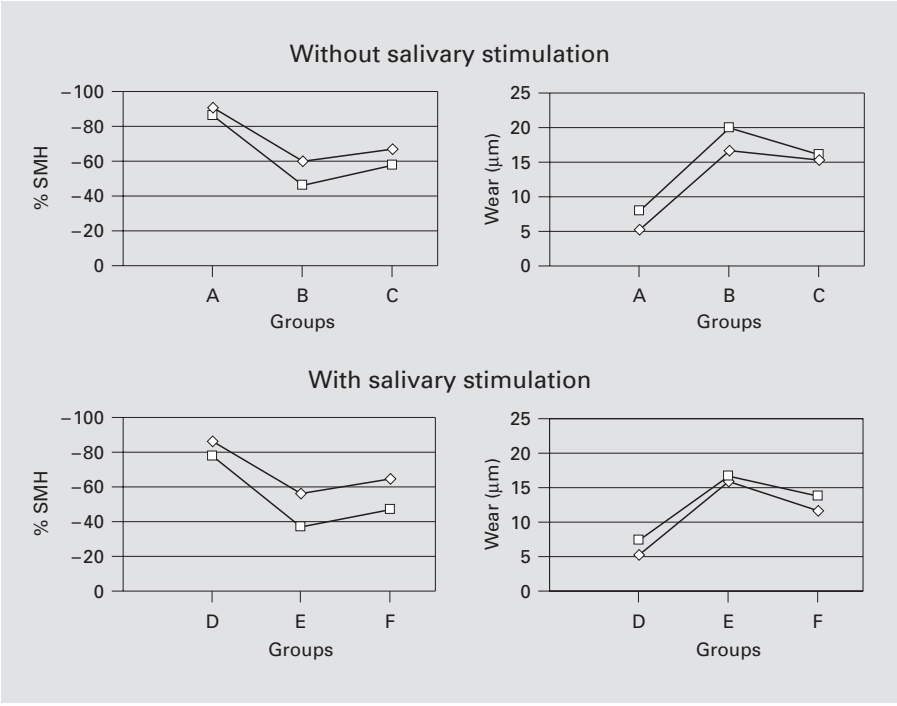

Fig. 1. Scatter plots illustrating the relation between %SMH or wear and the experimental groups: A and D = erosive challenge only; B and E = abrasive procedures 0 min after erosive challenge; C and F = abrasive procedures 1 h after erosive challenge. □ = Bovine enamel; ◇ = human enamel.

eroded enamel, thereby resulting in increased resistance to abrasion [Jaeggi and Lussi, 1999; Attin et al., 2000, 2001].

The findings of the present study are consistent with those available in the literature [Jaeggi and Lussi, 1999; Attin et al., 2000, 2001]. It was observed that an acidic beverage caused softening of the enamel surface, which presented smaller microhardness values, however without losing too much enamel in depth (wear). These results are in agreement with those of Amaechi and Higham [2001], who reported the occurrence of superficial loss of enamel and softening of the enamel layer underneath in the erosive phenomenon.

When the enamel surface, weakened by the action of the acidic beverage, was submitted to immediate brushing, there was loss of softened enamel, thus exposing a harder surface. When brushing was delayed (1 h) and the specimens were exposed to saliva, less enamel was mechanically removed during the brushing procedure, which resulted in higher %SMH and less wear compared to the immediate brushing condition ($r = -0.99$).

Several studies have investigated dental erosion and its association with tooth-brushing at different intervals [Jaeggi and Lussi, 1999; Attin et al., 2000, 2001; Hara et al., 2003]. However, comparison of the results should take into account the divergences regarding the types of beverages, times and periods of immersion, duration of the experiment, type of intraoral device and methodologies employed in each study. The values of human enamel wear obtained by Attin et al. [2001] after exposure to three erosive challenge protocols (similar to the conditions proposed in this study) were remarkably lower than those of the present study, due perhaps to differences in the methodologies. Nevertheless, the differences of wear behavior among the groups were similar when the findings of both studies are compared.

Flattening and polishing of specimens in this study possibly rendered enamel surfaces more susceptible to acid dissolution than would be the case under normal clinical conditions [Meurman and Frank, 1991; Ganss et al., 2000]. However, polishing provides a test surface with uniform composition and uniform erosion pattern [Mellberg, 1992], which is helpful for specimen standardization. A study design with 4 daily immersions of the intraoral device in the acidic beverage was established to simulate as closely as possible the regular intake of individuals considered at high risk for dental erosion [Hughes et al., 2002; Hunter et al., 2003]. It is known that during acidic beverage ingestion the pH drops to a level below the critical value, salivary flow rate increases [Millward et al., 1997] and the beverage is diluted by the saliva [Dawes and Macpherson, 1992]. Soft drink intake imme-

diately after placement of the device into the mouth attempted to simulate these interactions between acidic substances and saliva [Attin et al., 2001].

In view of the importance of the salivary parameters in decreasing the risk for erosion [Moss, 1998; Lussi et al., 2004], the in situ/ex vivo experimental model allowed assessment of the effect of stimulated salivary flow rate in the protective action against erosion, as observed for dental caries [Imfeld et al., 1995; Machiulskiene et al., 2001]. The erosive process is not totally reversible; nevertheless, the resting saliva is not able to yield complete rehardening of the eroded enamel [Gedalia et al., 1991]. On the other hand, saliva stimulated by the use of sugar-free chewing gum promoted a remineralizing action in the erosive/abrasive phenomena. Salivary flow stimulation yielded an increase in bicarbonate buffer and in salivary mineral content, which could facilitate calcium and phosphate redeposition onto the enamel surface and lessen enamel loss [Dawes, 1969]. It is important to point out, however, that chewing a sugar-free gum resulted in lesser mineral loss and lower enamel wear (table 1), but this did not prevent these alterations or promote complete rehardening of the softened zone underneath the completely dissolved enamel surface.

The results of this study (fig. 1) showed a direct relationship between human and bovine enamel for %SMH and wear. Featherstone and Mellberg [1981] found that artificial caries progression was 3 times greater in bovine than in human enamel. Human enamel is more compact than the bovine one, which is more porous and has less mineral content [Featherstone and Mellberg, 1981; Meurman and Frank, 1991]. The initial values for enamel hardness in the present study are consistent with these characteristics, as the human enamel hardness was approximately 10% higher than that of the bovine enamel. Eroded human enamel was more resistant to the mechanical efforts (brushing procedures), receptive to rehardening and showed little wear and low microhardness values (table 1). The bovine enamel lost substance as a result of the erosive action of the beverage (acidic dissolution). The remaining softening bovine enamel, supported by naturally more porous and fragile enamel, presented higher microhardness values than human enamel. This softened surface was easily removed when subjected to mechanical abrasive forces (tooth-brushing), thus exposing a subjacent harder mineralized enamel layer (table 1).

The findings of this study demonstrated the importance of combining both methods of analysis (microhardness and wear profile) for an accurate interpretation of the complex erosion/abrasion phenomena. Stimulated salivary flow was able to reduce mineral loss and enamel wear, and both human and bovine enamels proved adequate for erosion/abrasion investigations with a design similar to this study. After consuming acidic foods or drinks tooth-brushing should be delayed for at least 1 h. It is important to emphasize that the results of this in situ/ex vivo study provide only indications of what actually happens in the oral cavity. Before chewing gums are clinically recommended to prevent abrasion of eroded enamel, further studies should be conducted to evaluate the possibility of occurrence of adverse effects on tooth structure, such as the removal of softened enamel due to their physical action.

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