ENHANCING REMINERALIZATION OF PRIMARY ENAMEL LESIONS WITH FLUORIDE DENTIFRICE CONTAINING TRICALCIUM PHOSPHATE

Praphasri Rirattanapong[1], Kadkao Vongsavan[1], Chavengkiat Saengsirinavin[2] and Sumana Waidee[3]

[1]Department of Pediatric Dentistry, [2]Research Office, Faculty of Dentistry, Mahidol University, Bangkok; [3]Dental Department, Latbualuang Hospital, Lat Bua Luang, Phra Nakhon Si Ayutthaya, Thailand

Abstract. Fluoride dentifrice is effective in preventive dental caries but may cause fluorosis, especially in young children. Reducing the concentration of fluoride from the regular concentration of 1,000 parts per million (ppm) to 500 ppm can reduce the risk for fluorosis but increases the risk of caries. Adding tricalcium phosphate (TCP) to the dentifrices may improve the efficacy of remineralization possibly allowing for a lower concentration of fluoride to reduce the risk of fluorosis. We studied this to inform future caries prevention efforts in children. We immersed 40 sound primary incisors into demineralizing solution (pH=4.4) for 96 hours at 37°C to create demineralized lesions. The 40 teeth were then divided into 4 groups of 10 teeth each. Group A: control (treated with deionized water only); Group B: treated with fluoride dentifrice at a concentration of 1,000 ppm; Group C: treated with fluoride dentifrice at a concentration of 500 ppm and 500 ppm TCP, and Group D: treated with fluoride dentifrice at a concentration of 1,000 ppm and 500 ppm TCP. The teeth were each subjected to 7 days of pH-cycling and the studied dentifrice was applied for one minute, 3 times daily during the 7 day period. After the 7 day period the teeth were each sectioned and examined with polarized light microscopy. The depths of demineralized areas were measured using Image-Pro plus software. A pair *t*-test was used to compare lesion depths before and after dentifrice treatment. Differences in mean lesion depths within each group were analyzed using the One-way ANOVA and LSD tests; a 95% confidence intervals were calculated. The mean lesion depths in all the groups before dentifrice treatment were not significantly different (p=0.143). The mean demineralized lesion depths after dentifrice treatment were significantly different by group (p=0.00). The mean demineralized lesion depth in Group A significantly deeper than the other groups (p=0.00). Group D had the shallowest depth, significantly shallower than the other groups (p=0.006). There was no significant difference in the mean demineralized lesion depth between Groups B and C (p=0.478). The mean demineralized lesion depth changed significantly after dentifrice treatment in all the groups (p=0.00). Group A was significantly deeper (p=0.00) and groups B, C

Correspondence: Praphasri Rirattanapong, Department of Pediatric Dentistry, Faculty of Dentistry, Mahidol University, 6 Yothi Street, Bangkok 10400, Thailand.
Tel: +66 (0) 2200 7821 ext 30
E-mail: praphasri.rir@mahidol.ac.th

and D were all significantly shallow. Group D had the greatest reduction in mean demineralized lesion depth ($p<0.05$). The 1,000 ppm fluoride plus TCP dentifrice gave superior remineralization than the 500 ppm fluoride plus TCP and the 1,000 ppm fluoride dentifrice. The 500 ppm fluoride plus TCP gave the same remineralizing effect as the 1,000 ppm fluoride dentifrice. TCP enhances remineralization on primary enamel when added to fluoride dentifrice. Our results show if TCP is added to fluoride dentifrice a lower concentration of fluoride is needed to provide the same benefit as fluoride dentifrice with a higher concentration of fluoride, reducing the risk of fluorosis in children.

Keywords: fluoride, primary teeth, remineralization, tricalcium phosphate

INTRODUCTION

Despite advances in dental technology, public health preventive programs and dental health accessibility, dental caries still affect the Thai population (Petersen *et al*, 2015). The prevention of dental caries and the remineralization of initial enamel lesions are the goal of modern dentistry (Cochrane *et al*, 2010). Various products have been developed to promote remineralization and slowing progression of demineralization. Fluoride dentifrice is one of the most practical methods of promoting remineralization (Rao and Malhotra, 2011). However, the risk of fluorosis is concerning, especially among young children (Wright, 2014). In order to reduce the risk of fluorosis from fluoride dentifrice in young children, they should be supervised by caregivers during tooth-brushing to avoid swallowing the dentifrice. The majority of Thai children brush their teeth unsupervised. Sitthisettapong *et al* (2012) found more than half of children in their study brushed their teeth unsupervised. A low-fluoride dentifrice has been created for young children to reduce the risk of fluorosis (Evans and Dennison, 2009). Studies have found the benefit of using fluoride dentifrice for caries prevention among children and adolescents compared to placebo, but the concentration of fluoride should be 1,000 parts per million (ppm) or higher (Walsh *et al*, 2010). However, fluoride concentrations of 1,000 ppm or higher have been found to increase the risk of fluorosis among children aged less than 6 years (Wong *et al*, 2010). In Thailand, most commercial brands of children's dentifrice contain fluoride concentrations of 500 ppm.

Several studies have reported the benefit of combining fluoride with calcium in dentifrice to enhance remineralization (Pfarrer and Karlinsey, 2009; Amaechi *et al*, 2010). Studies have shown combining tricalcium phosphate(TCP) with fluoride can enhance remineralization better than fluoride alone (Karlinsey and Mackey, 2009; Karlinsey *et al*, 2009a). Several *in vitro* studies have found this to be a satisfactory method to evaluate dentifrices but none have used this method to directly compare the remineralizing properties of fluoride dentifrices with and without TCP on primary enamel lesion (Karlinsey *et al*, 2009b; Karlinsey *et al*, 2011).

The purpose of this *in vitro* study was to compare the remineralizing effect of fluoride dentifrice with and without TCP for remineralizing caries-like lesions in primary enamel at different concentrations of fluoride using a pH-cycling method to reflect oral conditions.

MATERIALS AND METHODS

Sample preparation

This study was approved by the Ethics Committee of Mahidol University. Forty human primary incisors were collected and coated with two layers of acid resistant nail varnish, leaving two square windows approximately 1x1 mm each on the sound, intact labial surface. The root apices were sealed with sticky wax and the teeth were immersed in deionized water until used.

Demineralizing and remineralizing solution preparation

Two demineralizing solutions and one remineralizing solution were prepared for this study. The first demineralizing solution [Demineralizing solution I (D1)] was used to create the demineralized lesions prior to treatment and the second demineralizing solution [Demineralizing solution II (D2)] was used with pH cycling to imitate oral conditions. These solutions were prepared using the method of Rirattanapong *et al* (2011). D1 consisted of 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4 and 0.05 M acetic acid at a pH 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. The remineralizing solution consisted of 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4 and 0.15 M KCl at a pH adjusted to 7.0 using 1M KOH.

Artificial caries lesion formation

Each tooth was immersed in 5 ml D1 at 37°C (Sheldon manufacturing, model 1545, Cornelius, OR) for 4 days to produce carious lesions 60-100 μm deep (Rirattanapong *et al*, 2011). Each tooth was then rinsed with 15 ml deionized water and dried with a piece of tissue paper. The teeth were then kept in artificial saliva modified from the technique of Amaechi *et al* (1999) until used.

Grouping and dentifrice preparation

After the initial artificial carious lesion formation, one of the two windows in each tooth was randomly chosen as the baseline demineralized lesion and coated with acid resistant nail varnish. The other window was used to evaluate the respective treatments. Forty demineralized study teeth were then randomly assigned to one of four groups of 10 teeth each.

The studied dentifrices were prepared by suspending 1 gram of the product in 3 ml of deionized water to give a ratio of 1:3 and then stirred using a hotplate (50°C) stirrer at 500 rpm until well mixed (Ekambaram *et al*, 2011). All dentifrice slurries were freshly prepared daily during the 7-day pH-cycling process as follows: Group A (control group) was comprised of deionized water; Group B was comprised of a 1 gram sample of fluoride at 1,000 ppm dentifrice; Group C was comprised of a 1 gram sample of fluoride at 500 ppm and TCP at 500 ppm and Group D was comprised of a 1 gram sample of fluoride at 1,000 ppm and TCP at 500 ppm.

pH-cycling process

The experimental process aimed to replicate the pH changes occurring in the oral environment for 7 days (Rirattanapong *et al*, 2011). Each cycle involved 3-hours in the D2 solution twice daily and 2 hours with remineralization solution twice daily. Each tooth was then placed in the respective studied dentifrice for 1 minute, three times daily; once before the first demineralization per day in D2 and once before and once after the second demineralization per day in D2. The teeth were then placed in remineralizing solution overnight. This twice daily demineralization at thrice-daily treatment with dentifrice was continued for 7 days.

This was intended to stimulate the normal oral environment with tooth brushing 3 times daily. The D2 and remineralization solution were created daily at the pH of the D2 and remineralizing solution were checked and adjusted to the desired level daily. After 7-day cycling process was complete, the nail varnish was removed with acetone and the teeth were cut into thin sections and examined under a polarized light microscope.

Thin specimen preparation

All specimens were cut longitudinal through the treatment and baseline windows in the labio-lingual axis using a low speed saw under copious water spray (BUEHLER IsoMet®, Lake Bluff, IL) to create a section approximately 400 μm thick. The sections were then ground and polished using wet 800, 1,000, 1,200, 2,000 and 2,500 grit silicon carbide paper. The thickness of each sections was measured using an electronic digital caliper (Mitutoyo model CD-6C, Kanagawa, Japan); all sections were 100-150 μm thick.

Polarized light microscopy measurement

All the sections were then immersed in deionized water, mounted on glass slides and photographed with a polarized light microscope (Nikon model eclipse E400 pol, Tokyo, Japan) at 10X magnification. The depths of the demineralized lesions on the photos were measured using Image-Pro® Plus software (Media Cybernatics, Rockville, MD). Three points on each section were measured and the average depth was calculated and used for analysis. The depths were measured by a single-blinded observer.

Intra-examination reliability

Eight sections (20% of all sections) were randomly selected and re-examined by the same examiner under the same conditions using the same equipment. Intra-examination reliability was calculated using the Pearson's correlation coefficient.

Statistical analysis

The means and standard deviations of the lesion depths were calculated for each group. A paired *t*-test was used to compare pre- and post-treatment lesion depths. One-way analysis of variance (ANOVA) and least significant difference (LSD) were used to test differences in mean lesion depth among the studied groups. SPSS, version 20.0 (IBM, Armonk, NY), was used to make statistical calculations. Significance was set at $p<0.05$.

RESULTS

Intra-examiner reliability was good with a Pearson's correlation coefficient of 0.998. After initial demineralization with D1 solution prior to pH-cycling, the demineralization lesion depths ranged from 90 to 111 μm but there were no significant differences among the groups ($p=0.143$). After the pH-cycling treatment with the studied dentifrice Group A treated teeth had significantly deeper demineralized lesions ($p=0.00$) than the other groups and Group D had significantly shallower demineralized lesions than the other groups ($p=0.006$). There was no differences in the mean demineralized lesion depth between Groups B and C ($p=0.478$). The mean demineralized lesion depths after pH-cycling were significantly different from before pH-cycling in all groups ($p=0.00$). Group A had a significantly deeper mean lesion depth after pH-cycling ($p=0.00$). Group B, C and D had significantly shallower lesion depths after pH-cycling and dentifrice treatment. Group D had the greatest improvement in mean demineralized lesion depth with dentifrice treatment ($p<0.05$) (Table 1, Fig 1).

Table 1
The means and standard deviations for lesion depth among various treatment groups.

Group	Treatment	Mean±SD lesion depth (μm)	
		Baseline lesion	Experimental lesion
A	Deionized water	100.25 ± 13.17[a]	143.85 ± 12.91[b]*
B	1,000 ppm F dentifrice	105.61 ± 7.33[a]	88.07 ± 6.38[c]*
C	500 ppm F+TCP dentifrice	98.83 ± 7.50[a]	84.59 ± 7.94[c]*
D	1,000 ppm F+TCP dentifrice	101.17 ± 8.15[a]	74.54 ± 12.86[d]*

*p<0.05 (t-test). Different superscript letters indicate significant differences (p<0.05, ANOVA, LSD test).

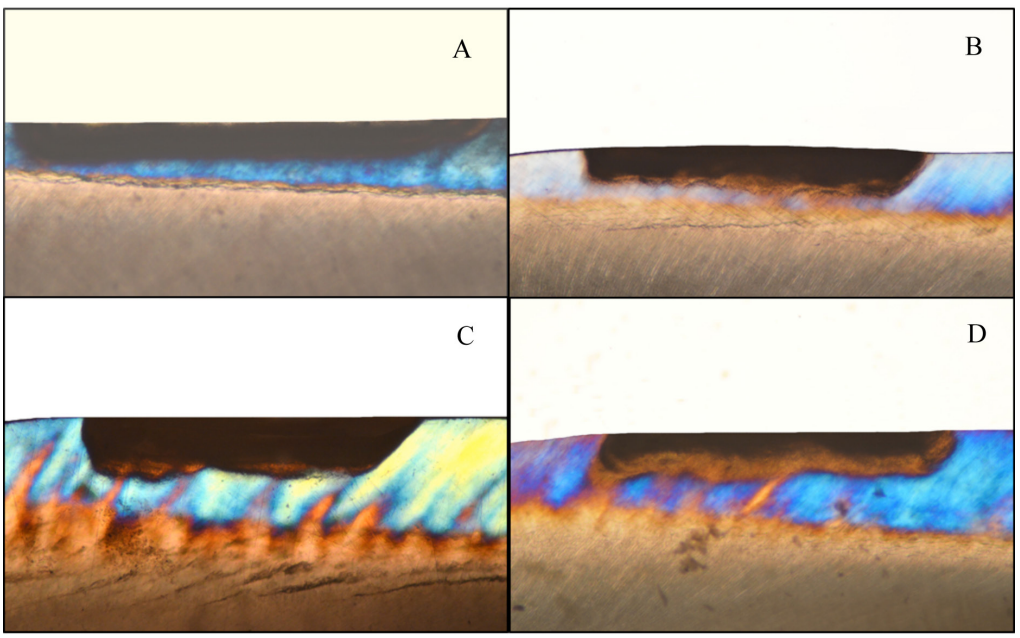

Fig 1–Polarized light photomicrograph at 10X magnification of lesions after treatment in deionized water (A), 1,000 ppm fluoride dentifrice (B), 500 ppm fluoride plus 500 ppm tricalcium phosphate dentifrice (C) and 1,000 ppm fluoride plus 500 ppm tricalcium phosphate (D).

DISCUSSION

Our lesion depths prior to pH-cycling were similar to previous studies (~100 μm) (Yimcharoen *et al*, 2011; Rirattanapong *et al*, 2015).

Our study results showed the combination of fluoride plus TCP gave greater remineralizing than fluoride alone, similar to studies by Karlinsey *et al* (2009b) and Karlinsey *et al* (2012). Based on our study fluoride at 1,000 ppm plus TCP and 500 ppm gave the greatest remineralizing effect, better than fluoride at 500 ppm plus TCP at 500 ppm, probably because of the higher fluoride content (Kapoor *et al*, 2016).

In our study, dentifrice containing fluoride at 500 ppm plus TCP at 500 ppm was significantly superior to the control group and equivalent to dentifrice containing only fluoride at 1,000 ppm, similar to a study by Mensinkai *et al* (2012). Our results confirmed adding TCP can improve remineraliztion of fluoride dentifrice. However, ours is the only study to evaluate this efficiency using pH-cycling and measurement of demineralized lesion depths using polarized light microscopy. Further studies are needed using other methods to determine remineralization. *In vitro* trials are also needed to confirm the efficacy of this dentifrice.

In conclusion, dentifrice containing fluoride at 1,000 ppm and TCP at 500 ppm gave the best remineralizing effect, but fluoride at 1,000 pm can increase the risk of fluorosis. Dentifrice with fluoride 500 ppm plus TCP at 500 ppm gave the same remineralizing benefit as dentifrice containing only fluoride at 1,000 ppm, suggesting using this dentifrice containing fluoride at 500 ppm plus TCP at 500 ppm is as effective in remineralization as dentifrice containing only fluoride at 1,000 ppm but has a lower risk of fluorosis in children.

REFERENCES

Amaechi BT, Higham SM, Edgar WM. Factors influencing the development of dental erosion *in vitro*: enamel type, temperature and exposure time. *J Oral Rehabil* 1999; 26: 624-30.

Amaechi BT, Karthikeyan R, Mensinkai PK, Najibfard K, Mackey AC, Karlinsey RL. Remineralization of eroded enamel by a NaF rinse containing a novel calcium phosphate agent in an *in situ* model: a pilot study. *Clin Cos Invest Dent* 2010; 2: 93-100.

Cochrane NJ, Cai F, Huq NL, Burrow MF, Reynold EC. New approaches to enhanced remineralization of tooth enamel. *J Dent Res* 2010; 89: 1187-97.

Ekambaram M, Itthagarun A, King NM. Comparison of the remineralizing potential of child formula dentifrices. *Int J Pediatr Dent* 2011; 21: 132-40.

Evans RW, Dennison PJ. The caries management system: an evidence-based preventive strategy for dental practitioners. application for children and adolescents. *Aust Dent J* 2009; 54: 381-9.

Kapoor A, Indushekar KR, Saraf BG, Sheoran N, Sardana D. Comparative evaluation of remineralizing potential of three pediatric dentifrices. *Int J Clin Pediatr Dent* 2016; 9: 186-91.

Karlinsey RL, Mackey AC. Solid-state preparation and dental application of an organically-modified calcium phosphate. *J Mater Sci* 2009; 44: 346-9.

Karlinsey RL, Mackey AC, Stookey GK. *In vitro* remineralization efficacy of NaF systems containing unique forms of calcium. *Am J Dent* 2009a; 22: 185-8.

Karlinsey RL, Mackey AC, Walker ER, Frederick KE. Spectroscopic evaluation of native, milled, and functionalized β-TCP seeding into dental enamel lesions. *J Mater Sci* 2009b; 44: 5013-6.

Karlinsey RL, Mackey AC, Walker TJ, *et al*. *In vitro* remineralization of human and bovine white spot enamel lesions by NaF dentrifices: a pilot study. *J Dent Oral Hyg* 2011; 3: 22-9.

Karlinsey RL, Pfarrer AM. Fluoride plus Functional β-TCP: a promising combination for robust remineralization. *Adv Dent Res* 2012; 24: 48-52.

Mensinkai PK, Ccahuana-Vasquez RA, Chedjieu I, *et al*. *In situ* remineralization of white-spot enamel lesions by 500 and 1,100 ppm F dentifrices. *Clin Oral Investig* 2012; 16: 1007-14.

Pfarrer AM, Karlinsey RL. Challenges of implementing new remineralization technolo-

gies. *Adv Dent Res* 2009; 21: 79-82.

Petersen PE, Hunsrisakhun J, Thearmontree A, *et al*. School-based intervention for improving the oral health of children in southern Thailand. *Community Den Health* 2015; 32: 44-50.

Rao A, Malhotra N. The role of remineralizing agents in dentistry: a review. *Compendium* 2011; 32: 26-33.

Rirattanapong P, Vongsavan K, Tepvichaisillapakul M. Effect of five different dental products on surface hardness of enamel exposed to chlorinated water *in vitro*. *Southeast Asian J Trop Med Public Health* 2011; 42: 1293-8.

Rirattanapong P, Vongsavan K, Saengsirinavin C, Phuekcharoen P. Efficacy of fluoride mouthrinse containing tricalcium phosphate on primary enamel lesions: a polarized light microscopic study. *Southeast Asian J Trop Med Public Health* 2015; 46: 168-74.

Sitthisettapong T, Phantumvanit P, Huebner C, DeRouen T. Effect of CPP-ACP paste on dental caries in primary teeth: a randomized trial. *J Dent Res* 2012; 91: 847-52.

Walsh T, Worthington HV, Glenny AM, Appelbe P, Marinho VC, Shi X. Fluoride toothpastes of different concentrations for preventing dental caries in children and adolescents. *Cochrane Database Syst Rev* 2010; 1: CD007868.

Wong MCM, Glenny AM, Tsang BWK, Lo ECM, Worthington HV, Marinho VCC. Topical fluoride as a cause of dental fluorosis in children. *Cochrane Database Syst Rev* 2010; 1: CD007693.

Wright JT. Fluoride toothpaste efficacy and safety in children younger than 6 years. *J Am Dent Assoc* 2014; 145: 182-9.

Yimcharoen V, Rirattanapong P, Kiatchallermwong W. Effect of casein phosphorpeptide toothpaste versus fluoride toothpaste on remineralzation of primary teeth enamel. *Southeast Asian J Trop Med Public Health* 2011; 42: 1032-40.