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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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ARTICLE INFO

*Article history:*
Accepted 1 August 2006

*Keywords:*
Tooth abrasion
Tooth Erosion
Demineralisation
Enamel
Dentine
Iron

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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0003–9969/$ – see front matter 
doi:10.1016/j.archoralbio.2006.08.010

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