

# Effect of bicarbonate on fluoride reactivity with enamel

## Efeito do bicarbonato na reatividade do fluoreto com esmalte

### Abstract

Purpose: Bicarbonate ( $\text{HCO}_3^-$ ) is an alkaline and buffering substance found in dentifrices, which could improve the anti-caries effect of fluoride (F). However,  $\text{HCO}_3^-$  could reduce the formation of calcium fluoride-like ( $\text{CaF}_2$ ), the most important product of F reactivity with enamel, whose formation is higher in low pH. The aim of this *in vitro* study was to evaluate if  $\text{HCO}_3^-$  interferes with the reactivity of F with human enamel.

Methods: Five dentifrice formulations were evaluated: placebo of F and  $\text{HCO}_3^-$  (pH 7.0);  $\text{HCO}_3^-$  (pH 9.0); F (pH 7.0); F (pH 9.0) and F+ $\text{HCO}_3^-$  (pH 9.0). F dentifrices contained NaF and all dentifrices were silica-based. The concentrations of total F (TF),  $\text{CaF}_2$  and firmly bound F (FA, fluorapatite-like) formed in enamel after 1-min reaction with dentifrice slurries (1:3) were determined.

Results: The formation of TF,  $\text{CaF}_2$  and FA was reduced in 22.1%, 47.9% and 4.8%, respectively, by the presence of  $\text{HCO}_3^-$  in the dentifrice formulation.

Conclusion: This *in vitro* data suggest that addition of  $\text{HCO}_3^-$  to a dentifrice may interfere with the reactivity of F with enamel, reducing mainly the concentration of  $\text{CaF}_2$  formed.

**Key words:** Dentifrice; fluoride; baking soda; enamel; reactivity

### Resumo

Objetivo: O bicarbonato ( $\text{HCO}_3^-$ ) é uma substância alcalina e tamponante encontrada em dentifrícios, que poderia melhorar o efeito anticárie do fluoreto (F). No entanto,  $\text{HCO}_3^-$  poderia reduzir a formação de fluoreto de cálcio ( $\text{CaF}_2$ ), o mais importante produto da reatividade do F com esmalte, cuja formação é maior em baixo pH. O objetivo deste estudo foi avaliar *in vitro* se o  $\text{HCO}_3^-$  interfere na reatividade do F com o esmalte humano.

Metodologia: Cinco formulações de dentifrícios foram avaliadas: placebo de F e  $\text{HCO}_3^-$  (pH 7,0);  $\text{HCO}_3^-$  (pH 9,0); F (pH 7,0); F (pH 9,0) e F+ $\text{HCO}_3^-$  (pH 9,0). Os dentifrícios fluoretados continham NaF e todos continham sílica como abrasivo. As concentrações de F total (FT),  $\text{CaF}_2$  e F firmemente ligado (FA, tipo flúorapatita) formadas no esmalte após 1 minuto de reação com as suspensões dos dentifrícios (1:3) foram determinadas.

Resultados: A formação de FT,  $\text{CaF}_2$  e FA foram reduzidas em 22,1%; 47,9% e 4,8%, respectivamente, pela presença de  $\text{HCO}_3^-$  na formulação do dentifrício.

Conclusão: Os resultados *in vitro* sugerem que a adição de  $\text{HCO}_3^-$  a um dentifrício pode interferir com a reatividade do F com o esmalte, principalmente reduzindo a concentração de  $\text{CaF}_2$  formado no esmalte.

**Palavras-chave:** Dentifrício; flúor; fermento químico para pão; esmalte; reatividade

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Received: October 20, 2008  
Accepted: November 27, 2008

## Introduction

When bacteria present in dental biofilm metabolize carbohydrates into acids, the pH falls and enamel may be demineralized. The biofilm pH stays low for some time, but returns to the baseline values and enamel can recover dissolved mineral due to the remineralizing action of saliva (1). This process of loss and gain of mineral is basically physicochemical and substances with anti-caries potential, like fluoride, may effectively reduce enamel demineralization and/or activate remineralization. The main action of topical fluoride (F) is attributed to its capacity of reacting with dental hard tissues, forming products that would interfere with the development and progression of dental caries (2). Among these products, the most important one is the loosely bound fluoride (CaF<sub>2</sub>-like), whose formation is higher in low pH (3).

Besides the recognized physicochemical effect of fluoride, other substances could act inhibiting and/or neutralizing the acids produced by bacteria. Among these, bicarbonate (HCO<sub>3</sub><sup>-</sup>) has been used in dentifrices in combination with F (4,5). At first, due to its alkaline and buffering properties, HCO<sub>3</sub><sup>-</sup> could neutralize the acids produced by bacteria in dental biofilm (6-8) enhancing the effect of F dentifrice. Also, the alkali action of HCO<sub>3</sub><sup>-</sup> would not favor *mutans streptococci* predominance in dental biofilm formed (4,9). Although there is no evidence about an additive anti-caries effect between F and HCO<sub>3</sub><sup>-</sup> (10), it seems that HCO<sub>3</sub><sup>-</sup> does not impair the effect of F (11,12). However, HCO<sub>3</sub><sup>-</sup> may interfere with the formation of CaF<sub>2</sub> on enamel due to its alkaline pH and not ideal conditions for chemical reaction. Since this subject has not been evaluated in controlled conditions, this study aimed at evaluating *in vitro* the effect of HCO<sub>3</sub><sup>-</sup> on the reactivity of F with enamel.

## Methods

### Enamel and dentifrices used

Impacted human third molars were stored in 2% formaldehyde solution, pH 7.0, and after dehydration, the crowns were powdered. Particles from 0.074 to 0.105 mm of diameter were obtained and enamel was purified according to Asgar (13). The dentifrices were formulated with silica as abrasive and had the following compositions: A (placebo of F and HCO<sub>3</sub><sup>-</sup>, pH 7.0); B (HCO<sub>3</sub><sup>-</sup>, pH 9.0); C (F, pH 7.0); D (F, pH 9.0) and E (F+HCO<sub>3</sub><sup>-</sup>, pH 9.0). The concentration of F (as NaF) in the formulations was 1100 µg/g and that of NaHCO<sub>3</sub> was 20%. The pH and concentration of F in dentifrices, as total F and ion F, were determined (14).

### Reactivity test

Enamel powder samples of 20 mg (n=12) were treated with the supernatant of a dentifrice slurry in stimulated human saliva (1:3) for 1 min and then evaluated for total fluoride (TF), alkali-soluble fluoride (CaF<sub>2</sub>) and firmly bound fluoride (FA, fluorapatite-like), according to the procedure described by Cury (15) and Franco and Cury (16). The treated enamel

was filtered under vacuum, washed three times with water and once with methanol, and dried for 1 h, at 90°C.

### Fluoride determination

For TF determination, 5 mg of enamel treated with the dentifrices was weighed (±0.01 mg) and 0.5 mL of 1 M HCl was added. After 1-h agitation, 2.0 mL of 0.5 M citrate was added and F determination was performed using an ion-selective electrode Orion 96-09 and an ion analyzer EA 940, previously calibrated with F standard solutions. For the analysis of CaF<sub>2</sub> and FA, 10 mg of enamel powder treated with the dentifrices was weighed and transferred to a centrifuge tube, to which 1.0 mL of 1 M KOH was added to extract CaF<sub>2</sub>, according to Caslavská et al (17). After 24-h agitation, the suspension was centrifuged at 2900 × g for 10 min. A 0.5-mL aliquot of the supernatant was neutralized with 1.0 mL of 1 M citrate and 1.0 mL of 1 M HCl and analyzed for F (as CaF<sub>2</sub>) as previously described. For FA determination, the supernatant was discarded and the precipitate was washed 3 times with deionized water and once with methanol. After the evaporation of methanol, 0.5 mL of 1 M HCl was added to the tube, and after 1-h agitation, 2.0 mL of 0.5 M citrate was added. The solution was then analyzed for F (as FA) as already described.

### Statistical analysis

Twelve repetitions were made and the data were analyzed by analysis of variance and test of Sheffé at 5% (18).

## Results

Fluoridated dentifrices showed concentrations ranging from 1098.3 to 1177.8 µgF/g for total fluoride and from 1168 to 1221.6 µgF/g as F ion (Table 1). In addition, dentifrice formulations containing HCO<sub>3</sub><sup>-</sup> showed the highest pH values, around 8.6-8.7.

**Table 1.** Concentrations (mean±SD) of total fluoride (TF), as ion (Fi) and pH (mean±SD) of the formulations.

Formulation	Fluoride (ppm)*		pH†
	TF	Fi	
Placebo	0.0795±0.09	0.031±0.04	7.0±0.4
HCO <sub>3</sub> <sup>-</sup>	0.10±0.14	0.035±0.04	8.6±0.1
F (pH 7)	1098.3±51.3	1168.3±50.9	6.8±0.4
F (pH 9)	1133.8±56.0	1181.5±18.6	7.8±0.3
F + HCO <sub>3</sub> <sup>-</sup>	1177.8±85.5	1221.6±48.0	8.7±0.1

\* Mean of 12 samples of each formulation.

† Mean of 6 samples of each formulation.

Human dental enamel powder treated with the fluoridated formulations presented statistically higher concentrations of fluoride, in the form of TF, CaF<sub>2</sub> and FA, than the enamel treated with the placebo and HCO<sub>3</sub><sup>-</sup> dentifrices (Table 2). However, it was observed that the enamel treated with the formulations containing only F, either with pH 7 or with

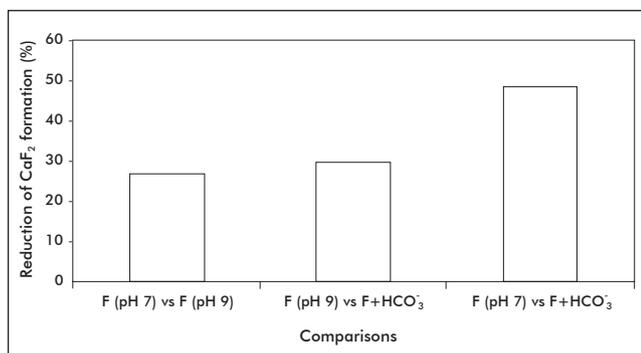
pH 9, showed higher concentrations of TF, CaF<sub>2</sub> and FA than the formulation that contained F and HCO<sub>3</sub><sup>-</sup>.

**Table 2.** Concentration (ppm; mean±sd; n=12) of total fluoride (TF), alkali-soluble fluoride (CaF<sub>2</sub>) and firmly bound fluoride (FA) in human dental enamel after reaction with the dentifrice formulations.

Formulation	Fluoride (ppm)*		
	TF	CaF <sub>2</sub>	FA
Placebo	72.6±3.9 <sup>c</sup>	0.84±0.24 <sup>d</sup>	66.6±1.8 <sup>c</sup>
HCO <sub>3</sub> <sup>-</sup>	74.8±3.3 <sup>c</sup>	2.38±0.45 <sup>d</sup>	72.7±2.2 <sup>cb</sup>
F (pH 7)	168.9±7.0 <sup>a</sup>	67.58±3.48 <sup>a</sup>	94.4±5.9 <sup>a</sup>
F (pH 9)	167.3±4.9 <sup>a</sup>	49.82±2.36 <sup>b</sup>	98.6±6.2 <sup>a</sup>
F + HCO <sub>3</sub> <sup>-</sup>	131.5±3.2 <sup>b</sup>	35.18±1.72 <sup>c</sup>	89.9±3.3 <sup>a</sup>

\* Means followed by distinct letters show significant difference among formulations (5%).

Figure 1 shows data related to the percentage of reduction of CaF<sub>2</sub> formation on enamel. The highest percentage of reduction was observed when comparing the F dentifrice (pH 7) versus the dentifrice containing F+HCO<sub>3</sub><sup>-</sup>.



**Fig. 1.** Comparison of the percentage of reduction of CaF<sub>2</sub> formation on enamel after reactivity with fluoridated dentifrices.

## Discussion

The results of the present study show at first that the addition of HCO<sub>3</sub><sup>-</sup> to fluoridated dentifrices may not improve their anti-caries effect, because there is a decrease in the reactivity and formation of CaF<sub>2</sub>, which is in agreement with the studies of Wefel et al (10) and Cury et al (12), who did not find any additive anti-caries effect of the dentifrice containing HCO<sub>3</sub><sup>-</sup>+F in comparison with the one only fluoridated.

Specifically in relation to TF in enamel as CaF<sub>2</sub> and FA, after the reactivity, their formation was statistically higher with the dentifrices containing only fluoride than the others. With regard to the formation of FA, there was no statistically significant difference between the dentifrices containing F and F+HCO<sub>3</sub><sup>-</sup>, which is supported by the literature, because the formation of FA is not largely influenced by the pH (3,19).

In relation to CaF<sub>2</sub>, the comparison between the dentifrices F (pH 7) and F (pH 9) was performed in order to verify only the influence of pH on CaF<sub>2</sub> formation on enamel. According to the results, it was confirmed that the higher the pH of the environment, the lower the formation of CaF<sub>2</sub> (3). The comparison between the dentifrices containing F (pH 9) and F+HCO<sub>3</sub><sup>-</sup> aimed to analyze the additional interference of HCO<sub>3</sub><sup>-</sup> on CaF<sub>2</sub> formation and, according to the results, a significant reduction was observed on CaF<sub>2</sub> formation due to the presence of bicarbonate. In the comparison between dentifrices containing F (pH 7) and F+HCO<sub>3</sub><sup>-</sup>, a more expressive decrease on CaF<sub>2</sub> formation was observed due to the effect of pH and presence of bicarbonate. Therefore, bicarbonate may interfere with the F reactivity with enamel due to its high pH and the effect of bicarbonate anion on the mineral formed.

Thus, the findings of this *in vitro* study show that HCO<sub>3</sub><sup>-</sup> reduces F reactivity with human enamel. Therefore, if a decrease in the anti-caries effect of F has not been observed in previous studies (10,12), it is probably because there must be a compensating effect, such as reduction of the acidogenicity of dental biofilm (6) and/or development of a less cariogenic microbiota (4,5), due to the alkaline and buffering effects of HCO<sub>3</sub><sup>-</sup>. The other explanation would be that the anti-caries effect of F dentifrice is not dependent of CaF<sub>2</sub> formation on enamel during toothbrushing.

## Conclusions

The *in vitro* results suggest that the addition of bicarbonate to a F-dentifrice reduces the amount of CaF<sub>2</sub> formed on enamel due not only to the pH increase, but also to a direct effect of bicarbonate anion.

## Acknowledgement

Dênis Santos received a scholarship from FAPESP (process 94/2794-1), during his undergraduation course in Dentistry at Piracicaba Dental School – UNICAMP.

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