EFFICACY OF FLUORIDE MOUTHRINSE CONTAINING TRICALCIUM PHOSPHATE ON PRIMARY ENAMEL LESIONS : A POLARIZED LIGHT MICROSCOPIC STUDY

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Abstract. The aim of this study was to evaluate the effect of fluoride mouthrinse containing tricalcium phosphate (TCP) on remineralization of primary teeth enamel lesions compared with fluoride mouthrinse alone to determine if the addition of TCP gives additional benefit. Thirty-six sound primary incisors were immersed in a demineralizing solution (pH 4.4) for 96 hours at 37°C to create demineralized lesions. After artificial caries formation, the specimens were randomly assigned to one of three groups (n=12): Group A: deionized water; Group B: 0.05% sodium fluoride (NaF) plus 20 ppm tricalcium phosphate mouthrinse and Group C: 0.05% sodium fluoride (NaF) only mouthrinse. A pH-cycling process was carried out for 7 days at 37°C. During pH-cycing, all the specimens were immersed for 1 minute; 3 times a day, in the respective mouthrinse. The specimens were then evaluated by polarized light microscopy with the computerized Image Pro Plus program. Data were analyzed using paired-t, one-way ANOVA and Tukey's multiple comparison tests at a 95% level of confidence. The depth of the lesions were significantly different between pre- and post-treatment for all groups (p=0.00). The lesion depth in the Group A (control) increased by 102%(±15), in Group B by 34%(±12) and Group C by 36%(±9). The lesion depths differed significantly between the control (Group A) and treatment groups (Group B,C) (p<0.05). Group A had a significantly greater increase in lesion depth compared to the other groups. There was no significant difference in the percent change in lesion depths between Groups B and C. We concluded that the fluoride mouthrinse containing tricalcium phosphate provides no additional benefit over the mouthrinse containing fluoride alone.

Keywords: fluoride mouhtrinse, polarized light microscopy, primary enamel, remineralization, tricalcium phosphate

INTRODUCTION

Dental caries are major public health problem among children in Thailand (Sutthavong *et al*, 2010). The current concept for treating dental caries is inhibition of demineralization and promotion of remineralization (Bansal *et al*, 2010). Detection

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and treatment of dental caries during the early stages is important to avoid loss of minerals and prevent the lesion from becoming cavitated. Early caries diagnosis allows the lesion to be treated by applying a remineralizing agent (Puig-Silla *et al*, 2009). With more than 50 years of clinical success, the application of fluoride is the gold standard for preventing dental caries. Fluoride mouthrinse is one of the most widely used forms of self-administrated caries prevention agents and is supported by dental research and accepted by practicing professionals worldwide (Marinho *et al*, 2003).

Besides fluoride, calcium-phosphate compounds have been reported to be effective for remineralization (Karlinsey and Pfarrer, 2012). Several studies claimed synergistic behavior between calcium-phosphate and fluoride which can lead to better remineralization (Karlinsey *et al*, 2009a; Mathews *et al*, 2012). Calcium-phosphate should not interfere with the action of fluoride and should enhance the fluoride's remineralization activity (Karlinsey *et al*, 2009a).

Recently, a preparation that combines fluoride and tricalcium phosphate (TCP) was introduced (Karlinsey *et al*, 2009b). This combination has been found to decrease caries in randomized controlled clinical trials (Karlinsey *et al*, 2010; Shen *et al*, 2011) but no one has studied the remineralization potential of fluoride and TCP containing mouthrinse on primary enamel lesions using polarized light microscopy. Thai mouthrinse developed by Mahidol University was expected to enhance the effectiveness of fluoride mouthrinse by adding TCP and is hoped to better enhance remineralization than mouthrinse containing fluoride only. It was developed to be a substitute for commercial products to provide a cost effective alternative, especially for school-based preventive programs.

The aim of this *in vitro* study was to evaluate the effectiveness of this fluoride and TCP containing mouthrinse on remineralization of caries-like lesions on primary enamel using polarized light microscopy and compare it with a fluoride only mouthrinse and a control.

MATERIALS AND METHODS

Specimen preparation

This study was approved by the Ethics Committee of Mahidol University. Thirty-six sound human primary incisors were obtained and stored in normal saline at room temperature until use. The teeth were coated with two layers of acid resistant nail varnish, leaving two square windows on each tooth of approximately 1x1 mm on an intact labial surface. The root apices were sealed with sticky wax. The teeth were then immersed in deionized water until use.

Demineralizing and remineralizing solution preparation

Two demineralizing solutions (D1, D2) and one remineralization solution (R) were prepared. D1 solution was used for the subsurface enamel demineralization test. The D2 and R solutions were used to simulate the supersaturation of apatitic mineral found in saliva. D1 consisted of 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, 0.05 M acetic acid and the pH was adjusted to 4.4 using 1M KOH. D2 consisted of the same components as D1, but the pH was adjusted to 4.7 using 1M KOH. R consisted of 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, and 0.15 M KCl and the pH was adjusted to 7.0 with 1 M KOH (Thaveesangpanich *et al*, 2005a). The demineralizing and remineralizing solutions were freshly prepared for each pH-cycling procedure.

Artificial caries lesion formation

Each tooth was immersed in 3 ml of D1 and incubated at 37°C (Sheldon Manufacturing, model 1545, Cornelius, OR) for 4 days to produce carious lesions 60-100 μm deep (Thaveesangpanich *et al*, 2005a). The teeth were rinsed with 15 ml deionized water and were then immersed in artificial saliva composed of 0.65 grams per liter KCl (British Pharmacopoeia, BP, Norwrick, UK), 0.058 g/l $MgCl_2$ (British Pharmacopoeia), 0.165 g/l $CaCl_2$ (British Pharmacopoeia), $K_2(HPO_4)_2$ (Pharmacopoeia, Rockville, MA), $KH_2(PO_4)_3$ (British Pharmacopoeia), 2 g/l $NaCO_2CH_3$ cellulose (Pharmacopoeia) and deionized water to make 1 liter as modified from Amaechi *et al* (1999) until use.

Grouping

After artificial carious lesion formation, one of two windows in each tooth (pre-treatment window) was coated with two layers of acid resistant nail varnish. The specimens were then ramdomly assigned into one (n=12) of three groups: Group A (control), deionized water; Group B, 0.05% sodium fluoride plus 20 ppm tricalcium phosphate; Group C, 0.05% sodium fluoride.

pH-cycling

The pH-cycling was conducted to imitate changes in the pH of the oral environment for seven days. The pH-cycling involved three hours of D2 solution twice daily, with two hours of R solution between each demineralizing procedure (Yimcharoen *et al*, 2011). The specimens were then immersed in R solution overnight at 37°C in a controlled environment incubator shaker (series25 incubator shaker at 150 rpm) (Series 25 Incubactor Shaker®, Ramsey, MN). After the week of pH-cycling, the nail varnish was carefully removed with acetone.

Thin specimen preparation

All the specimens were sectioned longitudinally through the lesion in the labio-lingual plane using a slow speed diamond saw with copious water spray (Accutom-50, Struers, Ballerup, Denmark) to create a thin section (approximately 400 μm thick). The sections were then ground with wet silicon carbide paper (800 and 1000 grit) until 100-150 μm thick as measured by an electric digital caliper (Mitutoyo® model CD-6C, Kanagawa, Japan).

Polarized light microscopy

A polarized light microscope (Nikon, model Eclipse E400 pol, Tokyo, Japan) at 10x magnification was used to measure lesion depth at 3 locations and photomicrographs were taken and analyzed using Image-Pro Plus Program (Media Cybernetics, Bethesda, MD). The microscopist was blinded to the treatment groups.

Intra-examination reliability

Seven sections (20% of the total sections) were re-examined by the same examiner under the same conditions using the same equipment. The intra-examination reliability was measured using the Pearson's correlation coefficient.

Statistical analysis

The mean and standard deviation lesion depths were calculated for each group. The paired-t test was used to compare the mean lesion depths pre- and post-treatment within groups. The one-way analysis of variance (ANOVA) and Tukey's multiple comparison test were used to compare differences in lesion depth and percent changes among the groups. Significance was set at $p<0.05$. Statistical analysis was performed using SPSS for Windows, version 20 (IBM, Armonk, NY).

RESULTS

The means and standard deviations

Table 1
The mean and standard deviation of lesion depth and percent change.

Group	Treatment	Mean lesion depth ± SD (μm)		% change
		Pre-treatment	Post-treatment	
A	Deionized water	88.30 ± 9.91	178.03 ± 9.93*	102.16 ± 15.12[a]
B	0.05%NaF plus 20ppm TCP	90.02 ± 7.09	120.55 ± 8.04*	34.52 ± 11.76[b]
C	0.05%NaF	89.29 ± 5.78	121.60 ± 10.58*	36.20 ± 8.60[b]

* shows significant difference pre- and post-treatment within groups (pair-*t* test) $p<0.05$.
Different letters indicate statistically significant differences among groups ($p< 0.05$, ANOVA, Tukey's test).

(SD) for lesion depth in all groups are shown in Table 1. The mean (±SD) baseline lesion depth for all 3 study groups ranged from 88.30(±9.91) μm to 90.02(±7.09) μm and there were not significant differences among the groups (p=0.864).

The mean (±SD) lesion depths post-treatment for the 3 study groups ranged from 120.55 (±8.04) μm to 178.03(±9.93) μm, all significantly greater (p=0.000) than baseline and the treatment groups were both significantly less than the control (p=0.000).

The percent changes for each group are shown in Table 1. The percent increase in lesion depth from baseline was significantly different for each group ($p<0.05$). Group A (control) was significantly deeper than Groups B and C (treatment groups); however there was no significant difference in percent change between group B and C (p=0.939). These findings showed no advantage of the fluoride plus TCP mouthrinse over the fluoride mouthrinse alone (Fig 1).

Results from duplicate examination showed the intra-examination reliability of the lesion depth as tested by Pearson's correlation coefficients was 0.914, which shows good reliability.

DISCUSSION

In the present study, an *in vitro* 7 days pH-cycling model was used to evaluate and compare the remineralization effect of fluoride alone versus fluoride with TCP mouthrinse using polarized light microscopy. Several studies have demonstrated the remineralization effect of fluoride with TCP products (such as toothpaste, cream, mouth rinse) on artificial caries lesion similar to this study (Rirattanapong *et al*, 2010; 2012). But none of these studies compared the demineralization and remineralization effect of fluoride mouthrinse containing TCP on primary teeth enamel lesions. For this study, all teeth were prepared with two 1x1 mm square windows on the labial surface. The advantage of this design is the depth of the artificial enamel lesion can be determined for any tooth at baseline and then compared with lesion post-treatment. The baseline lesion depths in our study are similar to those previous studies by Rirattanapong *et al* (2014) and Yimcharoen *et al* (2011). This technique was designed to minimize variation in initial lesion depth among the specimens. The results showed no significant differences in mean baseline lesion depth among the groups.

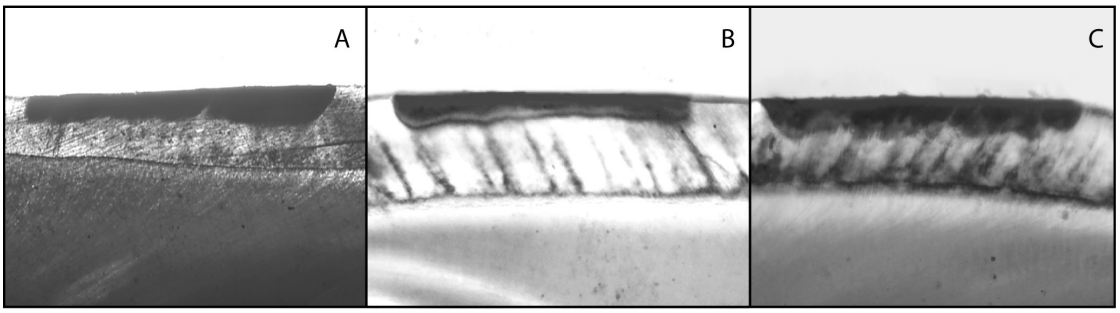

Fig 1–Polarized light photomicrograph at 10x magnification of lesion after treatment with 7 days pH-cycling: deionized water (control) group (A), 0.05%NaF plus TCP group (B) and 0.05%NaF group (C).

Our findings show fluoride mouthrinse significantly reduces further progression of caries lesions in primary enamel lesion. Both treatment groups (B and C) in our study reduce further progression of demineralization in primary teeth enamel lesions but neither one caused remineralization of those lesions. This could be due to the severity of the pH-cycling process on primary teeth. There are many possible explanations for this findings: thin enamel layer, low mineral content, high organic content and variations in the structure of the surface which could influence caries susceptibility in primary teeth enamel lesions (Thaveesangpanich *et al*, 2005b; Yimcharoen *et al*, 2011).

Fluoride is not the sole agent used for remineralization. TCP has been shown to have remineralizing effects *in vitro* and *in vivo* (Karlinsey *et al*, 2009a; Mathews *et al*, 2012). The remineralizing effect of fluoride plus TCP in mouthrinse has been reported by Karlinsey *et al* (2009b) and Amaechi *et al* (2010); however we did not find an advantage by adding TCP to fluoride in our study. One reason might be the high level of fluoride in our treatment groups. The concentration of fluoride (500 ppmF) in our study was higher than that used by Amaechi *et al* (2010) and Karlinsey *et al* (2009b). TCP has a low solubility, particularly in the presence of fluoride ions; insoluble TCP is not easily applied and does not localize effectively on the tooth surface and requires acid for solubility to produce ions capable of diffusing into enamel subsurface lesions (Reynolds, 2008). A lower fluoride concentration used with TCP might be more effective than a higher fluoride concentration. The fluoride plus TCP mouthrinse used in our study was not more beneficial than the fluoride only mouthrinse. Other reasons for why our study findings were different from other studies could be differences in study designs, different types of teeth used or methods of assessing remineralizing potential (Rirattanapong *et al*, 2011; Gonzales-Cabezas *et al*, 2012).

In this study, we used polarized light microscopy (Nikkon® model Eclipse E400 pol) and the Image-Pro Plus® computer program. Polarized light microscopy can give an accurate measurement of lesion depth; however, this method requires thin section preparation and is time consuming (Lo *et al*, 2010). A different method used for determining demineralization and remineralization of primary teeth enamel

may have contributed to the different results (Craig and Peyton, 1958).

In conclusion, fluoride mouthrinse containing TCP provides no additional benefit over mouthrinse containing fluoride alone.

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