Combination Concentration Effects of Calcium Hydrogenphosphate on Human Enamel Remineralization by Xylitol and Funoran

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Abstract

The combination of xylitol, funoran and calcium hydrogenphosphate have been shown to be effective in enhancing remineralization of human enamel. The aim of this research was to evaluate the effect of calcium hydrogenphosphate concentration combined on remineralization process by xylitol and funoran.

Remineralization solutions containing xylitol, funoran, and either four concentrations of calcium hydrogenphosphate were prepared. The initial caries-like enamel lesions were artificially made by demineralizing human enamel slabs in demineralizing solution (pH 4.7) for 3 days. Specimens immersed in the remineralizing solution containing of each concentration of calcium hydrogenphosphate, xylitol, and funoran at 37oC for 2 weeks. The degree of remineralization was evaluated by using Contact Microradiography with a Cu(Kα) x-ray source. Calcium hydrogenphosphate enhanced concentration-dependently the remineralization by xylitol and funoran in human enamel but there was no statistically different between the different concentration of calcium hydrogenphosphate.

It was confirmed that calcium hydrogenphosphate tend to enhance concentration-dependently the remineralization by xylitol and funoran. It can be concluded that the combination of xylitol, funoran and calcium hydrogenphosphate in appropriate concentration is effective to enhance remineralization process of initial human enamel caries lesions.

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Introduction

Xylitol is known to be effective as a non-cariogenic sugar substitute because it is not fermented by most oral microorganisms.[1] Furthermore, xylitol has been reported to enhance the remineralization of initial caries lesions by acting as a Ca-carrier, owing to its ability to form xylitol-Ca complex.[2-5]

Funoran is a high molecular weight sulfated polysaccharide composed of D-galactose and 3,6-anhydro-L-galactose. It is reported that funoran can strongly inhibit the adsorption of oral bacteria to saliva-coated hydroxyapatite (S-HA) beads as experimental salivary pellicle and had a strong desorptive activity against mutans streptococci preadsorbed to S-HA.[6-9] Funoran has been also reported to enhance remineralization, because it can bind with Ca^{2+} ions, and it is therefore expected to act as a Ca^{2+}-carrier.[10-12]

Calcium hydrogenphosphate ($CaHPO_4 \cdot nH_2O$; n=0-2) is a white powder without any smell or taste. It is thought that calcium hydrogenphosphate can provide a source of calcium and phosphate.[10,13] One of the chewing gum product marketed in Indonesia is chewing gum containing xylitol, funoran and calcium hydrogenphosphate.[14-16]

The aim of this study was to evaluate the concentration effect of calcium hydrogenphosphate combined on remineralization process of the subsurface

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enamel lesion or early caries by xylitol and funoran.

Materials and methods

Specimen preparation

The teeth used in this study were obtained under an informed consent protocol approved by the Faculty of Dentistry's Ethics Committee at the Faculty of Dentistry Universitas Indonesia (Number 102/Ethical Clearance/FKGUI/XI/2013). Fourteen mandibulary third molars without defects extracted from humans in order to treat wisdom tooth pericoronitis were sectioned into fifty six enamel slabs in mesio-distal and bucco-lingual direction. The outer enamel surface was done with 2000 grade in order to obtain a flat surface. A rectangular window (3 x 4 mm) test area was obtained on the center of flat enamel. One of the short side rectangular windows marked as a demineralization section. Each specimen was covered with sticky wax except for a rectangular window.

Demineralization and remineralization solution preparation

Demineralization solution contained carboxymethyl-cellulose sodium 10g, lactic acid 8.26 ml, and 5 mM hydroxyapatite 60 ml in 1000 ml distilled water. The pH adjusted to 4.7 with 5N NaOH. Remineralization solution contained carboxymethyl-cellulose sodium 10g, NaCl 8.78g, 5mM Hydroxyapatite 60ml, HEPES 4.76g, and 1% Thymol-EtOH 10ml in 1000ml distilled water. The pH adjusted to 7.0 with 5N NaOH.

Experiment materials

The experiment materials were the combination of xylitol, maltitol, funoran, and calcium hydrogenphosphate as showed in Table1.

Ingredient	Composition			
	I	II	III	IV
Xylitol	4.1	4.1	4.1	4.1
Maltitol	3.3	3.3	3.3	3.3
Funoran	0.01	0.01	0.01	0.01
Calcium hydrogenphosphate	0.0	0.01	0.02	0.05

Table 1. The Composition of remineralization materials (grams).

The fourth compositions were divided into group A, B, C, and D in a blind experiment method. The ingredients in each group diluted in 100 ml remineralization solution and use for remineralization process.

Demineralization and remineralization procedures

Fifty-six specimens with rectangular window were immersed in demineralization solution and stored at 37°C for 3 days. The demineralized specimens were washed gently with distillate water and dried in air. One half of window in demineralized specimen covered with sticky wax. All specimens divided in 4 group (n = 14), then immersed in 100 ml remineralization solution containing of each experiment material (group A, B, C, or D) at 37°C for 2 weeks. This remineralization solution for each group was change once every 2 days with a new fresh solution.

Contact microradiography (CMR) analysis

Wax removal

Each specimen was immersed in xylene and stored in a thermoregulator of 50°C for 40 minutes to dilute the sticky wax until all sticky wax remove from the specimen surface. All specimens then dehydrated gradually by immerse in 70% ethanol, 80% ethanol and 90% ethanol for 15 minutes, and then 100% ethanol twice for 1 hour.

Embedding

Pretreatment was done by immersing the specimen in styrene for 1 hour and follow by immersing in combination of styrene and Rigolac resin for 1 night. Then each specimen was embedded with Rigolac resin in a thermoregulator of 60 °C for 3 days.

Specimen preparation

Both side of the embedded specimen was cut parallel to the long side window using a diamond wire cutter. Demineralization section and middle of specimen marked with a pen marker as a guideline in grinding the specimen. The specimen was grinded with 2000 grade silicon carbide sandpaper to obtain 100 micrometer thickness.

Contact micro-radiography processing

The prepared specimens were placed directly on a X-ray film (PXHW Konica Minolta) adjacent to an 15-aluminum step-wedge (20 μm per step). The specimen was then placed in the center of x-ray source cabinet (Softex CMR-2, Tokyo, Japan) and exposed to Cu-Kα radiation through Ni filter for 10 minutes at a voltage of 17kV, a current of 3 mA, and a film focus distance of 100 mm. Exposed film was developed in developer

solution for 5 minutes at 20°C, rinsed with water, immersed in fixating solution for 5 minutes at 20°C, and then washed for 10 minutes in flowing tap water. The washed film was then air dried in room temperature.

Image and data analysis

Image analysis of specimen was carried out by aluminum stepwedge guidance. The analysis was done in 0-300 μm depth and 50 μm width of demineralization or remineralization areas each one at three sites. The vol% mineral profile of each group's enamel demineralized and remineralized areas was assessed for lesion depth (Ld; μm), mineral loss (ΔZ, vol%·μm) and maximum mineral value (Vmax; vol%)

Statistical analysis

The comparisons between 4 groups were analyzed using 1-way analysis of variance (ANOVA) and Tuckey test.

Results

Typical CMR images of enamel subsurface lesions after demineralization of all specimens represented in Figure 1. The images after treated with experiment material group A, B, C, and D. were shown in Figure 2.

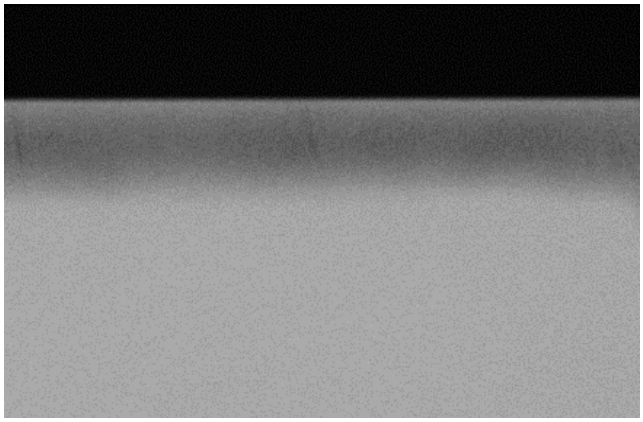
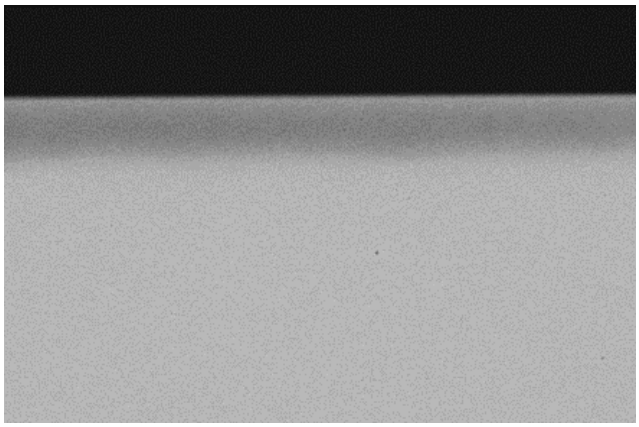
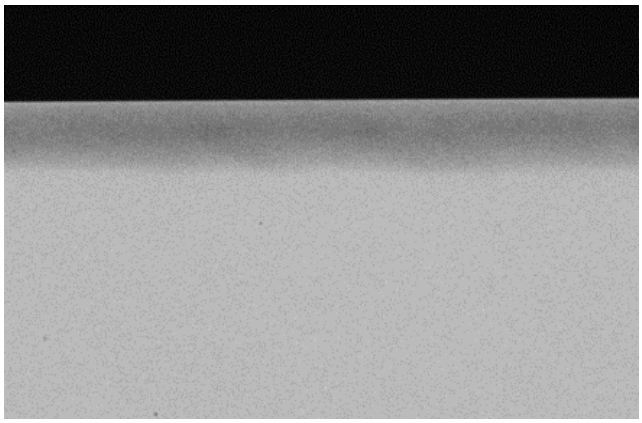


Figure 1. Typical CMR images of the enamel subsurface lesions after demineralization.

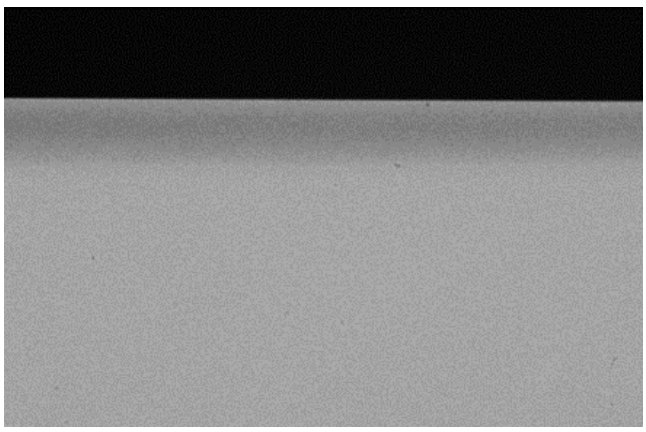
In group A, remineralization occured in the middle and the bottom of subsurface layer which the width of demineralized layer decreased, and the radioluscency image slightly decreased (Figure 2a). In group B, remineralization slightly occur in the middle and the bottom of the subsurface layer. The width of demineralized layer only slightly decreased. The radioluscency image was very slightly decreased (Figure 2b). In group C, remineralization occur in the middle and the bottom of the subsurface layer. The width of demineralized layer were decreased. The radioluscency image was decreased (Figure 2c). In group D, remineralization mostly occur on the outer, middle and at the bottom of the subsurface layer. The width of demineralized layer were highly decreased. The radioluscency image was highly decreased (Figure 2d).



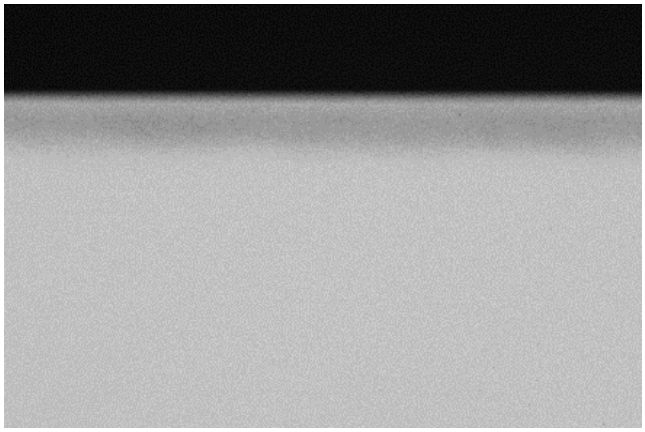
a. Group A



b. Group B



c. Group C



d. Group D

Figure 2. Typical CMR images of the enamel subsurface lesions after remineralization with material experiment in each group A, B, C, and D.

Mineral Loss (ΔZ) and Lesion depth (Ld) of human enamel subsurface lesions treated by 4 groups were shown in Table 2.

Group	Demineralization		Remineralization		Percent Remineralization
	ΔZ (vol%)	Ld (μm)	ΔZ (vol%)	Ld (μm)	
A	2881.0 ± 493.4	77.4 ± 7.5	2182.9 ± 473.6	67.2 ± 6.6	24.5 ± 6.4
B	2638.9 ± 635.5	75.3 ± 15.4	2122.8 ± 531.6	66.6 ± 13.3	19.7 ± 5.2
C	2456.5 ± 535.0	73.7 ± 7.5	1889.1 ± 548.1	64.1 ± 9.4	23.8 ± 11.5
D	2313.0 ± 502.4	70.2 ± 10.8	1709.0 ± 471.0	61.0 ± 13.2	26.6 ± 9.0

Table 2. Mineral Loss (ΔZ) and Lesion depth (Ld) of Human Enamel Subsurface Lesions Treated by 4 groups.

It was shown that remineralization occured in all demineralized enamel, although the mineral intake was not the same as mineral loss as in the demineralization process which was shown by the reduction of the lesion depth (Ld) in all groups as shown in Figure 2. and Table 2. The highest mineral intake was in group D and the lowest mineral intake was in group B.

After completing the data analysis above, the blind method was opened and the calcium hydrogenphosphate concentration of the remineralization solution in the experiment groups were revealed as shown in Table 3.

Ingredient	Group A	Group B	Group C	Group D
Xylitol	4.1	4.1	4.1	4.1
Maltitol	3.3	3.3	3.3	3.3
Funoran	0.01	0.01	0.01	0.01
Calcium hydrogenphosphate	0.02	0.0	0,01	0.05

Table 3. The Composition in experiment group (grams).

Remineralization also occured in demineralized enamel which were immersed in remineralization solution without calcium hydrogenphosphate (group B). The mineral intake percentage in the demineralized enamel was in line with the increase of calcium hydrogenphosphate concentration in the remineralization solution. The remineralization ratio of all groups were shown in Figure 3. Statistically, there was significant different of percent remineralization between all groups (p=.047).

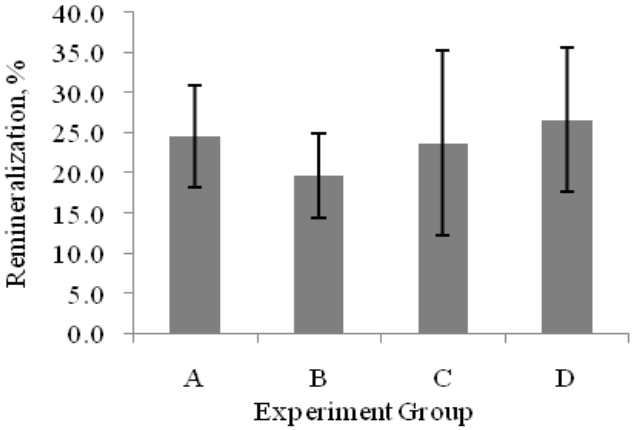


Figure 3. Remineralization ratio between experiment groups.

Discussion

In this study, CMR images showed that the enamel specimens demineralized homogenously with lesion depth of 70.2 – 77.4 □ m from the outer surface. These demineralized specimens showed the state of the clinical subsurface lesion of early caries which is marked as a white spot.

After immersion for 2 weeks in the remineralization solution containing different concentration of xylitol, funoran, and calcium hydrogenphosphate, the remineralization occured in all groups including immersion in the remineralization solution without calcium hydrogenphosphate. The remineralization effect of group B not containing calcium hydrogenphosphate was considered to be that of xylitol and funoran effect in the remineralization solution. Specimens in group B intaked only Ca mineral from hydroxyapatite in the remineralization solution that were showed as the lowest decrease in lesion depth. On the other hand, specimens of group D have higher

remineralization causing from the higher concentration of calcium hydrogenphosphate in its remineralization solution. It has been reported that 10% xylitol enhanced the remineralization of initial caries-like enamel lesion, and that the crystals in the demineralized enamel were repaired with application of remineralizing solution containing xylitol in making observation by transmission electron microscope.[2] It has been reported that xylitol forms various complexes with Ca^{2+} ions and will act as a Ca^{2+} ion carrier.[3-5] Xylitol as a sugar alcohol can not be fermented and used as energy by bacteria to grow and reproduce so that remineralization process can proceed without interference. It was reported that xylitol modifies the synthesis of polysaccharides from sucrose in *S. mutans* thereby decreasing the ability of the cells to adhere to hard surfaces.[17] It was thought that funoran combines with Ca^{2+} ions and that it also might act as a Ca^{2+} ion carrier.[11,12]

It is found that remineralization by funoran was restricted to surface layers because the size of its macromolecule prevents it from entering initial caries enamel lesions.[10] It has been also reported that funoran inhibits the adsorption of mutans streptococci to the surface of teeth and that it releases cariogenic mutans streptococci pre-adsorbed to teeth.[6-9] It is possible that funoran indirectly enhanced remineralization by these actions. Previous study investigated that regular chewing funoran-containing xylitol gum can significantly reduce the dental plaque volume and caries pathogen counts in the oral cavity.[18] It was also reported that chewing of xylitol gum over a long period may lead to decreased *gtfB gene* expression, which can negatively affect the synthesis of extracellular polysaccharides by *S. mutans,*and reduce the size and growth of *S. mutans* colonies resulting change of morphology.[17,19] It was suggested that the use of funoran-containing xylitol chewing gum may be useful for caries control. However, previous study had shown that there was no beneficial longterm effects of maternal xylitol gum exposure on their children's dental health when compared with gums containing chlorhexidine and fluoride.20

On the other hand, calcium hydrogenphosphate can act as a source of both calcium and phosphate, thereby, enabling remineralization.10,14 It was confirmed that calcium hydrogenphosphate tend to enhance concentration-dependently the remineralization by xylitol and funoran, but the remineralization rate will reach at the plateau as the concentration of calcium hydrogenphosphate rises.

Conclusions

It can be concluded that adding calcium hydrogenphosphate in appropriate concentration in combination with xylitol and funoran to chewing gum is effective to enhance remineralization of initial human enamel caries lesions.

Acknowledgements

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Declaration of Interest

The authors report no conflict of interest.

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