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Original

Effects of experimental xylitol varnishes and solutions on bovine enamel erosion *in vitro*

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Abstract: This *in vitro* study aimed to analyze the effects of application of xylitol varnishes and solutions to protect against enamel erosion. Twelve bovine enamel specimens were pre-treated with 5% NaF-Duraphat varnish, 10% xylitol varnish, 20% xylitol varnish, placebo varnish, 5% NaF solution, 10% xylitol solution or 20% xylitol solution. The varnishes and solutions were applied for 6 h and 1 min, respectively. Controls remained untreated (n = 12). Specimens were then subjected to erosive demineralization (Coca-Cola, 4 × 90 s/d) and remineralization (artificial saliva, 2 h) cycling for 10 days. After 5 days, the varnishes and solutions were reapplied. After reapplication, two specimens per group were analyzed by SEM. Enamel loss was measured profilometrically after the 5th and 10th days. Data were then analyzed statistically by ANOVA and Tukey's *post-hoc* test (n = 10, $P < 0.05$). After the 5th day, all varnishes and 20% xylitol solution significantly reduced the enamel loss when compared to the placebo varnish/control. After 10 days of erosive pH cycling, both xylitol varnishes and solutions significantly reduced the enamel erosion when compared with the control. However, 10% xylitol solution produced a smooth layer on eroded enamel and significantly reduced the enamel erosion when compared to the placebo varnish/control. Xylitol thus appears to be a good option to partially reduce enamel erosion. (J Oral Sci 52, 553-559, 2010)

Keywords: enamel; erosion; fluoride; xylitol.

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Introduction

Dental erosion is a common problem in modern societies, owing to the increased consumption of acid drinks, such as soft drinks, sport drinks, fruit juices and fruit teas, which in turn have a high potential to provoke dental demineralization (1,2). For the control of dental erosion, several preventive strategies have been proposal, one of these being fluoride application.

The application of fluoride has shown, in general, positive results in reducing the progression of enamel and dentin erosion *in vitro* and *in situ* over short experimental periods (3-7). The action of fluoride is mainly attributed to precipitation of CaF_2-like material on dental surfaces, which is assumed to act as a physical barrier hampering the contact between acid with the underlying enamel or as a mineral reservoir, which is attacked by the erosive challenge, thus buffering the acids or promoting remineralization. The formation of the CaF_2-like layer and its protective effects on demineralization depend on the pH, F concentration, type of F salt and vehicle (7,8). However, the maintenance of these treatment effects after successive erosive challenges is limited.

On the other hand, studies have shown that xylitol, a sugar

alcohol (non-acidogenic sweetener), when applied in high concentrations, is able to complex with calcium ions (9), penetrate into the demineralized surfaces (10,11) and reduce the diffusion coefficient of calcium and phosphate ions (10,12). In addition, Amaechi et al. (13) and Chunmuang et al. (14) reported that xylitol when added in orange juice, with or without fluoride, was able to reduce enamel erosion.

However, there have been no studies reporting the topical effects of xylitol varnishes on enamel erosion. The use of xylitol varnishes may enhance the effects of this sugar alcohol due to long-term contact with the enamel surface. Therefore, the aim of this *in vitro* study was to analyze the effects of application of 10% and 20% xylitol varnishes and solutions to protect against enamel erosion after 5 and 10 days of pH cycling. Positive results may allow the development of new preventive strategies for dental erosion. The null hypotheses tested were: (1) xylitol is able to reduce enamel loss to a similar degree as a commercial fluoride product, regardless of concentration and vehicle (varnish and solution); (2) there is no difference between the xylitol products, regardless of concentration and vehicle.

Materials and Methods

Specimen preparation

Ninety-six enamel specimens (4 × 4 × 3 mm) were prepared from the labial surfaces of bovine incisor crowns, which were stored in 0.1% buffered thymol solution (pH 7.0) during specimen preparation at 4°C. Specimens were cut at the middle of the crown surface using an ISOMET low-speed cutting machine (Buehler Ltd., Lake Bluff, IL, USA) with two diamond disks (Extec Corp., Enfield, CT, USA) separated by a 4-mm spacer. The specimen surfaces were ground flat with water-cooled silicon carbide discs (320, 600 and 1,200 grade papers; Buehler Ltd.), and were polished with felt paper wet with diamond spray (1 μm; Buehler Ltd.).

Enamel specimens were allocated by stratified randomization according to their surface hardness (HMV-2000; Shimadzu Corp., Tokyo, Japan, Knoop diamond, 25 g, 10 s) values (333 ± 18 KHN) into 8 groups of 12 corresponding to the following products: NaF-Duraphat varnish (2.26%F, pH 4.5); experimental 10% xylitol varnish (pH 5.0); experimental 20% xylitol varnish (pH 5.0); placebo varnish (pH 5.0); NaF solution (2.26%F, pH 4.5); 10% xylitol solution (pH 6.5); and 20% xylitol solution (pH 6.5). Controls remained untreated (n = 12).

Prior to the experiment, two layers of nail varnish were applied on 2/3 of the surface of each specimen in order to maintain a reference surface for loss determination after the experiment.

Treatments

Treatments were performed at the beginning of the experiment and after 5 days of erosive pH cycling.

Xylitol (10 g/100 ml and 20 g/100 ml; Danisco Ltd, Cotia, SP, Brazil) and NaF (5 g/100 ml) powders (Sigma-Aldrich, São Paulo, Brazil) were dissolved in deionized water immediately before application. The 5% NaF (Sigma-Aldrich) solution was adjusted to pH 4.5 by adding 12.6 g 5 M H_3PO_4/100 ml. These solutions were applied using a microbrush, and were left on the surface for 1 min (15). Excess solution was removed from the surface using a cotton roll. Subsequently, specimens were immersed in artificial saliva for 6 h.

With regard to varnishes, Duraphat contains 2.26% NaF, 33.1% alcohol, natural resins (colophonium, mastix, shellac), wax, saccharine and flavor. The other varnishes were prepared by FGM/Dentscare Company (Joinvile, Santa Catarina, Brazil), and contained colophonium, synthetic resin, thickening polymer, essence, artificial sweetener and ethanol (with or without xylitol). All varnishes had a soft consistency. The pH of all varnishes and solutions was measured using indicator paper (± 0.5 units) and electrodes (± 0.01 units), respectively.

Varnishes were applied in thin layers using a microbrush. Specimens remained in artificial saliva for 6 h (15). After this period, varnishes were carefully removed from the surface using acetone and a scalpel blade, taking care to avoid touching the dental surface. Complete removal of the layer was confirmed microscopically (× 40) (15).

Erosive challenge

All specimens were subjected to a 10-day demineralization and remineralization cycling. Erosion was performed with a freshly opened bottle of regular cola drink (Coca-Cola Company Spal, Porto Real, RJ, Brazil, pH 2.3, 30 ml/specimen, unstirred, 25°C) four times daily for 90 s each. After demineralization, specimens were rinsed with deionized water (5 s). Between erosive challenges, specimens were transferred into artificial saliva (30 ml/specimens, unstirred, 25°C) for 2 h. After the last daily erosive treatment, specimens were stored in artificial saliva overnight. The artificial saliva (pH 6.8) was renewed daily and consisted of 0.2 mM glucose, 9.9 mM NaCl, 1.5 mM $CaCl_2.2H_2O$, 3 mM NH_4Cl, 17 mM KCl, 2 mM NaSCN, 2.4 mM K_2HPO_4, 3.3 mM urea, 2.4 mM NaH_2PO_4 and ascorbic acid (16).

Profilometric measurement

Enamel loss (μm) was quantitatively determined by profilometry (accuracy: 0.5 μm; Hommel Tester T1000, VS, Schwenningen, Germany) after 5 and 10 days of pH

cycling.

For profilometric measurement, the nail varnish was carefully removed using a scalpel and acetone solution (1:1 water) and the specimens were dried (15). The diamond stylus was moved from the 1st reference to the exposed and 2nd reference areas (2.5 mm length). The differences in height between both reference areas and the exposed area were then calculated (μm). Three profile measurements were performed in the centre of each specimen and averaged. After the 5-day of erosive pH cycling and the retreatment, the reference area of the specimens was again covered with nail varnish. To assure that the nail varnish was placed over the original reference area, the position of the nail varnish was marked by carving with a scalpel at the borders of the sample.

Scanning electron microscopy (SEM)

After treatment and 5 days of pH cycling, two specimens per group were freshly retreated as described in the "Treatment" section. Specimens were carefully dried with paper, sputter coated with gold/palladium, dried under vacuum and examined by SEM (XL 30 FEG SEM; Philips, Netherlands; field emission gun at 15KV).

Statistical analysis

The software GraphPad InStat version 2.0 for Windows (GraphPad Software, La Jolla, CA, USA) was used. Assumptions of equality of variances and normal distribution of data were checked for all variables tested, using the Bartlett and Kolmogorov-Smirnov tests, respectively. As the assumptions were satisfied, data were analyzed by one-way ANOVA followed by Tukey's *post-hoc* tests, separately for both time points (5 and 10 days). The level of significance was $P \leq 0.05$.

Results

After the 5th day, all varnishes and 20% xylitol solution significantly reduced the enamel loss when compared to the control and placebo varnish. After reapplication plus 5 days of erosive challenge (total of 10 days of erosive challenges), both xylitol varnishes and solutions significantly reduced the enamel erosion when compared to controls. However, 10% xylitol solution performed better when compared to the other groups (Table 1).

SEM images of the eroded specimens untreated (control) and retreated with 20% xylitol solution, NaF solution and placebo varnish showed a demineralized surface (Fig. 1a-d). Eroded enamel specimens retreated with 20% xylitol and NaF solutions presented more surface irregularities than the other groups cited. On the other hand, eroded enamel specimens retreated with NaF and 10% xylitol varnishes presented some precipitates on the surface, with no signs of demineralization (Fig. 2 a, b), while 20% xylitol varnish and 10% xylitol solution, when applied on eroded enamel surfaces, produced a smoother layer when compared to specimens from the other groups (Fig. 2 c, d).

Discussion

In the present study, erosive demineralization was produced by a cola drink, as it is one of the most widely consumed soft drinks and exhibits erosive potential (17,18). Erosion was performed for 90s, 4 times daily, which is thought to be representative of the effects of rapid consumption of an acidic beverage (15). Between erosive challenges, specimens were remineralized in artificial saliva, in order to simulate the pH challenges that occur in the oral environment. However, artificial saliva is not able to simulate acquired salivary pellicle formation, which is thought to play an important role during erosive challenge and influence the interaction between fluoride and minerals (19). The pellicle could also be assumed to interact with

Table 1 Mean enamel loss (μm) ± SD of the enamel specimens according to treatment

Treatment	5th day	10th day
Control	$4.70 \pm 0.38^{a,b}$	7.19 ± 0.57^{a}
NaF solution	4.08 ± 0.59^{b}	$6.47 \pm 0.66^{a,b}$
10% xylitol solution	$4.28 \pm 0.31^{a,b}$	5.16 ± 0.61^{c}
20% xylitol solution	2.99 ± 0.36^{c}	$5.52 \pm 1.17^{b,c}$
Placebo varnish	4.77 ± 0.45^{a}	$6.39 \pm 0.87^{a,b}$
NaF varnish	2.73 ± 0.37^{c}	7.21 ± 0.85^{a}
10% xylitol varnish	3.09 ± 0.43^{c}	$5.43 \pm 1.15^{b,c}$
20% xylitol varnish	2.48 ± 0.67^{c}	$5.35 \pm 0.85^{b,c}$

Groups with different lower-case superscript letters significantly differed from one another on each day of analysis ($n = 10$, Tukey's test, $P < 0.05$).

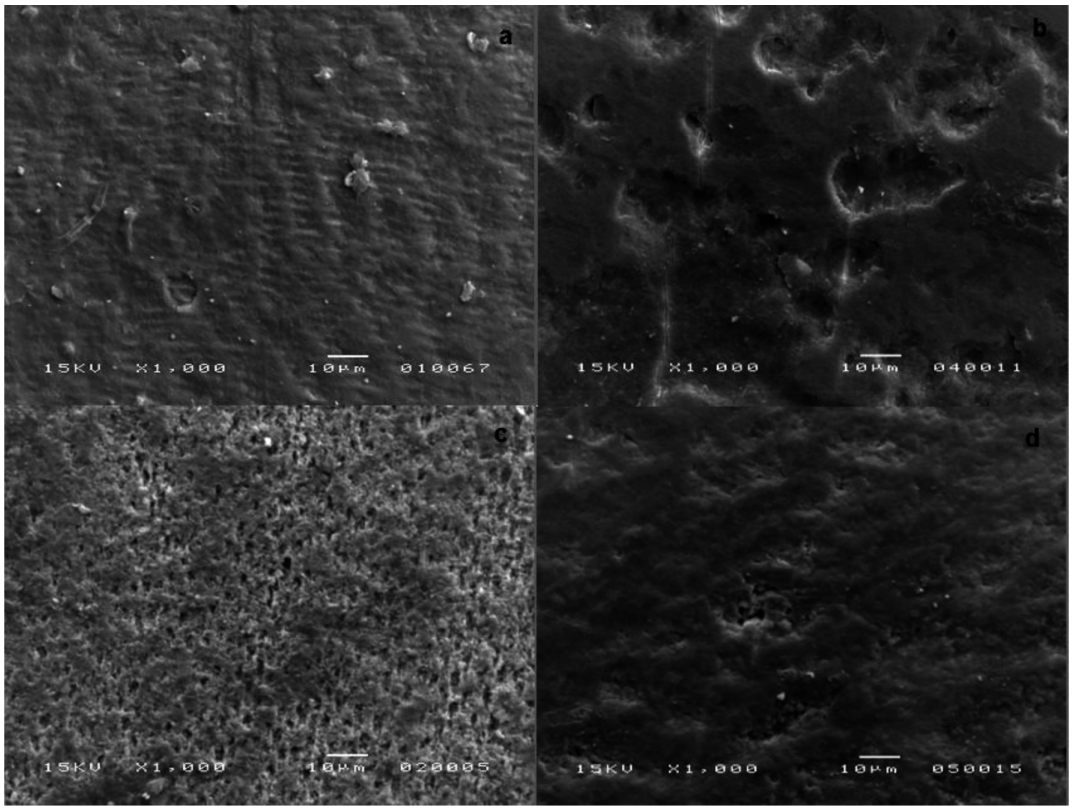

Fig. 1 SEM pictures of enamel specimens retreated as follows: a) control (untreated); b) 20% xylitol solution; c) NaF solution; d) placebo varnish.

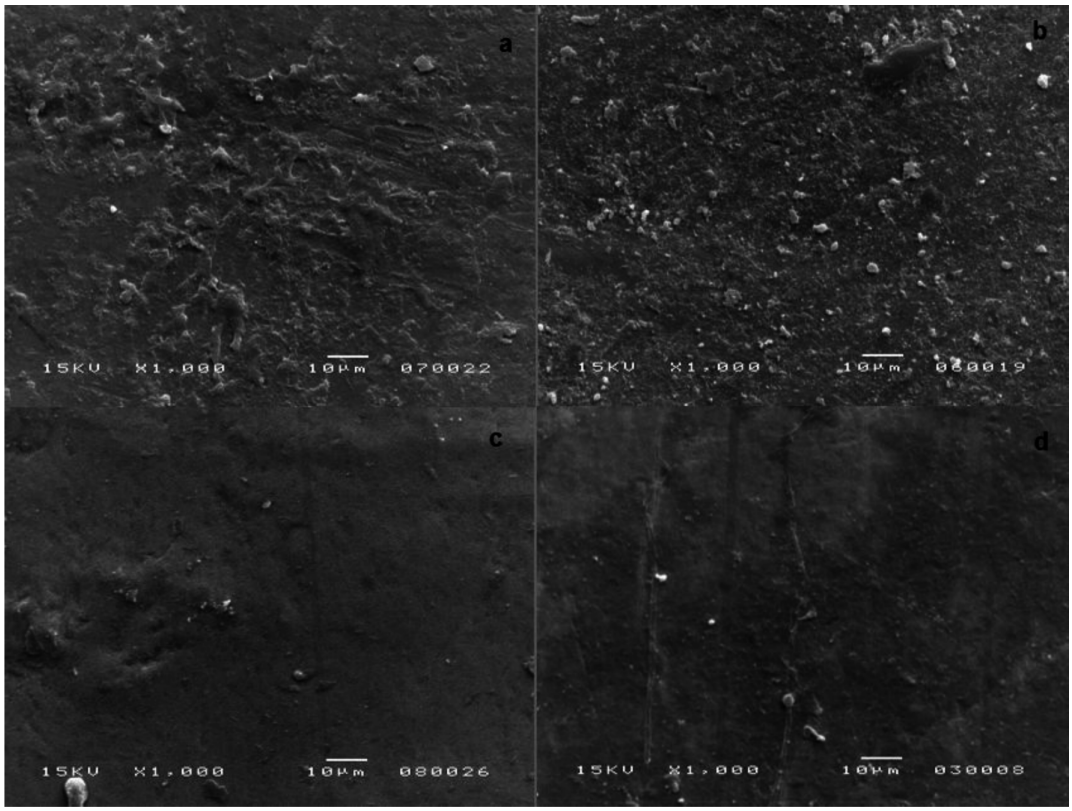

Fig. 2 SEM pictures of enamel specimens retreated as follows: a) 10% xylitol varnish; b) NaF varnish; c) 20% xylitol varnish; d) 10% xylitol solution.

xylitol. This limitation should be considered when the results are extrapolated to clinical situations.

All products were applied twice, at the beginning of the study and after 5 days of erosive challenge. The varnishes were completely removed after 6 h. This was performed in order to focus on the chemical effects of the varnishes rather than on the mechanical protection. Thus, varnishes were removed to simulate the clinical situation, in which the varnishes would probably be removed after some hours via toothbrushing or mastication. Although the basic composition of the varnishes was different, this appears to have had no influence on the results of the present study, as no differences were previously shown between Duraphat (Colgate) and a fluoridated varnish (Duofluorid) with the same basic composition of xylitol varnishes (all produced by FGM) on enamel erosion *in vitro* (15). On the other hand, the solutions were applied only for 1 min, simulating the clinical protocol, where it is recommended that fluoride solutions be applied for 1 to 4 min (15).

Contact profilometry is a commonly used method to measure dental loss by erosive and abrasive challenge produced in vitro and in situ, with an accuracy of around 0.3-0.5 μm for well-polished specimens (20). However, it must be remembered that although the stylus may be able to scratch acid-softened surfaces (21), this would occur in all groups, and so should not bias the results.

Based on the present results, the null hypotheses were partially rejected. With regard to the 1st hypothesis, on day 5, xylitol products (with exception of 10% xylitol solution) reduced enamel loss to the same degree as a commercial NaF varnish, which has previously shown potential to prevent enamel erosion (15). On the other hand, all xylitol products performed better than commercial NaF varnish on day 10. With regard to the 2nd hypothesis, xylitol varnishes appeared to perform better on sound surfaces than solutions and 10% xylitol solution on demineralized enamel surfaces. Some undetectable portions of varnish may have remained present on the surface after removal, and this fact could explain the differences in behavior between xylitol varnishes and solutions.

The results of the present study disagree with those of Chunmuang et al. (14), who found that application of 40% xylitol solution, with or without fluoride (227 ppm), between erosive challenge and remineralization was unable to significantly reduce human enamel erosion depth and surface softening after 14 days. The contrasting findings between the studies might be related to the erosive protocol. Even Chunmuang et al. (14) applied 40% xylitol solution 4 times daily (1 min), the application was performed between erosion and remineralization, and the total time of erosive challenge was 280 min (4 × 5 min, 14 days). It can thus be assumed that maintenance of the effects of treatment after successive erosive challenges is limited. After 10 days of erosive challenge (total time: 60 min), our study also showed reduced efficacy of xylitol products (except 10% xylitol solution), which were better than controls, but similar to placebo varnish.

The commercial NaF varnish was also unable to protect against enamel erosion after 10 days of the present experiment. CaF_2 globules that precipitated on the eroded enamel surfaces may have been unstable and unable to resist successive erosive challenges (7), even after reapplication.

On day 10, the 10% xylitol solution performed better than the other treatments, based on SEM images. From the profilometric data and SEM pictures, it might be assumed that the demineralized enamel surface allows better penetration of the solution when compared to a sound surface. The 20% xylitol varnish and 10% xylitol solution reapplication produced a smoother enamel surface when compared with other treatments. However, the 20% xylitol varnish could not significantly reduce enamel erosion when compared with placebo on day 10.

Xylitol activity is based on its ability to form complexes with calcium ions on the dental surface, inhibiting the translocation of dissolved calcium and phosphate, and the resultant demineralization (9,10,12). Therefore, based on the present results, xylitol solutions may perform better than varnishes on demineralized surfaces, due to the higher number of pores and the presence of dissolved calcium on the surface, allowing deeper penetration and reactions between fluid agents (solution vs. varnish) and enamel.

Miake et al. (11) showed that 20% xylitol added to remineralizing solution was able to induce remineralization of deeper layers of demineralized enamel by facilitating calcium movement and accessibility (Ca^{+2} ion carrier) into the lesion pores. However, in the present study, reapplication of 20% xylitol solution was unable to reduce enamel erosion as expected. SEM images showed a demineralized surface on the eroded specimen retreated with 20% xylitol solution. Miake et al. (11) produced an artificial caries lesion using a different method from that in the present study, in that an erosive lesion was created. Artificial caries present a subsurface lesion with a less-demineralized surface layer compared to erosion-like lesions (softening surfaces) (22). Therefore, the type of lesion might explain the differences in the findings.

However, the unsatisfactory results for 20% xylitol solution reapplication in the present study confirms the previously reported data of Chunmuang et al. (14), who found that daily application of 40% xylitol solution, between erosive challenge and remineralization, was unable to prevent enamel erosion. One explanation is that

the pores in the demineralized enamel surface do not allow penetration of high-concentration xylitol solutions (>20%). Pereira et al. (23) showed that 10% xylitol varnish, when applied on enamel specimens, was able to promote better xylitol release in artificial saliva than 20% xylitol varnish. The authors did not test xylitol solutions. However, from these data, it might be speculated that the interaction between xylitol and the environment (tooth or saliva) is modulated by the sugar concentration.

In order to better understand the present findings, further studies should test the effects of different concentrations of xylitol solutions and varnishes on both sound and demineralized surfaces. Another interesting point to be considered is that the pH of the xylitol solutions produced in the present study was highly unstable. The instability of the pH was mostly related to the concentration of xylitol. Immediately after preparation, the pH of the solutions was around 6.8. However, after some minutes, the pH dropped to 3.0. Therefore, in further studies the pH of solutions should be monitored and adjusted using a buffer in order to facilitate the use of the product in clinical settings.

Based on the results of the present study, it can be concluded that xylitol is a good option for partially reducing enamel erosion. However, maintenance of the effects of this treatment after successive erosive challenges is limited. The effects of xylitol plus fluoride varnishes/solutions, as well as buffering xylitol solutions (with or without fluoride) should be tested in further in vitro studies. Before clinical use, it is advisable to perform in situ studies to test the effects of these new products on human enamel and dentin erosion and abrasion.

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