

## AmF/NaF/SnCl<sub>2</sub> solution reduces *in situ* enamel erosion – profilometry and cross-sectional nanoindentation analysis

Thayanne Monteiro  
RAMOS-OLIVEIRA<sup>(a)</sup>  
Camila Vieira SILVA<sup>(a)</sup>  
Paula Mendes Acatuassu NUNES<sup>(a)</sup>  
Cecília Pedrosa TURSSI<sup>(b)</sup>  
Peter RECHMANN<sup>(c)</sup>  
Patricia Moreira de FREITAS<sup>(a)</sup>

<sup>(a)</sup>Universidade de São Paulo – USP, School of Dentistry, Department of Restorative Dentistry, São Paulo, SP, Brazil.

<sup>(b)</sup>São Leopoldo Mandic Institute and Dental Research Center, Department of Restorative Dentistry, Campinas, SP, Brazil.

<sup>(c)</sup>University of California San Francisco, School of Dentistry, Department of Preventive and Restorative Dental Sciences, São Francisco, CA, United States.

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**Corresponding Author:**  
Patricia Moreira de Freitas  
E-mail: pfreitas@usp.br

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**Abstract:** This *in situ* study aimed to investigate the effect of a tin-containing fluoride solution in preventing enamel erosion. Also, its effects on the partly demineralized zone were assessed for the first time. Thirteen volunteers participated in this 2-phase study, wearing removable intra-oral appliances containing four sterilized bovine enamel slabs, for 8 days, where 2 treatment protocols were tested using samples in replicas (n = 13): CO - no treatment (negative control) and FL - AmF/NaF/SnCl<sub>2</sub> solution (500 ppm F<sup>-</sup>, 800 ppm Sn<sup>2+</sup>, pH = 4.5). Samples were daily exposed to an erosive challenge (0.65% citric acid, pH 3.6, 4 min, 2x/day). In the 2<sup>nd</sup> phase, volunteers switched to the other treatment protocol. Samples were evaluated for surface loss using a profilometer (n = 13) and a cross-sectional nanoindentation (CSNH) test (n = 13) was carried out in order to determine how deep the partly demineralized zone reaches below the erosive lesion. The data were statistically analyzed by two-way ANOVA. Erosive challenges lead to smaller enamel surface loss (p < 0.001) in the FL group when compared to group CO. Data from CSNH showed that there was no significant difference in demineralized enamel zone underneath erosion lesions between the groups. An amorphous layer could be observed on the surface of enamel treated with tin-containing solution alone. Under the experimental conditions of this *in situ* study, it can be concluded that AmF/NaF/SnCl<sub>2</sub> solution prevents enamel surface loss but does not change the hardness of the partly demineralized zone near-surface enamel.

**Keywords:** Tooth Erosion; Fluorides; Dental Enamel.

### Introduction

Primary prevention of dental erosion is directly related to eating habits' change and dental hygiene, salivary stimulation and optimization of fluoride regimens. In situations where the aim is to limit the damage identified early, even if the individual is still asymptomatic, as well as for caries, fluoride is a widely-used strategy used to prevent dental erosion. However, its efficacy is still the object of controversy<sup>1,2</sup>, because little is known about the mechanism of action of fluoride on the prevention and/or control of dental erosion; erosive challenges are much more aggressive than cariogenic challenges and only a thin surface layer, partially demineralized, remains to provide structure for remineralization of eroded surface.<sup>1</sup>



Successful results on the reduction of dental demineralization by erosive acids have been shown with polyvalent metal ion-containing products, such as stannous, in several *in vitro* studies<sup>3,4,5,6,7</sup> and confirmed by other *in situ* studies.<sup>8,9,10,11</sup> Authors have reported the use of tin-ion in different presentation, such as solutions, toothpastes, gels and varnishes.

The mechanism of action of tin-based products relies mainly on tin deposition on the surface of the tooth, as well as possible incorporation of tin in the subsurface layer after prolonged immersion time in these products.<sup>12</sup> Apart from the deposition of CaF<sub>2</sub>-like globules on dental surface, which seem to be relatively less susceptible to acid dissolution, the reaction between tin and enamel hydroxyapatite can result in the formation of Sn-containing salts [(Sn<sub>2</sub>OHPO<sub>4</sub>, Sn<sub>3</sub>F<sub>3</sub>PO<sub>4</sub>, Ca(SnF<sub>3</sub>)<sub>2</sub>].<sup>3,13,14</sup> These tin deposits and its possible incorporation into the enamel surface form a covering “protective” stable layer, which results in a more acid resistant enamel surface.<sup>3,4</sup>

In a previous *in vitro* study, the use of AmF/NaF/SnCl<sub>2</sub> solution promoted significant protection against enamel loss.<sup>14,15</sup> Similarly, in an *in situ* model, the samples treated with AmF/NaF/SnCl<sub>2</sub> solution had a 67% reduction in enamel loss compared to the placebo/control group.<sup>16</sup> Because erosive demineralization can eventually result in an irreversible surface loss, prompt preventive measures, at early stages of dental erosion, are essential to reduce enamel tooth wear.

Therefore, the aim of this *in situ* study was to assess the efficacy of a stannous and fluoride-containing dental rinse on preventing initial dental enamel erosion involving softening and initial substance loss.

## Methodology

### Experimental design

The present two-phase *in situ* study was conducted with 13 volunteers that wore bi-lateral lower buccal intraoral appliances, each fitted with four bovine enamel samples per experimental phase (eight samples in total). The factor under evaluation was surface treatment at 2 levels (no rinse and rinse with tin-containing fluoride solution). The response variables were: surface loss (measured by profilometry) and depth of “near-surface demineralized” zone (measured by cross-sectional

nanohardness). Each group comprised 13 enamel slabs in replicas (26 enamel samples, n = 13), randomly assigned to the 13 volunteers, which were considered as statistical block.

### Sample size calculation

Sample size was determined based on previous study data<sup>17</sup>, with an enamel loss of 11 μm substance loss in the control group. This was substantiated with a group size of 11, under the assumption that the standard deviation was 4, α = 0.05, β = 0.2. To ensure that this group size was available at the end of the study, we included 13 volunteers at the start.

### Specimen preparation

Enamel slabs measuring 3 x 3 x 2 mm were cut from bovine incisors, which were stored in 0.1% thymol solution (4°C) for up to three months after the date of extraction. Slabs were mounted on acrylic rods with sticky wax and both surfaces of each slab were flattened and polished by using Al<sub>2</sub>O<sub>3</sub> papers with 1,200, 2,400 and 4,000 grade (Buehler Ltd., Lake Bluff, IL, USA). After each flattening and polishing procedure, the specimens were sonicated for 5 min in deionized water. Surface microhardness tests were performed (3 indentations, 100 μm distance apart, 0.49 N, 20s, Microdurometer HMV-2000, Shimadzu, Kyoto, Japan).<sup>18</sup> The selected fragments had an average surface microhardness of 342 ± 29 KHN. Afterwards the remaining samples were evaluated in an optical profilometer (3D PROSCAN 2100, Scantron, Eagan, MN, USA) for determination of the surface curvature. Only samples with surface curvatures < 0.3 μm were included in the study.

All selected samples were sterilized with gamma radiation and randomly distributed into 2 groups. All the samples had their surface protected by two strips of adhesive tape (UPVC, Graphic Tape; Chartpak, Leeds, USA) with 1.0 mm width each, leaving a central window of approximately 3 x 1 mm exposed with two lateral reference areas.

### Intraoral appliances preparation

An impression of the lower teeth of each volunteer was taken. Based on previously published studies<sup>19,20</sup>, two acrylic custom-made mandibular intraoral appliances were made. They consisted of a buccal segment

for each premolar area with 4 sites (4 x 4 x 3 mm), in which dental slabs were positioned and fixed with wax. In order to protect the slabs from the abrasive influence of the tongue or buccal mucosa, each slab was fixed approximately 0.5 mm below the surface of the appliance.<sup>21</sup> Within each side of the mandibular appliance, the positions of the specimens were randomly determined. The specimens were maintained in 100% humidity until the *in situ* experiment was conducted.

### Selection of volunteers

Ethical approval of the research protocol was granted by the Ethics Committee of the University of São Paulo (#206.317 - 02/22/2013). The study was designed and conducted in accordance with the guideline of the CONSORT statement. Thirteen volunteers had verbal and written information concerning the study. All of them agreed with the procedures and the purpose of the study and signed the informed consent and were extensively trained for all procedures. Subject inclusion criteria were: good general and oral health, good oral hygiene (no visible plaque, cavities, gingivitis or periodontal disease), no removable dentures or orthodontic devices, stimulated salivary flow > 0.7 ml/min. To collect the stimulated whole saliva, the participant was instructed to chew a piece of Parafilm (Pechiney Plastic Packaging, Menasha, WI, USA), measuring 5 x 5 cm for 30 s and the initial swallowing saliva. Subsequent, the participant excreted the saliva in a plastic cup during 5 min, whose mass (in grams) was previously measured in the digital scale. The mass of the container containing the saliva was measured to obtain the mass (in grams) and hence the volume of saliva. Total stimulated salivary flow was obtained by the ratio between the volume and the time, expressed in ml/min. Exclusion criteria were: gastroesophageal reflux disease, medication affecting salivation, pregnancy, breastfeeding, allergies to dental materials, use of tobacco and alcoholic beverages. Thirteen healthy adult volunteers (females, aged 23 to 35 years) with normal salivary flow rate took part in this study.

### Intraoral phase

Thirteen volunteers participated in this study wearing removable intra-oral appliances containing four sterilized bovine enamel slabs, for 2 experimental phase of 4 days each, with a washout time of 10 days

between the phases. Two treatments were tested using replicas (26 enamel samples, n = 13): CO - no treatment (negative control) and FL - AmF/ NaF/SnCl<sub>2</sub> solution (Elmex® Erosion Protection, GABA Int. AG, Switzerland).

In the 1st *in situ* phase, 7 volunteers were assigned to follow protocol A: negative control with no treatment (not using any fluoride rinse), while the other 6 volunteers followed protocol B: rinse with 10 mL of an AmF/NaF/SnCl<sub>2</sub> solution (500 ppm F- and 800 ppm Sn<sup>2+</sup>, pH 4.5). Volunteers were instructed to perform rinsing, before the daily erosive challenge (1½ hours before), once a day, for 30 seconds, always 30 minutes after the appliance insertion into the mouth, at the beginning of the daily use of the oral appliance. In the second phase of this *in situ* study, volunteers switched to the other protocol - they switched from protocol A to B, and from protocol B to A, respectively.

At the time the control group samples were being tested, the volunteers rinsed distilled deionized water (placebo solution) for 30 seconds, following the same protocol of treatment considered for the fluoride solution. Both placebo and fluoride solutions were delivered to participants without identification of each solution, then, the participants (dental students from the School of Dentistry of the University of São Paulo) did not know which solution they were using.

Twice daily, appliances were removed from the mouth and erosive demineralization was induced by *ex vivo* immersion of the appliances in 20 ml of 0.65% citric acid, pH 3.6, for 4 minutes, with no agitation at room temperature. The first erosive episode was performed 2 hours after the beginning of the daily cycling, to allow the formation of a salivary pellicle on the enamel surface. In order to increase the standardization of the experiment, the second acid challenge was carried out after five hours from the first acid challenge. Then, the devices were exposed again to oral environment for more five hours (remineralization process). After each erosive challenge, the appliance was gently dried with absorbent paper and immediately re-inserted into the mouth. The erosive solution was renewed after each exposure to acid solution. The volunteers were not allowed to brush the appliance. Plaque accumulation on all samples was prevented by the daily immersion

of the appliances in 0.12% chlorhexidine gluconate solution (pH 6.8, 1 minute, at the end of the day).

Volunteers wore their intra-oral appliances from 7 a.m. to 7 p.m. (times could vary  $\pm$  1 hr) continuously for 4 consecutive days of each treatment phase, removing them only to *ex vivo* acid challenge, during meals and for oral hygiene procedures. After meals or drinks, 15 minutes elapsed before reinsertion. Appliances were stored in closable plastic containers in humidity when removed from the mouth and overnight. Volunteers were instructed to refrain from using any fluoridated mouthrinses.

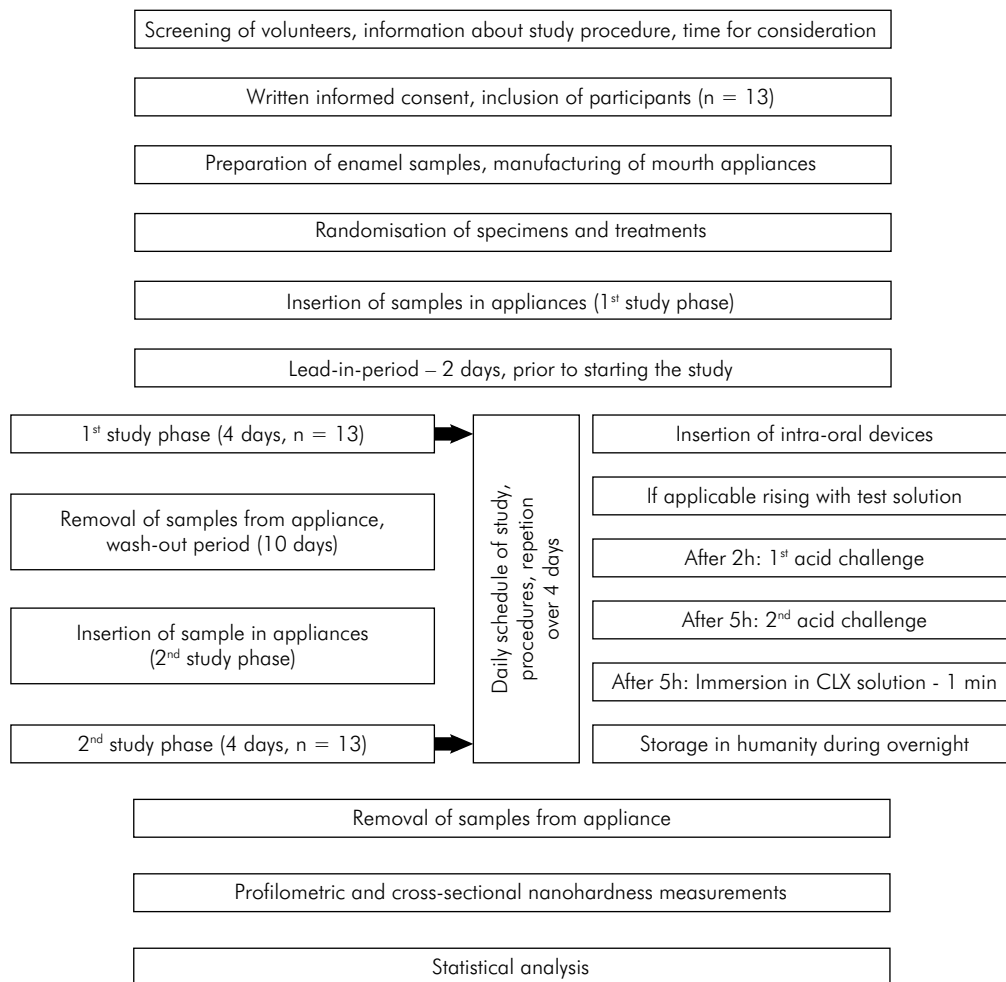
All procedures involved in the *in situ* phase of the present study were standardized and all volunteers were intensively trained, individually, for all procedures, in particular treatment processes with solutions,

cleaning and incorporation of intra-oral devices. Before starting the experimental phase, three meetings were done with each participant, where detailed explanations were performed and trained by each participant, individually. Moreover, they received a schedule containing all protocol of the experimental phase.

A flow-chart of the study procedures is presented in Figure.

### Analysis of surface loss

Surface loss of the enamel specimens in relation to the reference surfaces was quantitatively determined by an optical non-contact profilometer (PROSCAN 2100 3D Scantron, Eagan, MN, EUA), which captured a 3D topographical image of the enamel surface loss of each specimen. After the end of each experimental



**Figure.** Flow chart of study procedures.

phase, the enamel specimens were removed from the appliances and tapes were carefully removed. The surfaces of the specimens were profiled by sequential laser scans. The size of the area to be scanned was keyed in as  $x = 3 \text{ mm}$  and  $y = 1 \text{ mm}$ . The laser moved across the enamel perpendicularly to the erosive lesion, thereby recording 200 points on the  $x$  axis and 20 points on the  $y$  axis. An optic fiber camera positioned just behind the sensor transmitted the sample image to a monitor. Images were blindly analyzed using a specific software (Proscan Application software version 2,0,17), which calculated the average height of the two reference surfaces (in micrometer) and subtracted it from the height of the experimental area. The data were analyzed by a blinded-operator (operator C.V.S.).

### Cross-sectional nanohardness measurement

Following the analysis of surface loss, samples were cross-sectioned, exposing the inner surface of the enamel. One half of each enamel slab was randomly selected and embedded in acrylic resin. After, the enamel surface was exposed by flattening and polishing (800, 1,200, 2,400 and 4,000 grit of  $\text{Al}_2\text{O}_3$  papers). The polished specimen was then ultrasonically washed in deionized water for 3 min. Nanohardness measurements of the “near-surface demineralized zone” of the enamel section were performed with a Berkovich tip attached to an Ultra-Microhardness Tester (DUH-211S, Shimadzu, Tokyo, Japan) using a load of 25 mN, by a blinded-operator (operator P.M.A.N.). Loading and unloading speeds were set at 0.01mN/ $\mu\text{s}$ . For each specimen, three columns of 10 indentations each were made, one in the central region of the *in situ* eroded dental enamel and the other two columns at a 300- $\mu\text{m}$  distance from both sides of the central row of indentations (right and left). The indentations were made at 1  $\mu\text{m}$  (P1), 2  $\mu\text{m}$  (P2), 3  $\mu\text{m}$  (P3), 4  $\mu\text{m}$

(P4), 5  $\mu\text{m}$  (P5), 6  $\mu\text{m}$  (P6), 7  $\mu\text{m}$  (P7), 8  $\mu\text{m}$  (P8), 9  $\mu\text{m}$  (P9) and 10  $\mu\text{m}$  (P10) from the outer surface of eroded enamel. The mean values of all 3 measuring points at each distance from the surface were averaged.

### Statistical analysis

The assumptions of equality of variances and normal distribution of errors were, respectively, checked with Levene and Kolmogorov-Smirnov tests for the variables: surface loss ( $\mu\text{m}$ ) and near-demineralized area depth ( $\mu\text{m}$ ). The assumptions were satisfied and analysis of variance was performed for each variable, followed by Tukey’s post hoc test. In order to analyze the behavior of hardness along the depth of the near-surface demineralized zone, each treatment was analyzed separately using the one-way ANOVA test, and Tukey’s post hoc test, considering depth as a factor (10 levels: P1, P2, P3, P4, P5, P6, P7, P8, P9, P10). Significance was set at  $p \leq 0.05$ . The analyses were performed with the Minitab 16.1 Statistical Software.

### Results

All volunteers completed the experimental phases. Volunteers did not report adverse events or side effects. Table 1 shows that group CO (no fluoridated rinse solution -  $4.8 \pm 1.4 \mu\text{m}$ ) had a significantly higher surface loss than group FL (rinse with tin-containing fluoridated solution -  $1.9 \pm 0.9 \mu\text{m}$ ) ( $p < 0.001$ ).

The samples treated with AmF/NaF/SnCl<sub>2</sub> solution exhibited an average hardness of the near-surface demineralized zone (over the 10  $\mu\text{m}$ ) similar to the samples from the control group. The test revealed a statistically significant difference among groups ( $p < 0.001$ ) (Table 2) and the Tukey test suggested that both groups presented a partly demineralized zone of approximately 5  $\mu\text{m}$ , below the lesion.

**Table 1.** Results (mean  $\pm$  SD) of tukey test ( $p < 0.05$ ) with dependent variables cross-sectional nanohardness (CSNH) and tissue loss ( $\mu\text{m}$ ) for the study groups after *in situ* erosive challenging.

Experimentals groups (n = 13)	Surface treatments	Tissue loss	CSNH
CO	No treatment	$4,803 \pm 1,409^A$	$307,4 \pm 56,1^A$
FL	AmF/NaF/SnCl <sub>2</sub> solution	$1,892 \pm 0,954^B$	$312,2 \pm 49,4^A$

CO: no treatment (negative control group); FL: AmF/NaF/SnCl<sub>2</sub> solution (fluoride group); Means sharing the same superscript letter at the same collumm are not significantly different.

**Table 2.** Results of tukey test ( $p < 0.05$ ) of cross-sectional nanohardness values at different depths of partly demineralized zone.

Depth	n	Control group	Fluoride group
P1 – 1 $\mu\text{m}$	13	108,2 <sup>F</sup>	109,8 <sup>E</sup>
P2 – 2 $\mu\text{m}$	13	169,2 <sup>E</sup>	190,5 <sup>D</sup>
P3 – 3 $\mu\text{m}$	13	237,9 <sup>D</sup>	263,5 <sup>C</sup>
P4 – 4 $\mu\text{m}$	13	307,1 <sup>C</sup>	318,9 <sup>B</sup>
P5 – 5 $\mu\text{m}$	13	339,0 <sup>BC</sup>	347,0 <sup>AB</sup>
P6 – 6 $\mu\text{m}$	13	365,1 <sup>AB</sup>	364,3 <sup>A</sup>
P7 – 7 $\mu\text{m}$	13	378,8 <sup>A</sup>	376,3 <sup>A</sup>
P8 – 8 $\mu\text{m}$	13	386,6 <sup>A</sup>	383,4 <sup>A</sup>
P9 – 9 $\mu\text{m}$	13	393,2 <sup>A</sup>	385,7 <sup>A</sup>
P10 – 10 $\mu\text{m}$	13	389,2 <sup>A</sup>	382,3 <sup>A</sup>

Means sharing the same superscript letter are not significantly different ( $p > 0,05$ ).

## Discussion

This *in situ* experiment was designed for clinical simulation of early stages of dental erosion, which consists in the partial demineralization of the ultra structural components of the enamel surface, with no significant loss of substrate.<sup>22</sup> Therefore, this *in situ* study aimed to evaluate the effect of AmF/NaF/SnCl<sub>2</sub> on the prevention of enamel erosion.

Although many studies have investigated the effects of fluoride compounds containing tin in its composition in inhibiting dental erosion, most of them considered advanced stages of erosion, using relatively strong erosive challenges, with an average loss of enamel in order of 23  $\mu\text{m}$  – 147  $\mu\text{m}$ .<sup>1,8,10,11,16,23,24</sup> The *in situ* study design here using two erosive challenges per day (0.65% citric acid, pH 3.6) appears relatively slight but should reflect situations that can occur in patients with incipient erosion (2.7  $\mu\text{m}$  – 4.8  $\mu\text{m}$ ).<sup>25-27</sup>

As expected, in the present study, the profilometric analysis showed that the lowest tissue loss was observed in the fluoride-treated group (AmF/NaF/SnCl<sub>2</sub> solution containing 500 ppm F<sup>-</sup>, 800 ppm Sn<sup>2+</sup>, pH = 4.5) compared to the control group. The tin-containing solution was effective in inhibiting enamel surface loss in other *in vitro*<sup>4,5,28-30</sup> and *in situ*<sup>16,31</sup> studies. The erosive tissue loss reduction caused by the fluoride solution (AmF/NaF/SnCl<sub>2</sub>) compared to no treatment was 60%, which is very similar to the values

currently published in the literature. When the same tin containing fluoride solutions (AmF/NaF/SnCl<sub>2</sub> solution containing 500 ppm F<sup>-</sup>, 800 ppm Sn<sup>2+</sup>, pH = 4.5) as used here were tested, tissue loss reduction varied from 24 to 93%.<sup>14,16</sup>

*In vitro* and *in situ* studies show that pretreatment of enamel with tin-containing solutions can protect the enamel surface, inhibiting or reducing the erosive effect of acids through the formation of a superficial layer, containing Sn<sub>2</sub>OHPO<sub>4</sub>, Sn<sub>3</sub>F<sub>3</sub>PO<sub>4</sub>, Ca(SnF<sub>3</sub>)<sub>2</sub> and CaF<sub>2</sub>-like precipitates, with a low dissolution rate.<sup>1,3,4,12, 13, 24,32</sup>

It is believed that the treatment of the substrate with tin containing fluoride solutions favors the tin incorporation, and perhaps also the fluoride incorporation into the underlying enamel tissue, resulting in a broad structurally modified surface region, rich in tin and resistant to erosive acid challenge<sup>28</sup>. In addition, according to Hjortsjo *et al.*<sup>32</sup>, the mechanism by which metal fluorides inhibit dental erosion is at least partially associated with the presence of HF (hydrofluoric acid) molecules that are capable of penetrating into the enamel and possibly form CaF<sub>2</sub> molecules in the inner enamel substrate. According to these authors, this subsurface layer, rich in calcium fluoride, may exhibit greater resistance against erosive acids when compared to the outer layer of the enamel.

The cross-sectional nanohardness values showed that although the untreated (no fluoride) enamel may be more susceptible to demineralization, the subsurface (“near-surface demineralization”) evaluation did not reveal lower mineral content than the fluoride experimental group, which was indirectly assessed by nanohardness test. The data revealed that for both groups the demineralized area is the same, with nanohardness values ranging from 307.4  $\pm$  56.1 to 312.2  $\pm$  49.4. The near-surface demineralized area below the erosion lesion was approximately 4 to 5 micrometers in both groups (CO and FL). Given the above, the mineral content of the outer layer of the enamel (softened or demineralized enamel), exposed or not to the fluoride product, is similar and the difference on susceptibility to demineralization may rely on the presence of the subproducts of tin-solutions [Sn<sub>2</sub>OHPO<sub>4</sub>, Sn<sub>3</sub>F<sub>3</sub>PO<sub>4</sub>, Ca(SnF<sub>3</sub>)<sub>2</sub> and CaF<sub>2</sub>-like precipitates] in the fluoride-treated structure.

The low surface loss values observed in samples rinsed with the AmF/NaF/SnCl<sub>2</sub> solution can be also attributed to salivary factors. Previous studies have highlighted that the acquired saliva pellicle plays an important role in the development or not of dental erosion.<sup>33</sup> A dense and mature salivary pellicle works as a diffusion barrier inhibiting the direct contact of the acid with the tooth surface<sup>36</sup> and can favor the deposition of the tin salt and CaF<sub>2</sub>-like globules on the surface enamel by delivering calcium ions to the oral environment and further protecting the substrate during the acid challenge. However, studies evaluating the *in vitro*<sup>34,35</sup> and *in situ*<sup>10,24</sup> effect of tin containing fluoride solutions, which do not consider the presence of acquired pellicle, reported contradictory results regarding the effectiveness of the tin-containing solution. Hjortsjo et al.<sup>34</sup> reported that 0.87% SnF<sub>2</sub> solutions reduced enamel dissolution when specimens were exposed to citric acid. In 2014, the same authors<sup>35</sup> showed that 3.9% SnF<sub>2</sub> solution had negligible erosive effects on enamel. Experimental solution (pH 4,5) containing 1,900 ppm Sn<sup>2+</sup> (SnCl<sub>2</sub>) and 1,000 ppm F<sup>-</sup> (AmF/NaF) was very effective in inhibiting erosive mineral in enamel *in situ*, even under severe conditions and with short application times.<sup>10,24</sup>

A recent study carried out by the same working group (unpublished data) suggests that AmF/NaF/SnCl<sub>2</sub> solution has a better protective effect in the absence of an acquired pellicle, not interfering with the deposition of tin, fluoride, phosphorus as well as CaF<sub>2</sub>-like salts formed during the interaction between tin fluorides and hydroxyapatite. The authors verified that AmF/NaF/SnCl<sub>2</sub> solution was more effective in reducing tissue loss under erosive challenges when applied twice a day daily *in vitro* (without considering the formation of the acquired pellicle before acid cycling). However, in the *in situ* phase of the same study, the same solution applied twice a day showed greater potential to reduce the acid demineralization when compared to the control group, while the one-day application of the fluoride product did not differ from the control group. The authors believe that due to the fact that the second application of fluoride solution was made at the end of the daily erosive cycling, when most probably no or a thin layer of acquired

pellicle was on the enamel surface. At this time point the solution was more effective on reducing enamel loss, which is in agreement with other studies that do not consider the acquired pellicle formation for the experimental model.<sup>10,16,24</sup>

Another important point that might explain the different results is the type of substrate (bovine vs. human enamel). Bovine teeth were used instead of human teeth, because previous studies<sup>36,37</sup> have shown that bovine enamel have characteristics that make them suitable substitutes on human erosion/abrasion *in situ* models. Bovine teeth features include more uniform composition than human teeth (reduces the variability of responses), hardness, orientation of the enamel prisms and percentage by weight of calcium are equivalent to human dental enamel. They also have larger surface area and are easily obtained. Morphological differences between both substrates can be found – such as higher porosity and larger hydroxyapatite crystals – and a higher rate of progression of the demineralization of bovine substrate relative to the human enamel can be found. According to Zero<sup>38</sup>, differences between these substrates results in behavioral differences during experimental cariogenic *in situ* model.

Although this study here considered a light erosive challenging model (approximately 5 µm lesion), the results may be overestimated when compared to human teeth *in vivo*. As the present study was able to approach more of the biological variables of the oral environment, the results must be understood as an indicator of what possibly occurs under *in vivo* conditions.<sup>26</sup>

Until this moment, there are no studies comparing the anti-erosive effect of tin-containing solutions on bovine and human enamel. Additional studies are necessary to further elucidate the mechanism of action of the tin-containing fluoride solutions with the salivary pellicle and enamel.

Despite the fact that all procedures were standardized and that volunteers were intensively trained for all procedures, several factors could play a role in inter-individual variation when considering *in situ* models, mainly the differences within the individual biological factors, e.g. saliva composition and the different nature of the teeth that were used for specimens, as well as variations in studies protocols.

So, considering the findings and the limitations of this *in situ* study, it can be concluded that the enamel exposure to a tin-containing product (500 ppm F-, 800 ppm Sn<sup>2+</sup>, pH = 4.5) has a great potential to prevent enamel surface loss during erosive challenges.

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