Remineralisation of Enamel Subsurface Lesions by Xylitol Chewing Gum Containing Calcium Hydrogen Phosphate and Funoran

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Objective: *To investigate the effects of xylitol chewing gum containing calcium hydrogen phosphate and funoran (a polysaccharide extracted from seaweed) on initial enamel lesions.*
Methods: *The clinical trial of the study was double blind. Human enamel blocks with artificial subsurface lesions were mounted in oral devices in 15 subjects. The subjects served as their own controls and were instructed to chew the three different gums, containing: (1) 79% sucrose (S), (2) 40% xylitol (X) and (3) 40% xylitol plus calcium hydrogen phosphate and funoran (X+2), 7 times daily, for a period of 1 week each respectively. Mineral content within the lesions after chewing each gum was analysed by quantitative microradiography. All data were analysed statistically using a one-way ANOVA test.*
Results: *After chewing the gums, the amount of mineral at all depths of subsurface lesion was highest in the X+2 group, followed by the X group and then the S group. The difference between the groups was statistically significant ($p < 0.01$), especially in the lesion body.*
Conclusion: *Xylitol chewing gum containing calcium hydrogen phosphate and funoran significantly enhanced the remineralisation of initial human enamel lesions* in situ.
Key words: *calcium hydrogen phosphate, enamel subsurface lesion, funoran, remineralisation, xylitol chewing gum*

Xylitol is generally considered to be non-cariogenic as it is not fermented by oral bacteria. It has even been shown to help to prevent caries in animals and humans[1-5] and several mechanisms have been proposed to explain the caries-inhibitory effects of xylitol as a total or partial dietary substitute. For example, xylitol has been found to inhibit the growth and metabolism of the mutans group of streptococci, which are the prime causative agents of tooth decay[6,7], to reduce the quantity of dental plaque[8], and to reduce the population of mutants streptococci[9-11]. Xylitol also has passive effects on salivary stimulation and effects on buffer capacity, pH, and the concentration of calcium and phosphate in saliva[12,13]. Frequent use of xylitol chewing gum or toothpaste has been shown to enhance remineralisation of incipient white spot lesions and initial artificial enamel lesions[14-17]. Therefore, xylitol is recently more and more used in confectionery and oral care products, mostly in chewing gum.

However, the mechanisms underlying the inhibitory effects of xylitol on enamel subsurface lesions are not fully understood. It has been suggested that the xylitol

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molecules may participate in the remineralisation process by acting as carriers of calcium ions[18]. It was observed that remineralisation in artificially demineralised enamel in a remineralising solution containing 20% xylitol was significantly increased compared with the remineralising solution without xylitol[19]. Based on these considerations, it is believed that xylitol chewing gum may be used as an ion-carrier to deliver calcium and possibly other additives to promote remineralisation of mineral-deficient enamel sites[20]. Furthermore, xylitol chewing gum containing calcium lactate has been shown to enhance remineralisation of subsurface enamel lesions more than the same chewing gum containing xylitol only[21]. In addition, funoran (a sulphated polysaccharide extracted from the seaweed Gloiopeltis furcata), also a possible Ca^{2+}-carrier, has been added to xylitol gum; compared with xylitol gum only *in vitro*, the xylitol+funoran gum showed more mineral deposit on an enamel surface softened caries model with no surface-layer lesion[22]. Therefore, the purpose of the present study was to investigate the effects of xylitol chewing gum containing calcium hydrogen phosphate and funoran using an enamel caries model with a typical subsurface lesion, which is more similar to clinical early caries.

Materials and Methods

Artificial enamel subsurface lesions

An artificial enamel subsurface lesion model was made on enamel blocks (3 x 3 x 2 mm) from both the buccal and lingual surfaces of premolars extracted for orthodontic reasons as described previously[23]. Briefly, a total of 50 pairs of buccal and lingual enamel blocks were sterilised for 4 h in ethylene oxide and then covered by nail varnish, but leaving the natural enamel surface uncovered. The enamel blocks were placed in a demineralisation gel containing 3.0% hydroxyethylcellulose and 0.1 mol/l lactic acid, pH 4.0 for 48 h at 40°C. Upon removal from the demineralisation gel, the enamel blocks were rinsed thoroughly with distilled water. Ten demineralised enamel blocks selected randomly were examined by microradiography to evaluate the consistency of the subsurface lesion model, while the other enamel blocks were stored at 4°C under humid conditions until used.

Intraoral appliance

Removable lower-arch acrylic appliances were constructed for right premolars and first molars. Two enamel blocks with artificial lesions were mounted on cavities prepared in the lingual surfaces of the appliance. The appliance was put into the mouth of subjects day and night in all the experimental periods. At the end of each experimental period, the tested blocks were replaced with the next two enamel blocks.

Chewing gums

Chewing gums were obtained from LOTTE Co., Ltd, Tokyo, Japan, including: (1) sucrose gum (S), which served as the control and contained 79% sucrose; (2) xylitol gum (X), containing 40% xylitol, 35% maltitol, 4% mannitol and less than 0.1% aspartame; (3) xylitol + 2 gum (X+2), which contained 40% xylitol, 35% maltitol, 4% mannitol, less than 0.1% aspartame, 0.2% calcium monohydrogen phosphate and 0.1% *Gloiopeltis furcata* extract (funoran). The gum base and other ingredients of the three gums were the same. The gums were of the pellet type and weighed approximately 1.5 g per pellet.

Subjects and clinical trials

Fifteen volunteers (7 males and 8 females, aged 20–45 years, mean age 35 years) were recruited. All the subjects provided written informed consent. An intraoral examination was performed to confirm that subjects had no untreated caries, periodontal disease, or other obvious oral diseases. The subjects received a thorough prophylaxis before starting the trial.

The study was a double-blind, randomised, cross-over design, and conducted over a 5-week period. The subjects rotated between each of the three gums. Each subject served as his or her own control, and consumed one of the three different experimental chewing gums over a period of 1 week while wearing the intraoral appliance with the artificially demineralised human enamel blocks. The three 1-week chewing periods were separated by 1-week no-gum periods to minimise the risk of carry-over effects. The subjects and the clinical investigators were not aware of the order in which the experimental gums were used. The subjects were instructed to chew one gum pellet for at least 20 min and 7 gums per day for 7 days, at the following time points: 7:00 a.m., 9:00 a.m., 11:00 a.m., 1:00 p.m., 3:00 p.m., 5:00 p.m., and before sleeping. The total chewing time was at least 16.3 h for each kind of chewing gum. The experimental appliances were worn uninterrupted for 24 h per day during the 5-week study, except for oral hygiene. The subjects were also instructed not to use fluoride-containing products and tea 1 week before the trial and during the 5-week trial.

Microradiography and analysis of remineralisation

After the intraoral period, the enamel blocks were cleaned of debris and plaque by brushing them with a hairbrush. The enamel blocks, including 10 enamel blocks serving as control, were then embedded in epoxy resin and cut into thin sections (200 μm) with a cutting machine (Isomet 1000, Buehler, USA). The sections were further reduced to a thickness of approximately 100 μm using aluminium oxide abrasive. The sections were wrapped in a moist paper tissue until examined.

The sections were microradiographed (ISR-20, Japan) with high-resolution film (Konica high resolution plate 16 SN-2 2 x 2) in a special clamping jig. The X-ray generator was operated at 10 kV and 3 mA for 20 minutes. The films were developed in Kodak D-19. The images were scanned and transmitted into computer and analysed with an Image analysis system (Image-Pro Planetron, Inc USA system). During analysis, each section was identified by a random number, so that examiners could not identify the treatment. For each scanned image, a rectangular region of interest (ROI) was decided to cover the whole lesion from the surface through the lesion to sound enamel (from 0 to 200 μm). The ROI was repeated four times in different parts of the lesion. The signal intensities of the five ROIs were then measured by computer and averaged. The results were given in the percentage of mineral contents to avoid artefacts due to the different thickness of the enamel sections. The percentage of mineral contents of each layer of the artificial lesion was obtained by giving the sound enamel underlying the lesion a value of 100%. The percentage results were analysed by one-way ANOVA.

Results

Consistency of artificial enamel lesion

Ten demineralised enamel blocks were examined with microradiography to evaluate the consistency of the artificial enamel lesions. The enamel lesions had a relatively intact surface layer (about 20 μm), and a subsurface lesion body (about 90 μm), which had substantial loss of minerals (Fig 1). There was no difference in the lesion depths and mineral contents.

Increase of mineral contents in the subsurface lesion after chewing X and X+2 gums

The effects of chewing the xylitol and xylitol plus additives gums, for 1 week, on subsurface lesions are shown in Fig 1. The relative intensities of subsurface lesions after chewing the three different gums were also measured.

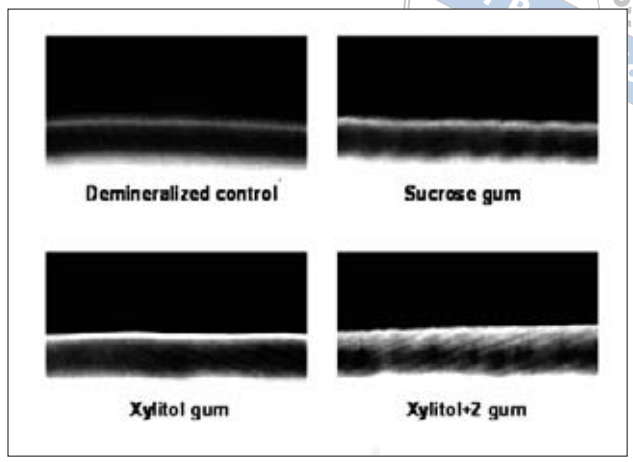

Fig 1 Representative microradiographic images of the artificial enamel subsurface lesions in demineralised control and the effects of 1-week of chewing the three kinds of gums on remineralisation of the lesions.

As shown in Fig 2A, the percentage of mineral contents at all depths of subsurface lesion was highest in X+2 group among the three groups, followed by the X group, and lowest in the S group. Compared with the sucrose group, as shown in Fig 2B, the percentage of mineral contents in surface layer (0–20 μm) was increased in the X group and significantly ($p < 0.05$) increased in the X+2 group. For remineralisation of the lesion body (20–110 μm), the mineral level was 54.03% in the X+2 group, 43.37% in the X group and 35.24% in the S group. The difference between these groups was statistically significant ($p < 0.01$).

Discussion

The present study showed that 1-week of consuming of the xylitol chewing gum or xylitol plus calcium hydrogen phosphate and funoran chewing gum could significantly increase the percentage of mineral contents of the subsurface lesions, compared with the control sucrose chewing gum (Figs 1 and 2). This indicates that consumption of xylitol chewing gum may enhance remineralisation of subsurface lesions. Furthermore, the gum containing xylitol plus calcium and funoran showed more effective remineralisation of the subsurface lesions than the gum containing xylitol only. The *in vivo* results support the observations by Miake et al[19] that xylitol-containing remineralising solution increased remineralisation of surface softened demineralised enamel *in vitro*, and also agrees with observations by Manning et al[14]. Moreover, the present subsurface demineralising model was more

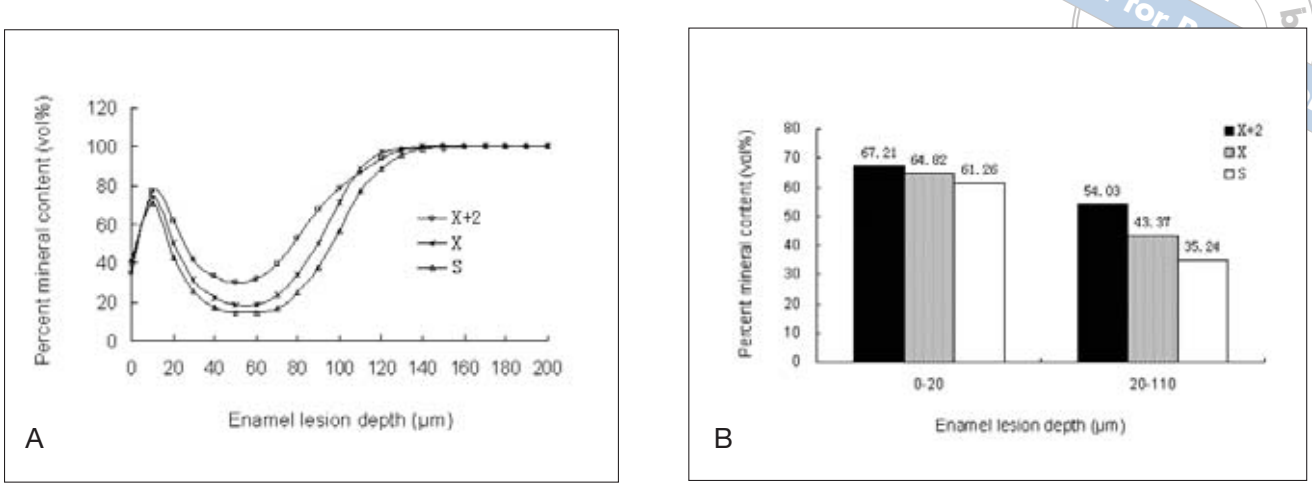

Fig 2 (A) Plots of percent mineral vs depth of enamel lesion after chewing the three kinds of gums containing (1) sucrose (S), (2) 40% xylitol (X) and (3) 40% xylitol plus calcium hydrogen phosphate and funoran (X+2).
(B) In the surface layer, the mineral level difference between X+2 and S is significant ($p < 0.05$). In the lesion body, each group is significantly different from every other group: X+2 vs S, $p < 0.01$; X+2 vs X, $p < 0.01$; X vs S, $p < 0.01$.

representative of clinical early caries than previous studies. Remineralisation occurred not only in the surface layer but also in the deeper subsurface lesion body after chewing xylitol or xylitol plus calcium and funoran. The remineralising effects of xylitol on subsurface lesions could further support the use of xylitol in chewing gum instead of sucrose. Also, the present observations of xylitol plus calcium and funoran on subsurface lesions encourage the integration of xylitol with calcium and funoran into chewing gum or oral care products. Further study is needed to evaluate whether xylitol-chewing gums containing calcium and funoran have clinical therapeutic effects on early caries or an epidemiological decrease in dental caries.

In the present study, mineral deposition also occurred in the surface layer of lesions in most of the sucrose gum chewing samples (Fig 1). Generally, it is believed that chewing sucrose gum should lead to demineralisation of enamel. In contrast, several studies have also observed that even chewing sucrose-containing gum could also to some extent enhance remineralisation of the artificial enamel lesions. Hall et al explained the phenomenon was due to the increased production of saliva from gum chewing, the increased local pH, and saturation of the local environment with respect to tooth mineral[21,24,25]. Therefore, the observation of mineral deposition occurring in the surface layer of lesions in the sucrose gum chewing samples in the present study may be caused by this mechanism.

Conclusively, the present results suggest that chewing gum containing xylitol plus calcium and funoran may be more effective in restoring initial dental caries or preventing dental caries.

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