

# REMINERALIZATION OF ENAMEL SUBSURFACE LESIONS BY XYLITOL CHEWING GUM CONTAINING FUNORAN AND CALCIUM HYDROGENPHOSPHATE

Sroisiri Thaweboon<sup>1</sup>, Siriruk Nakornchai<sup>2</sup>, Yasuo Miyake<sup>3</sup>, Takaaki Yanagisawa<sup>3</sup>,  
Boonyanit Thaweboon<sup>1</sup>, Surin Soo-ampon<sup>4</sup> and Duangjai Lexomboon<sup>5</sup>

<sup>1</sup>Department of Microbiology, <sup>2</sup>Department of Pediatric Dentistry, <sup>4</sup>Department of Physiochemistry, <sup>5</sup>Department of Community Dentistry, Faculty of Dentistry, Mahidol University, Bangkok, Thailand; <sup>3</sup>Department of Ultrastructural Science, Oral Health Science Center, Tokyo Dental College, Chiba, Japan

**Abstract.** The aim of the present study was to determine the remineralization effects of xylitol chewing gum containing funoran and calcium hydrogenphosphate on enamel subsurface lesions in humans. The study was a double-blind, randomized, cross-over design, with 4 types of gum: (1) xylitol gum, (2) xylitol gum containing funoran and calcium hydrogenphosphate, (3) sugar gum, and (4) gum base as a control. Seven subjects were instructed to wear removable lingual appliances, with half-slab insets of human enamel containing demineralized subsurface lesions. They were told to chew gum for 20 minutes 4 times per day for 7 days. Upon completion of each treatment the enamel half-slabs were paired with their respective demineralized control half-slabs, embedded, sectioned, and subjected to microradiography and densitometric image analysis, for measurement of the level of remineralization. The mean area of remineralization ( $\Delta Z_d - \Delta Z_r$ ) and mean percent remineralization (%R) in those chewing xylitol gum containing funoran and calcium hydrogenphosphate were significantly higher than the corresponding values for xylitol gum, sugar gum and gum base. Chewing xylitol gum containing funoran and calcium hydrogenphosphate has a significant effect on the remineralization of initial caries-like lesions of the teeth.

## INTRODUCTION

Chewing gum is known to have a beneficial effect on oral health and may be a useful adjunct to common oral hygiene. Gum chewing is a potent stimulant of salivary flow and calcium and phosphate levels, limiting demineralization and enhancing

remineralization (Imfeld, 1999; Dawes and Kubieniec, 2004). Chewing gum has the potential for being an effective vehicle for delivering therapeutic agents to the teeth because they allow prolonged contact between the agent and the teeth with minimal effort by the patient. A previous paper (Edgar and Geddes, 1990) reviewed studies that evaluated the effectiveness of a number of potential anticariogenic agents in chewing gum. Among the agents that have received considerable attention was calcium hydrogenphosphate, which was assessed for its effects on elevating the calcium and phosphate

Correspondence: Sroisiri Thaweboon, Department of Microbiology, Faculty of Dentistry, Mahidol University, 6 Yothi Road, Bangkok 10400, Thailand.

Tel: 66 (0) 2203 6410

E-mail: dtstw@mahidol.ac.th

concentrations in the saliva (Pickel and Bilotti, 1965).

The sugar alcohol xylitol has been claimed to have an effect on reducing dental caries (Kovari *et al*, 2003). The mechanisms of action include enhanced remineralization of the enamel by acting as a carrier for calcium ions into white spot lesions (Miake *et al*, 2003). The chewing of this kind of sugar-free gum after meals and snacks has been shown to promote remineralization (Imfeld, 1999).

Funoran, a high molecular weight sulfated polysaccharide extracted from the red seaweed *Gloiopeltis furcata* has been demonstrated to inhibit cariogenic and periodontopathic bacteria, and thereby contribute to the prevention of dental caries and periodontal disease (Saeki, 1994; Saeki *et al*, 1996 a,b). Chewing gum containing funoran and calcium hydrogenphosphate has a remarkable ability to enhance remineralization throughout all layers in initial caries-like enamel lesions *in vitro* (Takahashi *et al*, 2001). The purpose of the present study was to assess the remineralization effect of xylitol chewing gum containing funoran and calcium hydrogenphosphate on enamel subsurface lesions in a human *in situ* model in Thailand. The results from this study should provide information regarding the remineralization potential of these gum additives.

## MATERIALS AND METHODS

### Subject recruitment

The study was a double-blind, randomized, cross-over design. Seven healthy subjects were recruited from dental students (age 20-25 years) from Mahidol University. Before the beginning of the study, the volunteers gave their written informed consent to the study protocol, which was reviewed and approved by the Mahidol University Human Research Ethics Committee. An in-

tra-oral examination was performed to confirm that each subject had at least 22 natural teeth with no current caries activity, periodontal disease or other pathology. None of them were using antibiotics or medications which could have affected the salivary flow rate.

### Preparation of enamel subsurface lesions

Twenty-eight first premolars were extracted from the 7 patients during orthodontic treatment and prepared as described previously (Shen *et al*, 2001; Miake *et al*, 2003). In brief, the outer enamel surface was polished wet to a minor finish using Soflex™ (3M, St Paul, MN) disks on a slow-speed contra-angle dental handpiece. Each polished surface was then sawn from the tooth as an approximately 8 x 5 mm<sup>2</sup> slab, using a water cooled diamond blade saw and the whole slab was covered with acid-resistant nail varnish except for mesio-distal windows (approximately 6 x 4 mm).

Initial caries-like lesions were prepared artificially by the method modified from White (1987). In brief, the enamel slab was immersed in 40 ml of 0.1 mol/l lactic acid, 500 mg/l hydroxyapatite, and 20 g/l Carbopol C907 (BF Goodrich Cleveland, OH) at pH 4.8 for 5 days at 37°C. Each enamel slab was placed in the demineralizing buffer. After demineralization each enamel slab was sawn perpendicular to the windows into a 3 x 4 mm<sup>2</sup> slab and the cut surface was covered with nail varnish. One-half was retained as the demineralization control and stored in a tube with a humidified environment. The other half-slab was inset into an intra-oral appliance for the remineralization protocol. The enamel slab was changed every treatment period.

### Preparation of intra-oral appliance

A removable acrylic appliance was fabricated for each volunteer's lower jaw

covering the lingual surface from the first premolar to the second molar. The base was held in place by 2 stainless steel clasps.

### Experimental protocol

The protocols of the three *in situ* experiments were identical except for the composition of the chewing gums (Table 1). All the chewing gums were prepared by Lotte (Tokyo, Japan). A gum base without any additives was used as control. The gums were provided as coded products and were stored at room temperature. All chewing gum treatments were given double-blinded and randomized. For each experiment, all subjects were crossed over to each randomly assigned treatment, with at least 1 week between treatments.

All subjects used standard fluoride toothpaste for the duration of each experiment and chewed the gum at a natural chewing frequency for 20 minutes 4 times daily for 7 days at the following times: 8:00 AM, 12:00 AM, 4:30 PM and 8:30 PM. The appliances were worn during daytime except while eating, drinking or performing oral hygiene procedures. When the appliances were removed, they were stored in sealed moist plastic bags at room temperature. The subjects were instructed to rinse and clean their appliances using only a toothbrush. They

were informed not to brush the area containing the enamel slab. No alterations were made to the subjects' diets or oral hygiene procedures for the duration of each experiment. After completion of each treatment period, the enamel slabs were removed from the appliances for processing.

### Enamel sectioning and microradiography

At the completion of each treatment period, each remineralization enamel slab was dehydrated in graded alcohol and embedded in polyester resin (Rigolac; Nisshin EM, Tokyo, Japan). Ground sections (100  $\mu\text{m}$  thick) were taken from each enamel slab of the demineralized and remineralized areas and of the intact areas. Contact microradiograms (CMRs) were taken by means of a soft X-ray generator with a 20  $\mu\text{m}$  Ni filter (Softex CMR-3; Softex, Tokyo, Japan) operated at an accelerating voltage of 15 kV and a specimen current of 3 mA for 10 minutes with a Cu-K $\alpha$  line. An aluminium step-wedge was placed on the X-ray glass film plate (HRP-SN-2; KONICA MINOLTA, Japan) with the enamel sample for microradiography. The film was developed with a Kodak D-19 developer for 5 minutes at 20°C, rinsed with tap water and then fixed with Fujifix for 5 minutes at 20°C.

The CMR images were taken with an Image Analyze System (HDS-N1; HIROYA,

Table 1  
The composition of different gums used.

Type	Composition
Xylitol gum containing funoran and calcium hydrogenphosphate	Xylitol 45%, maltitol 35%, calcium hydrogenphosphate 0.2%, funoran 0.1%, flavor and gum base
Xylitol gum	Xylitol 44%, maltitol 35%, flavor and gum base
Sugar gum	Sucrose 79%, glucose syrup 0.6%, flavor and gum base
Gum base	Gum base

Japan) and digital images were made of each area (width 50  $\mu\text{m}$ ; depth 300  $\mu\text{m}$  from resin base to sound enamel) using a 256 level grey scale. The remineralization rate for each definite area was calculated by setting the photographic density of film not involving the specimen to 0% and that of intact enamel to 100%. An area free of artifacts or cracks was selected for analysis. Linear regression was used to convert the grey value data into values of equivalent thickness of aluminium. The section thickness was measured and the percent mineral data for the remineralization windows and demineralized control were computed using the equation by Angmar *et al* (1963). Lesion depth was defined as the distance from the surface to the location in the lesion where the mineral content was larger than 95% of the mineral content in sound enamel.

#### Remineralization data analysis

The percent mineral profiles for each enamel block's demineralized and remineralized lesions were compared with the median sound enamel between the lesions of the same section. The difference between the area under the densitometric profile of the demineralized lesion and the median sound enamel, calculated by trapezoidal integration, is represented by  $\Delta Z_d$  (Angmar *et al*, 1963). The difference between the areas under the densitometric profile for the remineralized lesion and the median sound enamel, calculated by trapezoidal integration, is represented by  $\Delta Z_r$  (Reynolds, 1997). These parameters were then converted to percent change in values after remineralization for each lesion. Percent remineralization (%R) represented the percent change in  $\Delta Z$  values:

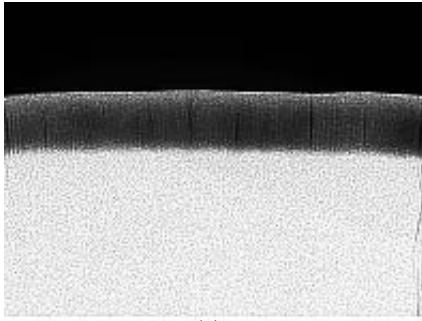
$$\%R = \frac{\Delta Z_d - \Delta Z_r}{\Delta Z_d} \times 100$$

The %R and lesion depth (Ld) data were analyzed using analysis of variance

with Scheffe's multiple comparison test. The critical level for alpha was set at 0.05.

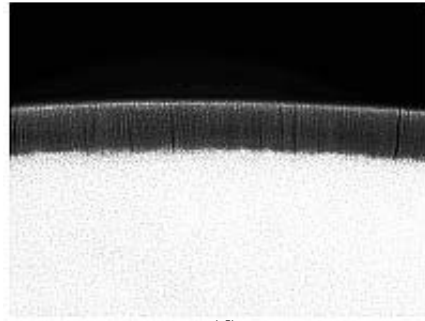
## RESULTS

Representative microradiographic images of the enamel subsurface lesion before and after *in situ* remineralization using the three different gum formulations and the gum base control are shown in Fig 1. A microradiograph of the artificially demineralized enamel slab was homogeneous and flat at the bottom of subsurface lesion. The enamel subsurface lesion used for this *in situ* study had a mean depth of  $129.77 \pm 14.88 \mu\text{m}$  and a mean volume of mineral loss of  $4,540 \pm 693.27 \text{ vol}\% \mu\text{m}$ . As shown in the microradiographs, the remineralization occurred in the surface and deep layers in all the gum groups. Chewing xylitol gum containing funoran and calcium hydrogenphosphate showed the lowest mean depth compared to the other gums (Table 2) and the demineralized control. However, a significant difference was observed between the xylitol gum containing funoran and calcium hydrogenphosphate and the demineralized control, and between the xylitol gum containing funoran and calcium hydrogenphosphate and the xylitol only gum. The mean area of remineralization ( $\Delta Z_d - \Delta Z_r$ ) and the mean percent remineralization (%R) of the xylitol gum containing funoran and calcium hydrogenphosphate were significantly higher than the corresponding values for xylitol gum, sugar gum and the gum base (Table 2). Chewing xylitol gum resulted in a significantly greater area of remineralization compared to the sugar gum and the gum base but the %R was not significantly different from the sugar gum. The addition of funoran and calcium hydrogenphosphate to xylitol gum produced a significantly greater enamel remineralization (xylitol gum containing funoran and calcium hydrogenphosphate had  $\Delta Z_d - \Delta Z_r = 651.69 \pm 155.41 \text{ vol}\% \mu\text{m}$



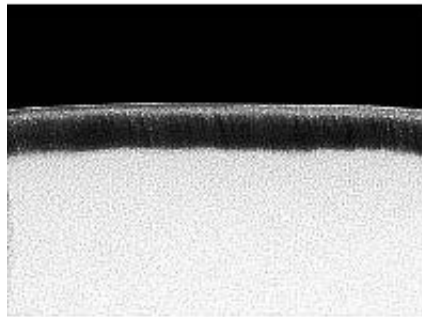
(a)

(a) Before gum chewing: enamel was demineralized to an almost uniform depth from the surface, but the surface layers remained slightly mineralized. Less mineralization was observed in the intermediate and deep layers.



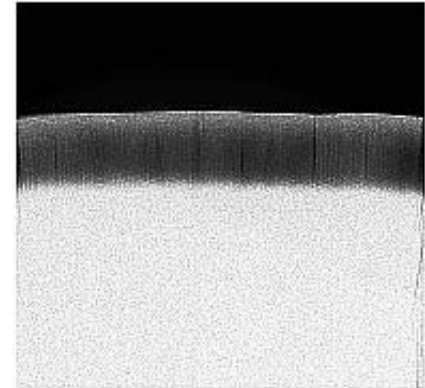
(d)

(d) Sugar gum: a thin, highly mineralized layer was seen in the surface layer of the enamel. The middle layer was uniformly remineralized. The deep layer became highly remineralized, and the width of the demineralized layer decreased compared with the enamel surface before gum chewing.



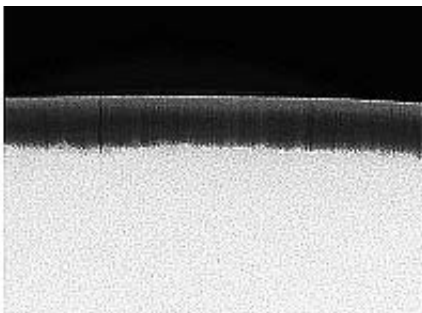
(b)

(b) Xylitol gum + funoran + calcium hydrogenphosphate: a wide, highly remineralized layer was seen in the surface layer of the enamel. Remineralization increased in the middle layer. The deep layer became highly remineralized, and the width of the demineralized layer decreased.



(e)

(e) Gum base: the remineralization layer was seen in the surface layer of the enamel. Remineralization was increased at the middle layer. The deep layer became highly remineralized, and the width of the remineralized layer decreased.



(c)

(c) Xylitol gum: A wide remineralization layer was seen in the surface of the enamel. The middle layer was slightly remineralized. The deep layer became highly remineralized, and the width of the demineralized layer decreased.

Fig 1—Representative microradiographic images of the enamel subsurface lesions before (a) and after chewing *in situ* remineralization by xylitol gum + funoran + calcium hydrogenphosphate (b), xylitol gum (c), sugar gum (d) and gum base (e).

Table 2  
Remineralization of enamel subsurface lesions by gum chewing.

	Xylitol + funoran + calcium hydrogenphosphate gum	Xylitol gum	Sugar gum	Gum base
Lesion depth, Ld ( $\mu\text{m}$ )	110.20 $\pm$ 13.06 <sup>a</sup>	124.03 $\pm$ 15.34 <sup>b</sup>	114.39 $\pm$ 12.62 <sup>a,b</sup>	110.22 $\pm$ 20.54 <sup>a,b</sup>
$\Delta\text{Zd}-\Delta\text{Zr}$ (vol% $\mu\text{m}$ )	651.69 $\pm$ 155.4	406.83 $\pm$ 188.13	295.08 $\pm$ 141.59 <sup>a</sup>	275.02 $\pm$ 110.78 <sup>a</sup>
% R (vol% $\mu\text{m}$ )	14.33 $\pm$ 3.77	9.76 $\pm$ 3.23 <sup>b</sup>	6.68 $\pm$ 3.49 <sup>a,b</sup>	5.83 $\pm$ 2.75 <sup>a</sup>

Values are mean  $\pm$  SD

<sup>a, b</sup> Values similarly marked were not significantly different ( $p > 0.05$ )

and %R = 14.33  $\pm$  3.77 vol%  $\mu\text{m}$ , xylitol gum had  $\Delta\text{Zd}-\Delta\text{Zr}$  = 406.83  $\pm$  188.13 vol%  $\mu\text{m}$  and %R = 9.76  $\pm$  3.23 vol%  $\mu\text{m}$ ). No significant difference was observed between sugar gum and gum base in the ability to produce remineralization.

## DISCUSSION

Chewing gum is a common habit in many countries. Chewing sugar-free gum has been reported to have beneficial dental effects, particularly in decreasing caries (Machiulskiene *et al*, 2001). It provides a masticatory and gustatory stimulus to salivation. Dawes and Macpherson (1996) showed that salivary flow is increased during gum chewing up to 10 times the unstimulated flow rate during the first minute of gum chewing, followed by a decrease to a level 3 times the unstimulated flow rate by 20 minutes. The remineralization observed in the lesions with all the gums in the present study may be attributed to an increase in salivary flow rate over the 20-minute chewing period.

Xylitol gum and xylitol gum containing funoran and calcium hydrogenphosphate showed greater remineralization than the sugar gum and the gum base. However, the percent remineralization with xylitol gum was not significantly different from that of sugar gum due to the high SD. This may be

explained by the different individual chewing habits and oral environment of the subjects, *eg*, flow rate and composition of saliva. In the present study, xylitol gum produced nearly 10% remineralization after chewing for 20 minutes, 4 times per day for 7 days. This is similar to the results reported by Iijima *et al* (2004) which showed that xylitol-containing sugar-free gum produced 9% remineralization when chewed for 20 minutes, 4 times per day for 14 days. The degree of remineralization after gum chewing has been demonstrated to vary among intra-oral sites (Amaechi *et al*, 1999) which could be related to the position of the enamel specimen mounted in the appliance. Some studies used mid-palate appliances (Shen *et al*, 2001; Iijima *et al*, 2004; Cai *et al*, 2007) while others used mandibular appliances (Koulourides *et al*, 1974; Buchalla *et al*, 2002; Itthagaram *et al*, 2005; Schirrmestu *et al*, 2007). In the present study, enamel specimens containing subsurface lesions were positioned lingually at the mandibular molar by means of a reversible appliance. This position imitated lingual lesions of the posterior teeth of the lower jaw, whereas those of the mid-palate appliances imitated palatal lesions of maxillary premolars and molars.

Xylitol has become a widely used non-cariogenic food additive. It is a natural sugar

alcohol which has been shown to be effective in dental caries prevention by the inhibitory effect on growth and metabolism of mutans streptococci (Hayes, 2001; Thaweboon *et al*, 2004). Xylitol is claimed to be a non-acidogenic sweetener that increases salivary secretion and the amount of calcium in plaque, while decreasing acid production (Makinen, 1988). In addition, it is thought to enhance the remineralization of carious lesions (Makinen *et al*, 1995). Although the mechanisms of action are not known, xylitol is believed to be associated with calcium in aqueous solution (Makinen and Soderling, 1984), inhibit the dissolution of calcium and phosphate ions from the enamel (Arends *et al*, 1990), and acts as a carrier of calcium required for enamel remineralization (Makinen and Soderling, 1984). Moreover, the use of xylitol with fluoride has been shown to increase remineralization and decrease demineralization (Amaechi *et al*, 1999).

The inclusion of funoran and calcium hydrogenphosphate significantly increases the remineralization ability (%R) of xylitol chewing gum to about 2 times that of sugar gum and gum base. Xylitol gum containing funoran and calcium hydrogenphosphate was demonstrated to enhance remineralization in initial caries-like enamel lesions *in vitro* (Takahashi *et al*, 2001; Yanagisawa *et al*, 2002). The remineralization effect is 1.7 times greater than that of xylitol gum containing casein phosphopeptideate-amorphous calcium phosphate. Recently, a similar *in situ* study in China showed significantly enhanced remineralization, using xylitol gum containing funoran and calcium hydrogenphosphate, of enamel lesions (Wang *et al*, 2007).

Funoran, a brownish-white polysaccharide powder, is extracted from the red seaweed *Gloiopeltis furcata*, which grows on rocks in shallow water along the Pacific coast line of Japan, China and Korea. The main

component of funoran is a sulfate agaroid (Hirase *et al*, 1958). Besides its use as food additives, funoran is employed for a variety of traditional Japanese and Chinese industries and arts owing to its excellent sizing and adhesive properties (Schachat and Glicksman, 1959). A histological study in mice revealed that funoran is nearly non-toxic (Akahane, 1998). No negative data has ever been reported regarding funoran (Saeki *et al*, 1996b). Previous studies showed that funoran strongly inhibited the colonization of cariogenic and periodontopathic bacteria on hydroxyapatite (Saeki, 1994; Saeki *et al*, 1996 a, b).

Calcium hydrogenphosphate or dicalcium phosphate ( $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ ) is a di-basic calcium phosphate which is usually found in the dehydrated form and is practically insoluble in water. It contains about 23% calcium and is mainly used as a dietary supplement in prepared breakfast cereals, enriched flour, and noodle products. Calcium hydrogenphosphate is commonly used as a tableting agent in some pharmaceutical preparations. In dentistry, dietary supplements of calcium and phosphate were proposed for the prevention of dental caries. At dose of 7.5% calcium hydrogenphosphate was found to elevate calcium and phosphate concentrations in saliva (Pickel and Bilotti, 1965). Two clinical studies performed with chewing gum containing calcium hydrogenphosphate showed a significant reduction in dental caries (Finn and Jamison, 1967; Richardson *et al*, 1972). The proposed remineralization process of calcium hydrogenphosphate was to involve the diffusion of  $\text{CaHPO}_4$  and associated calcium and phosphate ions through the protein/ $\text{H}_2\text{O}$  filled pores of the carious surface enamel into the body of the enamel lesion (Reynolds, 1997).

In conclusion, chewing xylitol gum containing funoran and calcium hydrogenphosphate results in significant reminerali-

zation of initial caries-like lesions of the teeth. The location of the dental device used to test mineralization varied by experiment, but both locations resulted in remineralization in the oral cavity, unrelated to tooth location.

#### ACKNOWLEDGEMENTS

We gratefully acknowledge the enthusiastic participation and compliance of all the subjects involved in the present study.

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