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The effect of xylitol and fluoride on remineralization for primary tooth enamel caries *in vitro*

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Abstract The effect of fluoride and xylitol on remineralization at the early stage of the enamel caries in primary tooth was studied. The samples were divided into four groups (control, 10% xylitol, 950 ppm NaF and 10% xylitol + 950 ppm NaF) and analyzed by the using single thin section method and pH-cycling model *in vitro*. The remineralizing ratio were control −8.9%, xylitol −0.4%, NaF 8.3% and xylitol + NaF 32.4%, respectively. Xylitol + NaF group particularly showed significantly smaller ΔZ value compared with 0 days ($P<0.05$). Therefore we assume that the effect of xylitol and fluoride are additive. We concluded that xylitol and fluoride treatment to the tooth enamel may be an effective caries-preventive measure in both primary and permanent tooth enamel.

Key words
Fluoride,
Primary tooth,
Remineralization,
Xylitol

Introduction

The sugar alcohol xylitol is a pentitol used as a sugar substitute in foods, chewing gum and confections to reduce potential cariogenicity. A major aspect of xylitol is that it cannot be fermented by plaque bacteria[1]. Several reports have suggested that xylitol have the capacity to promote remineralization of early caries lesions[2–4]. It is unclear whether these reported effects are attributable to direct action by xylitol, to the removal of readily fermentable carbohydrate from the diet, or to changes in the flow or composition of saliva. On the other hand, fluoride is the most effective agent in the prevention of dental caries, and it strongly reduces enamel demineralization while F^- ions also enhance remineralization[5–7]. The presence of fluoride reduces demineralization of enamel lesions, but most likely the mechanism of action is quite different from that of xylitol. Very few papers deal with the combined action of fluoride and xylitol on remineralization in primary tooth. The purpose of this study was to investigate the effect of fluoride and xylitol on remineralization at the early stage of the enamel caries in primary tooth using single thin section method and pH-cycling model *in vitro*.

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Materials and methods

1. Tooth preparation

1-1) Sample teeth

Ten crowns of primary molars collected from dentists in Hokkaido, Japan were utilized in the present study. All teeth were stored in thymol solution. Tooth crowns, which were caries-free by visual inspection, were cleaned with detergent and deionized water, were polished for 30 seconds using 5 μm alumina suspension (Alpha polishing alumina No. 1, Buehler) in order to expose fresh enamel surface.

1-2) Thin section preparation

A few thin sections about 200 μm thick were cut from the central part of the sample teeth. Fifteen thin

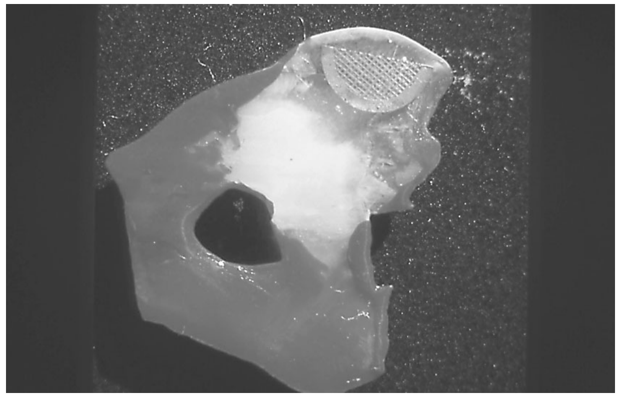

Fig. 1 Thin section sample with semicircular copper grid

Table 1 Chemical composition of demineralization and remineralization solutions

	Demineralization	Remineralization
$CaCl_2$	3 mM	3 mM
KH_2PO_4	10 mM	10 mM
$CH_3CH(OH)COOH$	50 mM	—
CH_3COOH	100 mM	100 mM
NaCl	100 mM	100 mM
pH	4.5	6.5

sections which were made from five teeth were utilized in this study. They were subsequently ground down to planoparallel thin sections (about 100μm thick) on wet abrasive paper (#300, #800, Matsunaga stone, Japan). Sample teeth sections were coated with light cured resin (Scotch bond 7533L, 7533R, Vitremer 3303FG, 3M Tokyo) with one window approximately 3 mm$\times$100μm of exposed enamel left on a surface in order to reinforce the thin samples. As shown in Fig. 1, a semicircular copper grid (Copper finder grids, Funakoshi, Japan) was attached with same resin mentioned above, on the thin section sample about 700–800μm depth from enamel surface in order to measure on same region in contact microradiogram.

2. Demineralization

In order to form the artificial enamel carious lesions, the sound thin section samples were immersed at 37°C and pH 4.5 for 1 week in a demineralizing solution (as shown in Table 1). The thin section samples which were made the artificial enamel carious lesions about 100μm from enamel surface, were utilized in this study.

3. pH-cycling

The pH-cycling model was used to produce lesions with a demineralization challenge and remineralization period alternating daily. The test regimen in each 24-hour period proceeded as follows:

(1) The samples were immersed for 5 minutes in a treatment solution which were (a) double-deionized water as control (b) 10% xylitol (c) 950 ppm NaF (d) 10% xylitol + 950 ppm NaF.
(2) The samples underwent 4 hours of demineralization at 37°C in a demineralization solution.
(3) The samples were removed from solution and thoroughly rinsed in double-deionized water.
(4) The samples were retreated for 5 minutes in a same treatment solution mentioned above.
(5) The samples were then immersed in a remineralization solution for 20 hours at 37°C.
(6) When the samples were removed from the remineralizing solution, they were rinsed in double-deionized water, and the pH-cycling regime was repeated for 14 days and nights of alternating

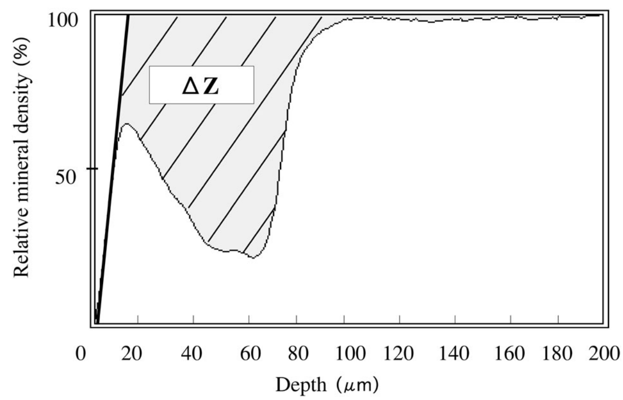

Fig. 2 Schematic mineral distribution and parameter assessed ΔZ (mineral loss value: vol% μm)

Table 2 Changes of ΔZ values in before and after pH-cycling

	n	0 days	7 days	14 days	Remineralizing ratio
(a) Control	5	102,298 ± 25,764	84,050 ± 22,685	111,401 ± 20,397	−8.9%
(b) Xylitol	3	103,946 ± 18,552	84,435 ± 21,355	104,358 ± 12,651	−0.4%
(c) NaF	4	98,162 ± 12,914	82,142 ± 17,250	90,060 ± 16,997	8.3%
(d) Xylitol + NaF	3	102,870 ± 22,532	76,475 ± 11,722	69,502 ± 17,315*	32.4%

*$P<0.05$

demineralization and remineralization treatment, as described above.

4. Assessment of demineralization/ remineralization

Contact microradiograms (CMR) were made to determine the mineral content of (1) sound enamel (2) carious enamel before pH-cycling (0 days). (3) Carious enamel after pH-cycling for 1 week (7 days). (4) Carious enamel after pH-cycling for 2 weeks (14 days). CMR of all samples were made together with an aluminum stepwedge on high sensitivity X-ray film (SRO-343, Kodac) using and X-ray generator (SOFRON, SRO-450C) at 15 kV and 15 mA for 30 minutes. The X-ray film was developed and fixed, then the 750 times magnified image was taken from it. Mineral profile curve, which was scanned from surface enamel to inner enamel on the magnified image, were measured by using image the analytical computer program (NIH image. v1.61). ΔZ, being the parameter of relative mineral loss of each lesion, were calculated from difference between ΔZ for sound enamel and ΔZ for carious enamel after pH-cycling. This parameter is schematically illustrated in Fig. 2. The degree of remineralization was evaluated by the changes of ΔZ.

The differences in average of ΔZ in each group and day were statistically analyzed by Mann-Whitney U-test.

Results

Table 2 summarize the results of the control group and treatment groups. The data are presented as means ± standard deviation of each group for the relative mineral loss (ΔZ) values. Remineralizing ratio is calculated as follows:

$$\text{Remineralizing ratio} = \{\Delta Z(0\text{ days}) - \Delta Z(14\text{ days})\}/\Delta Z(0\text{ days}) \times 100$$

As shown in Table 2, remineralizing ratio were control −8.9%, xylitol −0.4%, NaF 8.3% and xylitol + NaF 32.4%, respectively. ΔZ values between 0 days and 14 days did not differ significantly in groups of control, xylitol and NaF. Control group showed a slight progress toward demineralization, whereas NaF and xylitol+NaF groups showed tendency a trendtoward remineralization. Xylitol+NaF group particularly showed significantly smaller ΔZ compared with 0 days ($P<0.05$).

Discussion

Currently early caries lesions or precavitated lesions “so called white spot lesions” are more prevalent in the clinic of pediatric dentistry. This has been confirmed by recent epidemiological studies[8–9], although supporting evidence has been available for a long time[10]. Therefore the concept that remineralization can repair damage caused by demineralization is indispensable importance for the management of preventive care in the pediatric dentistry. Various managements are used in order to promote the enamel remineralization, *e.g.*, local fluoride application, professional mechanical tooth cleaning and use of sugar substitution recently. A large number of studies have been made on the human permanent tooth[1,4,7,11], little is known about effect of fluoride and xylitol on the primary tooth. For that reason, in this study, an attempt was made to use of fluoride and xylitol to treat primary tooth enamel caries using single thin section method and pH-cycling model *in vitro* as a preliminary study.

The technique employed in this study showed several advantages for single section use in de/remineralization experiments. Main advantages were (1) it is able to get several sample sections from one tooth. (2) Using same origin sample is able to avoid the influence the individual difference. (3) As the sample sections were not destroyed, it is able to make quantitative microradiographical evaluation possible repeatedly[12].

The study of sucrose substitutes during the past 30 years has clearly established that the group of sugar alcohols has turned out to be very interesting from the dental point of view. One member of this family of natural carbohydrates, xylitol, was shown to be especially effective in the prevention of caries[11]. Several long-term human clinical trials, some of them conducted under the auspices of the World Health Organization, have demonstrated the efficacy of xylitol in the prevention of caries in children and in young adults[11]. For effective caries prevention by xylitol, it is not necessary to replace sucrose totally in the diet. Several studies have shown that relatively small daily doses of xylitol can provide as effective protection against caries as full substitution of sucrose. Such impressive caries-reducing effects indicate the existence of a specific mechanism. Although, several chemical details of the mechanism are still unknown, the xylitol effect can be explained as the concerted involvement of three separate phenomena: (1) salivary effects; (2) microbiological effects; (3) bioinorganic effects. Because it is sweet, xylitol stimulates the flow rate of saliva and the secretion of and/or formation of bicarbonate ions, the most important buffer of saliva. And xylitol is not utilized by the cariogenic microorganisms to any significant extent. On the contrary, several studies have shown that the growth of *S. mutans* can be inhibited by the xylitol[1,11]. In this study, xylitol group did not show the tendency of not only demineralization but also remineralization. A possible explanation of this phenomenon may be that the *in vitro* experiment system without saliva, microorgans and plaque was employed in the present study. It is suggested that saliva, microorgans and plaque have important roles in progress of remineralization by xylitol.

As shown in Table 2, remineralizing ratio were control −8.9%, xylitol −0.4%, NaF 8.3% and xylitol+NaF 32.4%, respectively. NaF and xylitol+NaF groups showed tendency a trendtoward remineralization. Xylitol+NaF group particularly showed significantly smaller ΔZ value compared with 0 days ($P<0.05$). These results are in accord with the findings of Arends *et al.*[13] Therefore we assume that the interaction between xylitol and fluoride is synergistic effect. Fluoride ions in solutions most likely affect the dissolution rate of enamel mineral or form fluorapatite-like precipitates[14]. On the other hand, xylitol interaction with the enamel mineral can be explained by the ion-solvent interaction between Ca^{2+} and xylitol. Xylitol is known to penetrate into demineralized enamel[15]. Xylitol forms weak complex or chelates with Ca^{2+} [15]. Such complex may play a role in the overall control of calcium utilization in caries lesions. Arends *et al.*[13] describe the interaction between xylitol and Ca^{2+} ions as in a high xylitol situation the Ca^{2+} ions are not only hydrated by water but partly solvated by xylitol molecules. Because hydrogen is a more electronegative radical than carbon-hydrogen groups, one may expect the hydroxyl groups in xylitol to be more effective than water in solvating particularly positive ions. It is presumed that the above effect lower the diffusion coefficient of Ca^{2+} ions out of the demineralizing enamel.

Sakurai *et al.*[7] reported the effect of xylitol and fluoride on mineralization on the permanent tooth at same conditions of this study and they showed the remineralizing ratio of −5.1%, −35.9%, 48.4% and 59.5% for control, xylitol, NaF and the xylitol + NaF respectively. These results agree with our findings except xylitol and fluoride groups. It is possible, therefore, that xylitol and fluoride treatment to the tooth enamel may be an effective caries-preventive measure in both primary and permanent tooth enamel. But, we have only limited information on the interaction between xylitol and fluoride for primary tooth enamel. Further studies will be necessary to understand about the cariostatic effects by xylitol for the primary tooth enamel.

In conclusion, this *in vitro* model study utilized primary tooth showed that (1) remineralizing ratio were control −8.9%, xylitol −0.4%, NaF 8.3% and xylitol + NaF 32.4%, respectively, and (2) the interaction between xylitol and fluoride is synergistic effect.

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