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Short communication

# Effect of xylitol varnishes on remineralization of artificial enamel caries lesions in situ

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ABSTRACT

*Objectives:* Analyze the effect of varnishes containing xylitol compared to commercial fluoridated varnishes on the remineralization of artificial enamel caries lesions *in situ*.
*Methods:* Twenty subjects took part in this crossover, double-blind study performed in four phases of 5 days each. Each subject worn palatal appliances containing four predemineralized bovine enamel specimens. Artificial caries lesions were produced by immersion in 30 ml of lactic acid buffer containing 3 mM $CaCl_2 \cdot 2H_2O$, 3 mM $KH_2PO_4$, 6 μM tetraetil metil diphosphanate (pH 5.0) for 6 days. The specimens in each subject were treated once with the following varnishes: 20% xylitol (experimental); Duofluorid™ (6% NaF, 6% $CaF_2$), Duraphat™ (5% NaF, positive control) and placebo (no-F/xylitol, negative control). The varnishes were applied in a thin layer and removed after 6 h. Fifteen subjects were able to finish all phases. The enamel alterations were quantified by surface hardness and transversal microradiography. The percentage of surface hardness recovery (%SHR), the integrated mineral loss and lesion depth were statistically analyzed by Friedmann and Dunn's tests test ($p < 0.05$).
*Results:* Enamel surface remineralization was significantly increased by Duraphat™, Duofluorid™ and 20% xylitol formulations. Significant subsurface mineral remineralization could also be seen for the experimental and commercial varnishes, except for Duraphat™, when the parameter "lesion depth" was considered.
*Conclusions:* 20% xylitol varnish seem to be a promising alternative to increase surface and subsurface remineralization of artificial caries lesions *in situ*. Clinical significance: effective vehicles are desirable for caries control. Xylitol varnishes seem to be promising alternatives to increase enamel remineralization *in situ*, which should be confirmed by clinical studies.



## 1. Introduction

Xylitol has proved to have an important role in the prevention of dental caries, as many studies report a reduction in salivary levels of *Streptococcus mutans*, especially due to the prolonged and continuous exposure to xylitol by chewing gums [1–4]. The use of xylitol in chewing gums showed a decrease of decay between 30 and 60% in Finnish children [5]. However, the clinical relevance of reduced intra-oral levels of this microorganism is still unclear [6] as not all studies confirm the inhibitory effect of xylitol on *S. mutans* and reduction of dental caries [7,8]. Also, there is still uncertainty about the real mechanism of action of xylitol involved in caries control.

A probable mechanism of action of xylitol is on enamel remineralization, as described in previous studies [9,10]. A study involving high-resolution electron microscopy and microradiography revealed a higher remineralization in intermediate and deep layers of enamel samples immersed in 20% xylitol solution compared with control [11].

The frequency of use of xylitol has been recognized to be more important than its amount in the prevention of dental caries [2,12]. Taking into account the price and the number of times that vehicles as chewing gums should be used every day to offer a xylitol salivary concentration that is able control tooth decay [13–15], dental

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varnishes seem to be good alternatives due to their long-term contact with the enamel surface. Furthermore, varnish is a professional product whose application does not depend on the daily patient's compliance, which could favor its effect. Considering this, a recent *in vitro* study performed by our group, showed that a xylitol varnish containing 20% xylitol with fluoride was able to improve the surface enamel remineralization as much as the commercial fluoride varnishes. This might suggest that the effect was due to fluoride rather than xylitol content, as the mechanism of action of fluoride varnish is based on the formation of a $CaF_2$-like layer on enamel surface that releases fluoride during the cariogenic challenges [16].

However, the varnish containing 20% xylitol without fluoride was able to improve the surface enamel remineralization as much as the commercial fluoride varnishes and, for the subsurface region, the combination of xylitol and fluoride was ineffective in improving remineralization, suggesting that fluoride may have hampered xylitol diffusion into the enamel and consequently its remineralizing effect.

Thus, the present study aimed to analyze the remineralizing effect of experimental varnish containing 20% xylitol without fluoride, compared to commercial fluoridated varnishes *in situ*. The null hypothesis was that the varnishes containing xylitol are not effective on enamel surface and subsurface remineralization compared to the commercial fluoride varnishes.

## 2. Materials and methods

### 2.1. Preparation of bovine enamel specimens and artificial caries formation

Four hundred and fifty enamel specimens (4 mm × 4 mm × 2.5 mm) were prepared from incisor bovine teeth, freshly extracted and disinfected by storage in 2% formaldehyde solution (pH 7.0) for 30 days at room temperature. After visual inspection, stained and/or cracked teeth were excluded. Besides, soft tissues were removed from the coronal and root surfaces with the aid of a periodontal curette (Duflex™, SSWhite, Rio de Janeiro, RJ, Brazil). The specimen was obtained, after two double sections of the widest portion of the dental crowns, and polished, as described by Magalhães, et al. [17].

Four hundred and twenty enamel specimens were selected by using the baseline surface hardness (Mean KHN 351.7 ± 20.6). They had 1/3 of the surface protected (control area) with nail varnish and they were further subjected to the formation of artificial caries lesion by immersion in 30 ml of buffer containing 50 mM lactic acid, 3 mM $CaCl_2{\cdot}2H_2O$, 3 mM $KH_2PO_4$, 6 μM tetraetil metil diphosphanate and traces of thymol (KOH to adjust pH to 5.0) [18] for 6 days. After demineralization, the other outer 1/3 of the surface was protected with nail varnish (demin control area), leaving a central band of demineralized enamel. The surface hardness of the predemineralized enamel (SH lesion) was measured immediately after demineralization but before protection with nail varnish.

### 2.2. Ethical aspects and experimental design

Twenty healthy adults (17 women and 3 men, 18–30 yr of age) were enrolled according to the study inclusion and exclusion criteria. Inclusion criteria were as follows: stimulated physiological salivary flow rate of >1 ml/min; non-stimulated physiological salivary flow rate of >0.25 ml/min; and good oral health (i.e. no frank cavities or significant gingivitis/periodontitis). Exclusion criteria were: systemic illness; pregnancy or breastfeeding; use of fixed or removable orthodontic appliances; use of fluoride mouthrinse or professional fluoride application in the last 2 months; and hyposalivation. A sample size of 11 subjects was previously calculated considering an α-error level of 5% and a β-error level of 20% (www.ddsresearch.com) according to the results of a previous *in situ* study [19]. Due to the possibility of subject dropout, 20 volunteers were initially recruited.

The study was performed following the guidelines of good clinical practice and conformed to the Declaration of Helsinki. Ethical approval for the study involving human subjects was granted by the local Ethics Committee (no. 543.634, CAAE 22763113.3.0000.5417/2014; Ethics Committee of the Bauru School of Dentistry, University of São Paulo, SP, Brazil). Two varnishes (control and containing 20% xylitol with the same basic composition as the commercial varnish Duofluorid™), were especially manufactured by FGM/Dentscare (Joinville, SC, Brazil). Xylitol concentration was determined by the maximum incorporation of that polyol into the varnish that would not lead to precipitation. The varnishes contained colophonium, synthetic resin, thickening polymer, essence and ethanol (informed by manufacturer). Xylitol was supplied by Danisco (Xylitab™ 300, Danisco Brasil Ltda, Cotia, SP, Brazil).

This prospective cross-over, double-blind study was performed with a washout interval of 7 d before each of the four, 5-d-long experimental phases. The subjects were randomly allocated to the different treatments in each phase. At the first phase, five subjects were allocated to Treatment A (Duofluorid™ (6% NaF, 2.71% F, 6% $CaF_2$, pH 8.0, FGM/Dentscare)), five to Treatment B (Duraphat™ (5% NaF, 2.26%F, pH 5.0, Colgate, São Bernardo, SP, Brazil), and five to Treatment C (20% xylitol, pH 5.0, experimental; FGM/Dentscare) and five to Treatment D (control varnish, no xylitol or fluoride, pH 5.0, experimental; FGM/Dentscare). At the second, third and fourth phases, the enamel samples and the treatments were changed in order to provide a cross-over design, in which all subjects randomly participated in all treatments. The subjects received written instructions including the schedule and were extensively trained for all procedures required during the study. Informed consent was obtained from all subjects before starting the study.

### 2.3. Treatment of specimens

Before treatment, the specimens were disinfected by dipping in 70% alcohol solution for 30 min in addition to the previous disinfection in formaldehyde solution.

The specimens were treated with 6% NaF, 6% $CaF_2$ varnish (Duofluorid™); 5% NaF, varnish (Duraphat™); "20% xylitol varnish (experimental) no xylitol or fluoride varnish (control) *in vitro*. The varnishes were applied onto the enamel surface using a microbrush and were allowed to dry for 1 min before storage in artificial saliva (0.2 mM glucose, 9.9 mM NaCl, 1.5 mM $CaCl_2{\cdot}2H_2O$, 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 traces of ascorbic acid, pH 6.8; 30 ml per sample) [20] at 25 °C for 6 h (17). The varnishes were then carefully removed using a surgical blade and cotton swabs soaked in 50% acetone solution [21]. The nail varnish was reapplied after this procedure to protect the control areas.

### 2.4. In situ protocol

Seven days before and throughout the *in situ* phases, the subjects brushed their teeth with a 1500 ppm fluoride toothpaste (Sorriso Fresh, Colgate-Palmolive®), in order to standardize the amount of fluoride in the oral reservoirs. Two cavities, 5-mm wide × 5-mm wide × 4-mm depth, were made on the left side and on the right side (i.e. four cavities in total) of each acrylic palatal appliance. The predemineralized and previously treated with varnish specimens were fixed with wax and a new acrylic palatal appliance was made in each phase. During the *in situ* phases, the