

THE EFFECT OF CASEIN PHOSPHOPEPTIDE TOOTHPASTE *VERSUS* FLUORIDE TOOTHPASTE ON REMINERALIZATION OF PRIMARY TEETH ENAMEL

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Abstract. This study evaluated the effect of a CPP-containing toothpaste and compared it with fluoride-containing toothpastes on remineralization of caries-like lesions in primary teeth enamel, using polarized light microscopy. Forty-eight sound primary incisors were coated with nail varnish, leaving two 1x1 mm windows before being placed in a demineralizing solution for 4 days. After demineralization, all the specimens were coated with nail varnish over one window and were randomly assigned to 4 groups (A to D; $n = 12$). Group A teeth were exposed to distilled water. Group B teeth were exposed to a CPP-containing toothpaste (Hi Herb[®]). Group C teeth were exposed to a 260 ppm fluoride-containing toothpaste (Smile baby toothgel[®]). Group D teeth were exposed to a 500 ppm fluoride-containing toothpaste (Oralmed[®] Kid). Polarized light microscopy was used to evaluate lesion depth, before and after a 7-day pH cycle. Lesion depth was measured using a computerized method with the Image-Pro[®] Plus program. Differences in mean lesion depth within groups and between groups were analysed using the paired *t*-test, Kruskal-Wallis test and Mann-Whitney *U* test at a 95% level of confidence. Mean lesion depths in Groups A, B, C and D significantly increased by 110.1, 36.1, 40.2 and 18.2%, respectively. The mean lesion depths for all the toothpaste groups (B, C and D) were significantly different from the control group (A). Comparisons made among treatment groups showed Group D was significantly different from Groups B and C. All toothpastes were effective for inhibiting progression of carious lesions. However, a 500 ppm fluoride-containing toothpaste inhibited lesion progression better than a CPP-containing toothpaste and a 260 ppm fluoride-containing toothpaste.

Keywords: casein phosphopeptide, fluoride, remineralization, toothpaste

INTRODUCTION

One of the most important concepts that has evolved in cariology over the past

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several decades is demineralization and remineralization of enamel (Casamassimo and Warren, 2005). Early caries can be arrested and the tooth surfaces remineralized through appropriate treatment. It is widely accepted fluoride is one of the most important agents for promoting remineralization, especially with the aid of topical fluorides (Itthagarun *et al*, 2000; Thaveesangpanich *et al*, 2005a).

Fluoride-containing toothpaste was introduced in industrialized countries during the late 1960s and is today the most common vehicle delivering fluoride to the oral cavity. Systematic reviews of the effectiveness of fluoride-containing toothpastes have been published. Twetman *et al* (2003) reviewed 905 studies, of which 54 met the criteria for inclusion in their meta-analysis. They found daily use of fluoride-containing toothpaste provided a decayed, missing and filled surfaces (DMFS) prevention fraction of 25% in young permanent dentition. They found incomplete evidence for effectiveness of fluoride-containing toothpaste on primary dentition.

Fluoride-containing toothpastes are the most widely used method to prevent caries. Fluoride can enhance the process of remineralization when used properly. However, repeated ingestion of fluoride can result in chronic fluoride toxicity, the most common manifestation of which is dental fluorosis (Nowak and Crall, 2005). Dental fluorosis occurs when there is a disruption of the mineralization of tooth enamel while the enamel is forming before tooth eruption (McDonald *et al*, 2004), especially during late secretion or early maturation of enamel. Milsom and Mitropoulos's study (1990) indicated fluoride-containing toothpaste was the only major source of fluoride to cause fluorosis. Mascarenhas and Burt (1998) found the fluorosis from swallowing fluoride-containing toothpaste prior to age 6. Hong *et al* (2006) showed significant positive association between fluorosis prevalence and levels of fluoride intake during the first 3 years.

Due to the potential risk of fluorosis described above, fluoride containing toothpaste should not be used in small

children who are unable to expectorate the toothpaste or who are allergic to fluoride. However, fluoride is not the sole agent in remineralization. Remineralization may be achieved by simultaneously supplying calcium and phosphate to the teeth (Torrado *et al*, 2004).

Reynolds *et al* (1992) investigated the calcium phosphate salt of casein phosphopeptide (CPP) for anti-caries activity in a rat model. They found at levels of 0.5 to 1%, CPP was as effective at inhibiting caries formation as 500 ppm fluoride.

Dundon *et al* (1994) compared 0, 250, 500 and 1,000 ppm fluoride and CPP-containing toothpastes. CPP-containing toothpaste was about one-third as effective as 1,000 ppm fluoride at inhibiting the surface-softening of enamel *in situ*.

Many investigators have studied de/remineralization of enamel lesions in permanent teeth using fluoride-containing toothpaste and non-fluoride-containing toothpaste. However, few investigators have studied primary teeth, and there are no reports of studies of de/remineralization of enamel lesions in primary teeth using CPP-containing toothpaste and fluoride-containing toothpaste.

The purpose of this *in vitro* study was to evaluate the inhibition of demineralization by CPP-containing toothpaste compare with two concentrations of fluoride-containing children's toothpaste on primary teeth enamel *in vitro*.

MATERIALS AND METHODS

Sample selection

Forty-eight human primary incisors were collected from teeth extraction or natural exfoliation. Only teeth with sound enamel were selected to be used in this study.

Table 1
Types of toothpaste used in the study.

Active ingredients	Trade name	Manufacturer
Casein phosphopeptides (concentration not indicated)	Hi Herb®	Sahaphattanaphiboon Company, Thailand Lot No. 74/08
0.20% w/w Sodium monofluorophosphate (260 ppm)	Smile baby toothgel®	The Boots Company, England Lot No. 2466708/09
0.38% w/w Sodium monofluorophosphate (500 ppm)	Oralmed® Kid	Ouiheng Company, Thailand Lot No. 427018/08

Toothpaste used

All the toothpaste used is commercially available and ubiquitous in Thailand (Table 1). Toothpaste for each of the treatment groups was prepared as a slurry solution by mixing with 30 ml deionized water (Rirattanapong *et al*, 2010).

Demineralizing and remineralizing solutions preparation

The demineralizing and remineralizing solutions were prepared using the method of Rirattanapong *et al* (2010). Demineralizing solution 1 (D1) was comprised of 2.2 mM CaCl₂, 2.2 mM NaH₂PO₄ and 0.05 M acetic acid at a pH adjusted to 4.4 with 1M KOH. Demineralizing solution 2 (D2) was comprised of the same components as D1, but the pH adjusted to 4.7 with 1M KOH. The remineralizing solution (R) was comprised of 1.5 mM CaCl₂, 0.9 mM NaH₂PO₄, and 0.15 M KCl at a pH adjusted to 7.0 with 1M KOH. The demineralizing and remineralizing solutions were freshly prepared for each cycle and kept in separate plastic containers.

Specimen preparation

Forty-eight intact extracted or naturally exfoliated human primary incisor teeth were stored in 0.01% Thymol solution at room temperature. All the teeth were cleaned of soft tissue debris and

inspected for cracks, fluorosis, enamel hypoplasia, white spots/brown spots and tetracycline discoloration. Then, all the teeth were polished with fine pumice to remove organic contaminants and then kept in normal saline until used.

Upon removal from the saline, the teeth were blotted dry with tissue paper and coated with acid resistant nail varnish (Revlon, USA) in two layers, leaving two square windows of approximately 1x1 mm on the buccal surface. The root apices were sealed with sticky wax. The teeth were immersed in deionized water until used.

Caries-like lesion formation

Each tooth was immersed in 3 ml demineralizing solution (D1) and incubated at 37°C (Sheldon Manufacturing, Model 1545, Oregon, USA) for 4 days to produce carious lesions of 60-100 μm deep (Rirattanapong *et al*, 2010). Then, the tooth was rinsed in 15 ml deionized water and dried with tissue paper.

Test groups

After artificial carious lesion formation, one of two windows in each tooth was randomly assigned to be used as a "before" treatment window and was coated with acid resistant nail varnish (Revlon, USA) in two layers. The forty-eight

Table 2
Tooth lesion depths and percentage changes for in each tested group ($n = 12$).

Group	Treatment	Mean lesion depth \pm SD ()		Percentage change
		Pre-test	Post-test	
A	Distilled water(control)	86.76 \pm 8.29 ^a	182.10 \pm 21.25 ^b	110.12 \pm 17.53 ^b
B	Hi Herb [®]	90.85 \pm 4.39 ^a	123.62 \pm 9.99 ^c	36.09 \pm 8.94 ^c
C	Smile baby toothgel [®]	87.29 \pm 9.44 ^a	121.61 \pm 12.35 ^c	40.23 \pm 15.29 ^c
D	Oralmed [®] Kid	87.65 \pm 4.65 ^a	103.56 \pm 10.40 ^d	18.17 \pm 10.03 ^d

The same letters indicate no statistically significant difference ($p > 0.05$).

specimens were pooled and randomly assigned to one of four groups, comprised of 12 specimens each group. The specimens were then placed in a self-cured acrylic block and immersed in deionized water until use. A toothpaste slurry was freshly prepared during the pH-cycling process as follows: Group A (control group) was comprised of distilled water; Group B was comprised of a pea-sized (0.32 grams) sample of CPP-containing toothpaste (Hi Herb[®]); Group C was comprised of a pea-sized (0.32 grams) portion of 260 ppm fluoride-containing toothpaste (Smile baby toothgel[®]); Group D was comprised of a pea-sized (0.32 grams) portion of 500 ppm fluoride-containing toothpaste (Oralmed[®] Kid).

pH-cycling process

The experimental process imitated the changes in pH of the oral environment for 7 days. All specimens were subjected to a pH-cycling procedure. Each cycle involved three hours of demineralization twice daily, with two hours of remineralization in between. A one-minute toothpaste slurry treatment was given before the first demineralizing cycle and before and after the second demineralizing cycle; then, all the specimens were placed in remineralizing solution overnight at 37°C

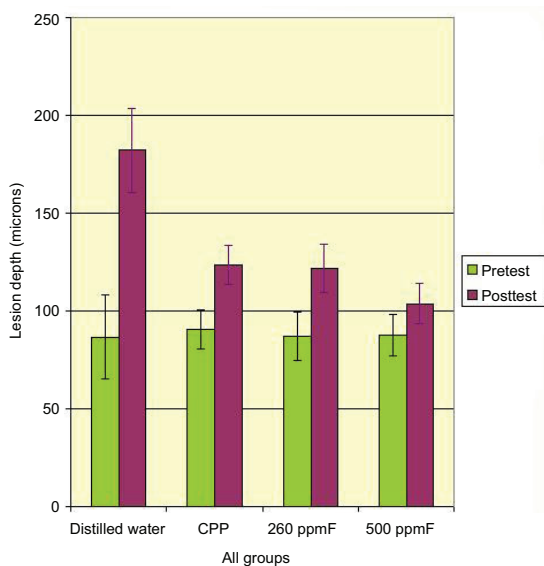
in an incubator shaker (Series 25 Incubator Shaker[®], USA) (150 rpm).

Thin section preparation

Seven days after completion of the pH cycles, each specimen was removed from the block. All remaining acid-resistant nail varnish was removed with acetone solvent. All specimens were cut longitudinally through the lesion (bucco-lingual axis) using a slow speed diamond saw under a copious water spray (Accutom-50, Struers, Denmark) to create a thin section (approximately 400 μ m thick). The thin sections were then ground with wet 800 and 1,000 grit silicon carbide paper. The thickness of each of the thin sections was measured with an electronic digital caliper (Mitutoyo[®] model CD-6C, Japan). Sections with a thickness of 100-150 μ m were used.

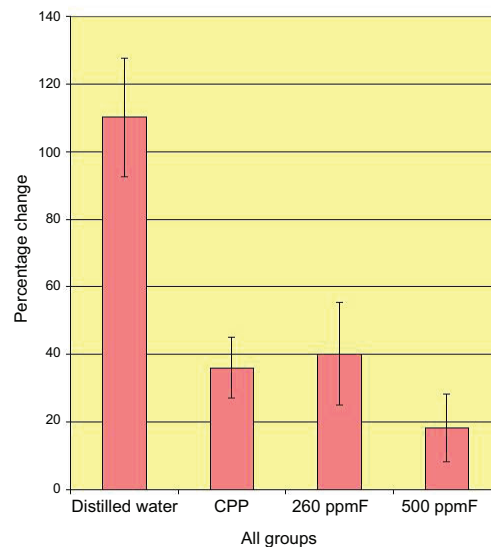
Polarized light microscopic measurement

The sections were covered with deionized water and photographed using polarizing light microscopy at 10x magnification (Nikon[®] model eclipse E400 pol, Japan) to evaluate the lesions on each enamel section. The average of three points was used to measure lesion depth. The pictures were then analyzed using Image-Pro[®] Plus Program (Media Cyber-



CPP, Casein phosphopeptide
 260 ppmF, Fluoride at 260 ppm
 500 ppmF, Fluoride at 500 ppm

Fig 1—Mean lesion depth of control and treatment groups before and after pH-cycling.



CPP, Casein phosphopeptide
 260 ppmF, Fluoride at 260 ppm
 500 ppmF, Fluoride at 500 ppm

Fig 2—Percentage change of lesion depth in control and treatment groups after 7-day of pH-cycling.

netics, MD, USA). Lesion depths were recorded using a single-blind technique.

Intra-examination reliability

The microscopic measurements were calibrated. Twenty sections (20% of all sections) were randomly selected and re-examined by the same examiner under the same conditions using the same equipment. Examination reliability was tested using Pearson’s correlation coefficients.

Statistical analysis

Means and standard deviations for lesion depths before and after 7 days of pH-cycling were calculated for each group. Mean values for pre-test, post-test and percent change in lesion depth were checked for normal distribution using the Kolmogorov-Smirnov test (K-S test). Differences between pre-test and post-test

lesion depths within the same group were compared using the paired-samples t-test. The Kruskal-Wallis and Mann-Whitney *U* tests were used to test differences between pre-test and post-test lesion depths and percentage change. Significance was set at $p < 0.05$ for all statistical tests.

RESULTS

Results from the duplicate examination showed good reliability with a Pearson’s correlation coefficient of 0.978.

The mean and standard deviation (SD) for changes in lesion depths after the 7-day pH cycle are shown in Table 2 and Fig 1. The pre-test lesion depths for each group ranged from 86.76 ± 8.29 m to 90.85 ± 4.39 m. No significant differences were seen among the tested groups

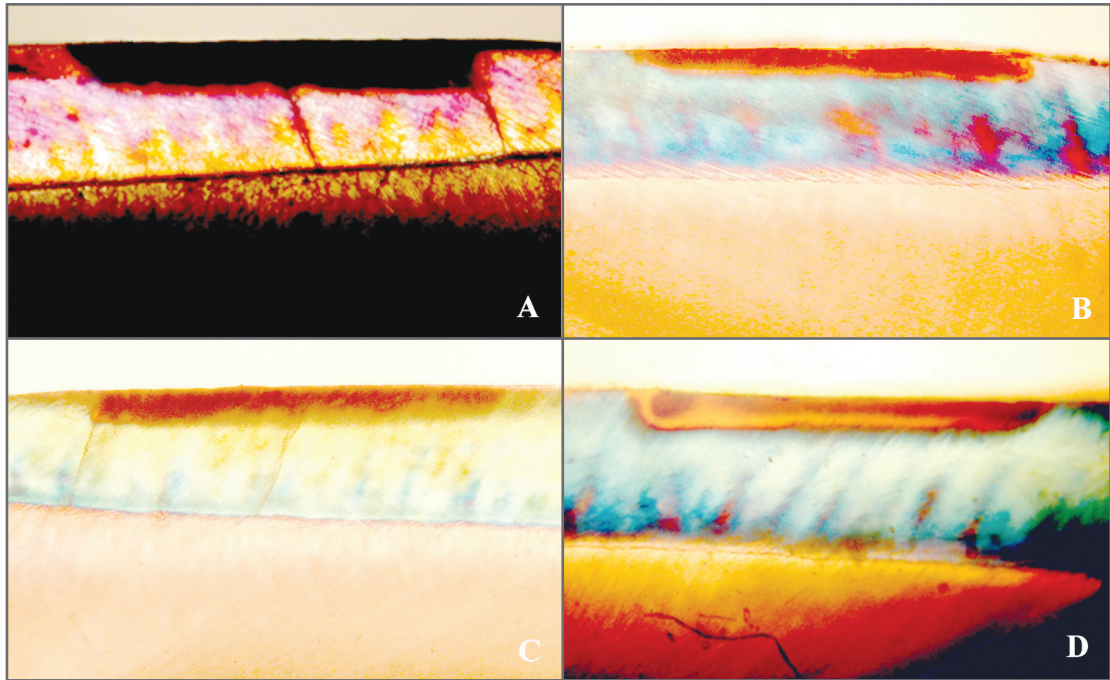


Fig 3—Polarized light photomicrograph at 10x magnification of one enamel lesion after treatment from Groups A-D.

($p=0.312$) for pre-test lesions. Comparisons between pre-test and post-test lesion depths in all groups were significantly different ($p=0.000$). The mean lesion depths in Groups A, B, C and D increased by 110.1, 36.1, 40.2 and 18.2%, respectively (Fig 2).

The post-test lesion depth for each group ranged from 103.56 ± 10.40 μm to 182.10 ± 21.25 μm . The results show the post-test mean lesion depths for all the treated groups were significantly different from the control group (Fig 3) ($p=0.000$). Comparison among the treatment groups showed Group D was significantly different from Groups B and C ($p=0.000$ and 0.001 , respectively).

DISCUSSION

In the present study, the pre-test

lesion depths did not vary significantly by group. Although caries were produced artificially, there were no major variations among the pre-test teeth.

After the 7-day pH cycle, the mean lesion depths for all the treated groups were significantly different from the control group. All treated groups inhibited demineralization progression of the carious lesions. However, none of the treated groups had remineralization of the carious lesions. This may be due to the severity of the demineralization with the pH-cycling model when used with primary teeth. Possible reasons for this are: primary teeth enamel is thin, has a low mineral content, has a high organic content and has variations in the structure of the surface that may influence caries susceptibility (Tha-veesangpanich *et al*, 2005a,b).

A meta-analysis of 70 trials regarding the effectiveness of fluoride-containing toothpaste for preventing dental caries in children found fluoride-containing toothpaste significantly reduces the incidence of caries in the permanent dentition, sufficient to warrant a strong recommendation. However, the review provided little information about the effectiveness on primary dentition (Twetman *et al*, 2003; Itthagarun *et al*, 2007). From our study it can be concluded fluoride-containing toothpaste has the ability to reduce lesion depth progression in primary teeth. This study confirms the protective effect of fluoride-containing toothpaste. A similar study by Thaveesangpanich *et al* (2005b) found a pea-sized portion of 500 ppm fluoride-containing toothpaste inhibited demineralization progression in primary teeth better than non-fluoride toothpaste *in vitro* in both 7-day and 10-day pH-cycling models.

Itthagarun *et al* (2007) found the use of toothpaste containing fluoride at 500 ppm promoted remineralization among primary teeth using an *in vitro* 7-day pH-cycling model. However, Thaveesangpanich *et al* (2005a) found toothpaste containing fluoride at 500 ppm did not result in remineralization, possibly due to small sample size, the aggressiveness of the artificial caries system used, the fluoride content of the enamel specimen used or the calcium composition of the non-fluoride toothpaste control group. Toothpaste manufactured in Korea failed to show remineralization even with varying fluoride levels (Itthagarun *et al*, 2007). The fluoride content may be insufficient to remineralize some carious lesions or the sample size may have been too small (Itthagarun *et al*, 2007).

Toothpaste with low fluoride concentrations has been designed primarily to

reduce the risk of fluorosis and therefore are intended for use by young children. The fluoride content of toothpaste varies from 250 to 500 ppm (Thaveesangpanich *et al*, 2005b). Children under 8 years old should consume no more than 0.1 mg of fluoride/kg body weight if an undesirable degree of fluorosis is to be avoided (Levy *et al*, 1995). Products with low fluoride content are less likely to cause fluorosis and less effective in promoting remineralization. In our study the mean lesion depth in teeth treated with 260 ppm fluoride-containing toothpaste was significantly less than the control group. Therefore, a 260 ppm fluoride-containing toothpaste is more effective than non-fluoride-containing toothpaste.

Comparison for post-test percentage changes in lesions showed the 500 ppm fluoride-containing toothpaste group was significantly different from the 260 ppm fluoride-containing toothpaste group and the CPP-containing toothpaste group. The 500 ppm fluoride-containing toothpaste group had the lowest post-test mean lesion depth. It appears 500 ppm fluoride-containing toothpaste had a better protective effect than 260 ppm fluoride-containing toothpaste and CPP-containing toothpaste. The effect of the 260 ppm fluoride-containing toothpaste was similar to the CPP-containing toothpaste.

The results of our study correspond to those of Damato *et al* (1990) who concluded remineralization was significantly higher in the 500 ppm fluoride-containing group than the 250 ppm fluoride-containing group. In our study, higher fluoride concentrations did not produce significant increases in remineralization. Our results are in contrast to those of Rirattanapong *et al* (2010) who found fluoride-containing toothpastes (250, 500 and 1,000 ppm)

resulted in significantly more remineralization than non-fluoride-containing toothpaste; however, no differences in lesion depths occurred among the 250, 500 and 1,000 ppm fluoride-containing toothpastes. This might be due to the different types of fluoride used; in Rirattanapong's study sodium fluoride with carboxymethylcellulose as stabilizer was used (Rirattanapong *et al*, 2010), but in our study sodium monofluorophosphate was used with a silica abrasive system.

Fluoride is not the sole cause of remineralization. Another method of remineralization is attained by supplying calcium and phosphate to the teeth (Torrado *et al*, 2004). CPP have been shown to inhibit enamel demineralization and promote remineralization (Dundon *et al*, 1994). No investigators have studied remineralization of enamel lesions in primary teeth using CPP-containing toothpaste. A CPP-containing toothpaste had the same efficacy for inhibiting demineralization progression in primary teeth enamel as a 260 ppm fluoride-containing toothpaste. Reynolds *et al* (1992) reported CPP in toothpaste can significantly inhibiting enamel demineralization, similar to 500 ppm fluoride toothpaste. Dundon *et al* (1994) found CPP-containing toothpaste had the same efficacy as about one-third the amount of 1,000 ppm fluoride toothpaste at inhibiting surface-softening of enamel. Thus, CPP may be an alternative for fluoride. CPP-containing toothpaste may be used for preventive treatment of caries in children who are allergic or sensitive to fluoride or in small children who are unable to expectorate. Toothpaste containing 260 ppm fluoride should be used in children who are unable to expectorate to reduce risk of fluorosis.

In conclusion, CPP-containing toothpaste, 260 ppm fluoride-containing tooth-

paste and a 500 ppm fluoride-containing toothpaste all had significant efficacy for inhibiting demineralization of carious lesions; 500 ppm fluoride-containing toothpaste inhibited lesion progression better than CPP-containing toothpaste and a 260 ppm fluoride-containing toothpaste.

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