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Effect of an iron mouthrinse on enamel and dentine erosion subjected or not to abrasion: An *in situ/ex vivo* study

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ABSTRACT

Objectives: This *in situ/ex vivo* study evaluated whether a rinse with an iron solution could reduce wear and the percentage of microhardness change of human enamel and dentine submitted to erosion followed by brushing after 1 or 30 min.

Design: During 2 experimental 5-day crossover phases (wash-out period of 10 days), 10 volunteers wore intraoral palatal devices, with 12 specimens (6 of enamel and 6 of dentine) arranged in 3 horizontal rows (4 specimens each). In one phase, the volunteers immersed the device for 5 min in 150 mL of cola drink, 4 times a day. Immediately after immersion, no treatment was performed in one row. The other row was brushed after 1 min using a fluoride dentifrice and the device was replaced into mouth. After 30 min, the remaining row was brushed. In the other phase, the procedures were repeated, but after immersion the volunteers rinsed for 1 min with 10 mL of a 10 mM ferrous sulphate solution. Changes in surface microhardness (%SMH) and wear (profilometry) of enamel and dentine were measured. Data were tested using ANOVA and Tukey's tests ($p < 0.05$).

Results: The enamel presented more wear than dentine, under all experimental conditions. The iron solution caused a significant reduction on the %SMH in enamel, and a significant reduction on the wear in dentine, regardless the other conditions.

Conclusions: Rinsing with an iron solution after an erosive attack, followed or not by an abrasive episode, may be a viable alternative to reduce the loss of dental structure.

1. Introduction

A major factor in tooth wear is the interaction between erosion of dental hard tissues by dietary or endogenous acids and intraoral abrasive forces. The softening effect of acids, caused by partial demineralisation, renders enamel or dentine vulnerable to physical forces, which might have little or no effect on the intact tissue.[1–6] Thus, abrasion of softened enamel enhances considerably the loss of hard tissue that is caused by exposure to acid alone.[1,7] Because these erosive and abrasive processes are frequently observed, efforts have been made to elucidate how erosive/abrasive lesions can be prevented. Among the preventive strategies, it has been suggested that toothbrushing after an erosive attack should be delayed to allow the saliva to exert its natural remineralising action on the eroded enamel, thereby resulting in increased

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resistance to abrasion.[4,6,7] The salivary stimulation by chewing gum after an erosive or erosive/abrasive attack has also been suggested.[8] Studies on the influence of fluoridation measures on tooth wear of erosive damaged tooth substance are scarce. Attin et al.[3] showed *in vitro* a protective effect of an acidified fluoride gel on enamel abrasion. On the other hand, the *in situ* study by Lussi et al.[9] showed that a single fluoride rinse had no significant effect on the prevention of toothbrush abrasion of softened enamel. Recently, Magalhães et al.[10] reported that fluoride dentifrice had a protective effect on eroded enamel subjected to brushing abrasion.

The effect of iron on reducing enamel demineralisation by acids has been suggested.[11] Some *in situ* studies have shown that iron reduces the demineralisation of enamel in a situation of high cariogenic challenge.[12,13] The possibility that iron could be used to reduce an erosive or erosive/abrasive challenge was arisen based on studies using abiotic models, which showed that iron was effective on inhibition of enamel dissolution.[14,15] Thus, the aim of this study was to evaluate *in situ* the effect an iron mouthrinse on the reduction of the erosive action and synergistic effect between erosion and abrasion, in human enamel and dentine specimens, when exposed to a soft drink.

2. Materials and methods

This study was approved by the Institutional Review Board of Bauru Dental School, University of São Paulo, Brazil (Process 029/2004). Ten adult volunteers (five male and five female) with average age of 23.2 years (range 19–30 years), and normal stimulated salivary flow rate (>1 mL/min) took part in the study after signing an informed, written consent. The volunteers were not smokers, did not have active carious lesions and did not receive topical application of agents with high fluoride concentration at least 2 weeks prior to the beginning of the study. They did not have systemic diseases such as xerostomia and gastro-esophagic disorders. The number of volunteers was calculated based on the study by Rios et al.[8].

2.1. Experimental design

This study used a randomised design, performed in two crossover phases of 5 days. The factors under evaluation were treatment in two levels: no rinse (control) and rinse with a ferrous sulphate solution (groups NR and R, respectively); dental substrate in two levels: human enamel and dentine (subgroups E and D, respectively); and time elapsed between erosive and abrasive procedures in three levels: 1 min, 30 min and erosive challenge only (experimental conditions 1 min, 30 min and Ero, respectively). The volunteers wore acrylic palatal appliances each containing 12 dental slabs of each substrate (6 enamel and 6 dentine). A new appliance was constructed for the volunteers in each phase. The response variables were depth of enamel surface wear (μm) and percentage of superficial microhardness change (%SMH).

2.2. Preparation of the enamel specimens

Enamel and dentine slabs (4 mm × 4 mm) were obtained from recently extracted, caries free, unerupted human third permanent molars, which were stored and sterilised in 2% formaldehyde solution pH 7.0 for 30 days at room temperature. All tooth surfaces were used for preparation of the specimens (crown and root for enamel and dentine, respectively). The enamel surface of the slabs was ground flat with water-cooled carborundum discs (320, 600 and 1200 grades of Al_2O_3 papers; Buehler, Lake Bluff, IL, USA), and polished with diamond spray (1 μm; Buehler). The same procedure was used for dentine surfaces, except for 320 grade Al_2O_3 papers. A surface Knoop microhardness test was performed (five indentations in different regions of the slab, 50 g, 10 s for enamel and 25 g, 5 s for dentine, HMV-2000; Shimadzu Corporation, Tokyo, Japan) to select 120 enamel (KHN 319-367) and 120 dentine (KHN 80-97) slabs.

2.3. Palatal device preparation

Custom-made acrylic palatal devices were made with six sites (10 mm × 6 mm × 3 mm) recessed into the polished surface of each appliance. Two slabs (one enamel and one dentine) were randomly assigned to each of the six 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 specimens' surfaces. To minimise the contact between the tongue and the specimens, these were positioned posterior to the incisive papillae.

2.4. Intraoral phase

A 5-day lead-in period was used. During this period and throughout the experimental phase, the volunteers brushed their teeth with a fluoride dentifrice (1030 ppm F as NaF, pH 6.8; Crest, USA). The palatal device was worn for two phases of five consecutive days with an interval of 7 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.[16] During the following 5 days, erosive/abrasive challenges were carried out extraorally four times/day (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 (Coke; Companhia Fluminense de Refrigerantes, Porto Real, Rio de Janeiro, Brazil) for 5 min. The device was removed and the volunteers were instructed to take one sip of the beverage, before reinserting the device into the mouth. For group NR no rinse was done. For group R, the volunteers rinsed for 1 min with 10 mL of a 10 mmol L^{-1} ferrous sulphate solution. Subsequently, for experimental condition 1 min, the device was again removed and the corresponding slabs were brushed by the volunteers. The brushing procedure consisted of 10 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 (approximately 0.3 g). Volunteers were trained and instructed to carefully perform this procedure, avoiding contact of the toothbrush and dentifrice with the remaining specimens. Experimental conditions Ero were submitted only to the

erosive process. The brushed specimens were washed under running tap water and the device was replaced into mouth. For experimental condition 30 min, after the intraoral device had been worn for 30 min, the corresponding slabs 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. In addition, the volunteers were instructed not to touch the enamel blocks with the tongue, in order to avoid the abrasive effect of the tongue.[23] 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.[17]

2.5. Microhardness analysis

By the end of 5th day, the volunteers stopped wearing the palatal devices. The nail varnish over the reference surfaces was carefully displaced using a Hollenback spatula 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 Corporation, Japan) with a Knoop diamond under a 50-g load for 10 s for enamel and a 25-g load for 5 s for dentine. Ten indentations were made on each specimen, five on the previously protected enamel surface (SMH) and five on the experimental areas (SMH_1). The change of surface microhardness (%SMH) was calculated as a percentage of the initial hardness.

2.6. Wear analysis

The enamel wear was determined in relation to the reference surfaces, by profilometry using a profilemeter (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.

2.7. 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, two-way analyses of variance (ANOVA) were performed, for enamel and dentine, separately. The factors evaluated were groups and experimental conditions. Individual comparisons were made by Tukey's test. In order to compare enamel and dentine, three-way ANOVA and Tukey's test were performed. An alpha value of 5% was considered as indicator of statistical significance.

3. Results

Table 1 shows the percentage of microhardness change and the wear of enamel and dentine surfaces of the factors under study, as evaluated by two-way ANOVA. For enamel, considering the response variable % SMH, ANOVA revealed a significant effect of the factors group ($F = 9.21$) and experimental conditions ($F = 4.29$). The interaction between these factors was also significant ($F = 4.11$). The %SMH was significantly smaller for group R when compared to NR, for Ero condition, but not for 1 and 30 min conditions. For group NR, a significant difference between the conditions Ero and 30 min was seen, while for group R, no significant differences among the experimental conditions were observed. As for the response variable wear, despite the group R had lower values than group NR, this difference was not statistically significant ($F = 3.01$, $p = 0.099$). Also the difference between the experimental conditions and the interaction between the factors were not significant ($F = 0.09$, $p = 0.915$ and $F = 1.21$, $p = 0.310$, respectively). For dentine, an inverse relationship for the response variables, when compared to enamel, was observed. For the %SMH, no significant differences between the groups ($F = 0.01$, $p = 0.909$) and experimental conditions ($F = 1.15$, $p = 0.327$) were detected.

Table 1 – Wear and the percentage of surface microhardness change (%SMH) in enamel and dentine after erosion only, or associated with abrasion 1 or 30 min after

Variables	Groups	Experimental conditions		
		Erosion	Erosion + abrasion after 1 min	Erosion + abrasion after 30 min
Enamel				
%SMH	No rinse	$-80.8 \pm 3.6^{a,A}$	-76.1 ± 6.5^{AB}	-70.8 ± 7.7^{B}
	10 mM iron rinse	-68.4 ± 7.4^{b}	-69.9 ± 6.6	-68.3 ± 8.4
Wear (μm)	No rinse	9.3 ± 6.1	10.7 ± 4.0	10.3 ± 4.6
	10 mM iron rinse	8.6 ± 4.1	7.1 ± 2.6	6.8 ± 3.3
Dentine				
%SMH	No rinse	-46.1 ± 14.4	-35.1 ± 11.9	-40.3 ± 11.9
	10 Mm iron rinse	-41.7 ± 17.5	-41.2 ± 13.4	-40.2 ± 11.3
Wear (μm)	No rinse[a]	8.0 ± 5.2	6.8 ± 3.4	6.1 ± 1.8
	10 mM iron rinse[b]	5.1 ± 2.5	5.7 ± 2.2	3.4 ± 1.7

Mean ± S.D. ($n = 10$). Different lower case letters in the same column indicate statistical significance between the groups. Different upper case letters in the same line indicate statistical significance among the experimental conditions ($p < 0.05$).

There was no significant interaction between these factors ($F = 0.96$, $p = 0.393$). The iron rinse significantly reduced the wear for group R, compared to NR ($F = 5.91$), regardless the experimental conditions. There was no significant difference among the experimental conditions ($F = 2.41$, $p = 0.104$).

For the response variable %SMH, three-way ANOVA detected a significant difference between the subgroups ($F = 139.65$). Enamel presented a significantly higher %SMH when compared to dentine. For the factors groups and experimental conditions, no significant differences were detected ($F = 2.56$, $p = 0.127$ and $F = 2.45$, $p = 0.100$, respectively). The interactions between the factors were not significant. Considering the response variable wear, three-way ANOVA detected a significant difference between groups ($F = 5.11$) and subgroups ($F = 21.38$), but not among the experimental conditions ($F = 1.04$, $p = 0.361$). No significant interactions between the factors were found. The iron rinse significantly reduced the wear. As observed for the response variable %SMH, enamel presented a significantly higher wear when compared to dentine.

4. Discussion

In the initial stage of erosion there is a softening of the surface due in part to the demineralisation of the surface. At this stage of the process, repair (remineralisation) is in theory still possible as the remaining tissue could act as a scaffold. In a second, more advanced stage, repair is not possible, while the remaining softened enamel beneath the lost hard tissue is remineralisable.[18] The findings of this study demonstrated the importance of associating both methods of analysis (microhardness and wear profile) for accurate interpretation of the complex erosion/abrasion phenomena. The use of the iron rinse significantly reduced the %SMH of enamel subjected to erosion only. However, for dentine, the results were considerably different. The iron rinse significantly reduced the wear, regardless the experimental conditions, but had little or no influence on %SMH.

In order to explain the role of iron on enamel and dentine specimens after an erosive challenge, it is important to understand its mechanism of reaction with these dental substrates. Torell [11] reported that when enamel is incubated with ferrous salt solutions, acid-resistant enamel surfaces are established due to the precipitation of ferric phosphates on the enamel surface, due to the combination of ferric ions with phosphate ions dissolved at the enamel surface. The formation of this ferric phosphate barrier was also suggested in a recent *in situ* study simulating a high cariogenic challenge, in which a 15 mM iron solution was dripped on enamel blocks prior to dripping a 20% sucrose solution.[13] Based on this mechanism, we can hypothesise that iron may have reacted with phosphate dissolved from enamel after the erosive challenge. This ferric phosphate may have precipitated on the enamel surface, thus influencing the surface microhardness values. This is consistent with the fact that when the iron rinse was used, the values of %SMH were similar for all the experimental conditions (Table 1). In previous studies,[1,7,8] it has been reported that when an abrasive challenge is conducted after an erosive challenge, the enamel wear is significantly higher, when compared to the erosion condition only. In our protocol, however, this relationship was not observed. A possible explanation for this observation is the fact that the abrasive condition adopted here (10 brushing strokes) may have not had a great impact when compared to the high erosive challenge (immersion in the cola drink for 5 min). In addition, for the R group, the ferric phosphate layer mentioned above may have protected the partially demineralised enamel layer, and may have been removed firstly during the following abrasive challenge, which helps to explain why the enamel wear was reduced for the experimental conditions 1 and 30 min, despite this difference was not significant.

In contrast to enamel, erosion in dentine seems to be more than a surface phenomenon.[19] SEM, time-related atomic force and X-ray tomographic microscope studies have shown that demineralisation of dentine is first apparent at the interface between inter- and peritubular dentin, and – with increasing exposure time – results in a hollowing and funnelling of the tubules. Finally, the peritubular dentine is completely dissolved. The innermost sound dentine is then followed by a zone of partly demineralised dentine until a layer of completely demineralised collagen amounting up to one third of the total etching depth is reached.[20,21] The presence of this demineralised organic layer may hamper ionic diffusion influencing demineralisation.[19] It has been shown that in demineralised bovine root dentine presenting a completely demineralised collagen layer, further mineral loss is increased in collagenase-treated samples. Additionally, the demineralisation rate decreases as the amount of demineralised organic matrix increases.[22] This was confirmed in an *in vitro* study by Ganss et al.,[19] which showed that in dentine the erosive mineral loss was relatively high at the beginning of the experimental period and continued with somewhat lower dissolution rates. A subsequent study by Gregg et al.[23] reinforced this assumption, since a non-linear erosion pattern over time for dentine was shown, with less erosion occurring in successive 5-cycle periods. After 15 cycles, with resembles the protocol of our study (16 cycles), the wear found for dentine was considerably lower when compared enamel, subjected to the same experimental conditions, which was also observed in the present study. Even in root caries, an initially high increase in lesion depth followed by a reduced lesion progression was observed and interpreted as a result of a collagen layer serving as a diffusion barrier.[24] It has also been suggested that this organic layer may also exhibit buffering properties.[25] During an erosive challenge from the outer surface, it may adsorb H^+ ions, thus preventing the inner dentine from reaching low pH values.[22,26] Thus, this collagen diffusion barrier may have been responsible for the different response patterns of enamel and dentine to the erosive and erosive/abrasive challenges. On the other hand, previous *in situ* and *in vitro* studies when erosion was associated with abrasion have questioned the influence of this collagen layer, due to physical influences.[27–29] Nevertheless, Gregg et al.[23] have shown that dentine specimens licked by the tongue after exposure to acid lost less tissue after 15 cycles (but not after 5 or 10 cycles) than enamel specimens submitted to the same condition. This finding is comparable to the present study, despite we tested abrasion by brushing strokes. It is important to highlight that the numbers of cycles used in the different

studies must be taken into account when comparing the wear patterns of enamel and dentine.

In this study, the wear of dentine was significantly reduced in the presence of iron, regardless the experimental conditions. Thus, it seems likely that the ferric phosphate layer described above for enamel may have been deposited onto the collagen layer, rendering it more resistant to abrasive forces. The ultrastructural analysis of the dentine eroded and eroded/abraded in the presence of iron would be instructive in order to confirm these hypotheses. By other hand, the lack of difference in hardness for dentine could be explained by the fact that the specimens were soaked once a day in chlorhexidine, which has been reported that to occlude dentine tubules due to the deposition of calcium phosphate.[30]

In conclusion, the present *in situ/ex vivo* study suggests that an iron rinse after an erosive attack, followed or not by an abrasive challenge, may be a viable alternative to reduce the loss of dental structure. Before iron can be used clinically to control dental erosion, additional studies are necessary in order to establish the ideal dose that would have the maximum protective effect with minimum side effects.

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