EFFECT OF FLUORIDE VARNISHES CONTAINING TRI-CALCIUM PHOSPHATE SOURCES ON REMINERALIZATION OF INITIAL PRIMARY ENAMEL LESIONS

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Abstract. The aim of this study was to evaluate the effect of fluoride varnishes containing tri-calcium phosphate on remineralization of primary enamel lesions. Forty-eight sound primary incisors were coated with nail varnish, leaving two 1x1 mm windows before being placed in a demineralizing solution for four days. After demineralization, all the specimens were coated with nail varnish over one of the windows and were randomly assigned to one of four groups: Group A: deionized water; Group B: Duraphat® Fluoride Varnish; Group C: Clinpro™ White Varnish; Group D: TCP-fluoride varnish. Polarized light microscopy was used to evaluate initial lesion depth and after a 7-day pH cycle. Lesion depth was measured using a computerized method with the Image-Pro Plus Program. The differences in mean lesion depths were compared among the groups using the One-Way ANOVA and Tukey's multiple comparison tests at a 95% confidence interval. Group A had a significant increase in lesion depth compared to the other groups. No significant differences were found among Groups B, C and D. We concluded fluoride varnishes containing tri-calcium phosphate inhibit progression of initial primary enamel lesions, and the brands tested were not significantly different from each other in efficacy.

Keywords : fluoride varnish, primary teeth, remineralization, tri-calcium phosphate

INTRODUCTION

Dental caries are a major oral health problem worldwide in spite of improvements in oral health (Ismail *et al*, 2013). Dental caries are a pathological condition resulting from an imbalance in pathological processes and preventive factors (Featherstone, 2006). Topical fluoride delivered via various vehicles is a standard effective anti-caries treatment (Miller *et al*, 2012). Fluoride varnish is a standard remineralizing agent developed to prolong the contact time between fluoride and the tooth surface acting as a slow-releasing reservoir of fluoride (Ramaswami, 2008; Azarpazhooh and Main, 2009).

Karlinsey *et al* (2009) showed that

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adding calcium phosphate salts, such as tri-calcium phosphate (TCP), to the varnish may improve the mineralization of bovine dentin, but few studies have addressed the effect of tri-calcium phosphate on primary teeth.

Mahidol University has developed a fluoride varnish product. To enhance the effect of the varnish, we have added calcium and phosphate. It is hoped this product will enhance remineralization better than products not containing calcium and phosphate. It could be used as a substitute for imported products and help reduce cost.

This *in vitro* study evaluated the effect of fluoride varnishes containing tri-calcium phosphate on remineralization of initial primary enamel lesions using polarized light microscopy.

MATERIALS AND METHODS

Specimen preparation

This study was approved by The Ethics Committee of Mahidol University. Forty-eight sound extracted or naturally exfoliated human primary incisor teeth were obtained and stored in normal saline at room temperature until used. All the studied teeth were coated with acid resistant nail varnish, leaving two square windows of 1x1 mm each on the labial surface. The root apices were sealed with sticky wax. The teeth were immersed in deionized water after preparation until use.

The caries like lesion formation

Each tooth was immersed in 3 ml demineralizing solution (Rirattanapong *et al*, 2010) and incubated at 37°C (Sheldon manufacturing, model 1545, Cormelius, OR) for 4 days to produce initial enamel lesions of 60-150 m deep (Itthagarun *et al*, 2007). All teeth were immersed in artificial saliva modified from Amaechi *et al* (1999) until use.

Grouping

After artificial carious lesions formation, one of the two windows in each tooth was randomly assigned to be used as a "baseline lesion" window and was coated with acid resistant nail varnish and the other one was used as an "experimental lesion" window, exposed to the test products and pH-cycling process. Forty-eight specimens were pooled and randomly assigned to four groups, comprised of 12 specimens in each group. The fluoride varnish in group D was freshly prepared. Details of the fluoride varnish used in this study are described in Table 1. The fluoride varnish products were applied according to the manufacturers' instructions; the treated teeth were stored for 24 hours in a moist environment (Santos *et al*, 2009). All the specimens were then brushed and rinsed with deionized water to remove the varnish.

pH-cycling

All the specimens were subjected to a 7-day-pH-cycling procedure (Yimcharoen *et al*, 2011). Each cycle involved three hours of demineralization twice daily with two hours of remineralization in between. Demineralizing and remineralizing solutions were modified from Rirattanapong *et al* (2010) and were freshly prepared for each pH cycle. All the specimens were then placed in remineralizing solution overnight at 37°C in a controlled environment incubator shaker (Series 25 Incubator Shaker®, Hauppauge, NY).

Thin section preparation

After the 7-day-pH-cycling procedure, all remaining acid-resistant nail varnish was carefully removed with acetone. All the specimens were longitudinally cut through the lesion (inciso-gingival axis)

Table 1
Fluoride varnishes used in this study.

Active ingredients	Trade mark	Manufacture company
5% sodium fluoride	Duraphat® Varnish	Colgate Oral Pharmaceuticals, New York, NY, lot no.1C37/50
5% sodium fluoride with TCP	Clinpro™ White Varnish	OMNI Preventive Care, A 3M ESPE Company, West Palm Beach, FL, lot no.37717/12
5% sodium fluoride with TCP	TCP-fluoride varnish	Faculty of Dentistry, Mahidol University

TCP, tri-calcium phosphate.

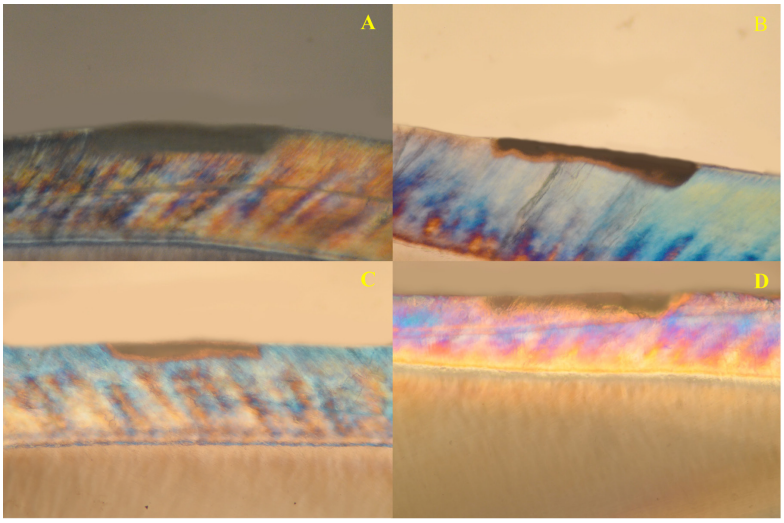

Fig 1–Polarized light photomicrograph at 10x magnification of lesion from deionized water group (A), Duraphat® group (B), Clinpro™ White Varnish group (C), TCP-fluoride varnish group (D).

using a slow speed diamond saw with copious water spray (Accutom-50, Strues, Ballenep, Denmark) to create a thin section (approximately 400 m thick). All the thin sections were then ground with wet 800 and 1,000 grit silicon carbide paper. The thickness of each section was measured with an electronic digital caliper (Mitutoyo® model CD-6C, Kanagawa, Japan). Sections with a thickness of 100-150 m were used.

Polarized light microscopy

All sections were placed in deionized water, mounted on glass-slides and the artificial caries lesion depth was measured with a polarized light microscope (Nikon® model eclipse E400 pol, Tokyo, Japan) at 10x magnification. The maximum depth of the lesion was measured at three points and the results averaged. Photographs of the caries were taken, and analyzed using a computerized calculation method with Image-Pro® Plus (Media Cybernetics, Silver Spring, MD). Lesion depths were recorded using a single-blind technique.

Intra-examination reliability

Ten sections (20% of all sections) were randomly selected and re-examined by the same examiner under the same conditions using the same equipment. The intra-examination reliability was

Table 2
Mean and standard deviations of lesion depths and the percent change for all groups.

Group	Treatment	Mean lesion depth ± SD ( )		Percent change
		Baseline lesion	Experimental lesion	
A	Deionized water (control group)	128.07 ± 16.62 [a]	429.97 ± 37.84 [b]	255.68 ± 53.85 [d]
B	Duraphat®	130.54 ± 16.41 [a]	229.43 ± 14.50 [c]	80.47 ± 26.66 [e]
C	Clinpro™	133.70 ± 21.14 [a]	222.42 ± 21.17 [c]	73.60 ± 20.41 [e]
D	TCP-fluoride varnish	125.98 ± 5.09 [a]	215.21 ± 18.81 [c]	74.47 ± 22.40 [e]

Same letters indicate no statistically significant difference ($p \geq 0.05$).
TCP, tricalcium phosphate.

evaluated using the Pearson's correlation coefficient.

Statistic analysis

Means and standard deviations for the lesion depth were calculated for each group. One way-analysis of variance (ANOVA) and Tukey's multiple comparison test were used to test differences in means and percent changes in lesion depth among the groups (SPSS version 20.0 for Windows, IBM, Armonk, NY). Significance was set at $p<0.05$.

RESULTS

The intra-examination reliability of the lesion depth tested by the Pearson's correlation coefficient was 0.934, which shows good reliability.

The means and standard deviations (SD) for the baseline lesions and experimental lesions are shown in Table 2. The mean ± SD baseline lesion depth for each group ranged from 124.28 ± 8.69 m to 133.73 ± 21.18 m. No significant differences were found for the baseline lesion depth ($p = 0.416$).

The mean ± SD of the experimental lesion depth for each group ranged from 215.97± 15.74 m to 431.23 ± 31.99 m. The experimental mean lesion depth in all treated groups were significantly different from the control group ($p = 0.000$). The comparison among the treatment groups showed no significant differences among groups B, C and D (Fig 1).

The percent changes calculated for each group are shown in Table 2. The mean experimental lesion depth for all the treated groups were significantly different from the control group ($p = 0.000$). Comparisons among the treatment groups showed no significant differences among groups B, C and D.

DISCUSSION

In this study, all teeth were prepared with two square windows of approximately 1x1 mm to labial surface. The advantage of this design is the depth of the lesion can be determined in any tooth at baseline, minimizing variations in initial lesion depth among specimens. No significant differences in mean baseline lesion depth were present. This implies that even though the specimens produced artificial carious lesions in different teeth, using different teeth did not have a major

effect of demineralization progression.

None of the fluoride varnishes (Duraphat®, Clinpro™ White, and TCP fluoride varnish) had a different effect in inhibiting progression of the initial primary enamel lesion but all were significant different from the control group.

Several studies have shown that fluoride varnish can promote enamel remineralization (Castellano and Donly, 2004; Marinho *et al*, 2013). These studies found the evidence that a fluoride varnish can reduce the incidence of caries in permanent dentition is high and sufficient to warrant a strong recommendation for use. However, the review provided little evidence regarding effectiveness in primary dentition. From this study, we can conclude fluoride varnish can reduce lesion depth progression in primary teeth. Santos *et al* (2009) also found fluoride varnish (Duraphat®) reduced lesion depth compared to the control group in primary teeth *in vitro* after using a 7-day pH-cycling model.

Fluoride is not the sole agent used for remineralization. Another remineralizing agent is the application of calcium and phosphate to the teeth (Hick and Flaitz, 2000; Schemehorn *et al*, 2011).

TCP interacts with demineralized enamel to promote remineralization (Karlinsey *et al*, 2009). The above study found TCP fluoride varnish (Clinpro™ White varnish) had better efficacy in inhibiting progression of initial primary enamel lesions than Duraphat® but the difference was not significant. The TCP fluoride prepared by the researchers of Mahidol University was not different in efficacy to Clinpro™ White varnish. It is unclear if the efficacy of the varnish was due to the effect of the fluoride, the TCP or the combination.

The cost of fluoride varnish is an important factor in developing countries with limited budgets. In comparison to imported products, the cost of the TCP fluoride varnish prepared by researchers at Mahidol University is cheaper. Further *in vitro* and *in vivo* studies are needed to compare efficacy.

In conclusion, fluoride varnishes containing tri-calcium phosphate inhibited progression of initial primary enamel lesions and to various brands tested were not significantly different from each other.

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