EFFECT OF FLUORIDE VARNISHES ENHANCED WITH DIFFERENT CALCIUM PHOSPHATE ON MICROHARDNESS OF PRIMARY TEETH

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Abstract. Adding calcium and phosphate may enhance the remineralization effect of fluoride varnish on teeth. There are few studies of the remineralizing effect of this combination on primary tooth; therefore we aimed to determine this. We developed demineralized lesions on 50 primary incisors by immersing them in demineralizing solution for 96 hours and then divided the teeth into 5 groups of 10 teeth each: Group A: control group (no treatment), Group B: 5% sodium fluoride (NaF) (Duraphat®) varnish, Group C: 5% NaF enhanced with tricalcium phosphate (TCP) (Clinpro™ White) varnish, Group D: 5% NaF enhanced with amorphous calcium phosphate (ACP) (Enamel Pro®) varnish, and Group E: 5% NaF enhanced with TCP varnish (Mahidol). The fluoride varnish products were applied according to the manufacturer's instructions and then the teeth were stored for 24 hours in a moist environment. The specimens were carried out through pH-cycling procedure for 7 days. Each cycle involved three hours of demineralization twice daily with two hours of remineralization in between and placed in remineralizing solution overnight at 37°C. The surface microhardness was examined using a Vickers hardness tester (100 grams for 15 seconds) at baseline, before and after pH-cycling. One-way ANOVA and Tukey's multiple comparison at a 95% level of confidence were applied. After pH-cycling procedure, the differences in microhardness values between the treatment groups and the control group were statistically significant (p=0.000). The control group had the lowest mean microhardness value of the study groups (p=0.000). There was no significant difference among the treatment groups (p>0.05). Adding calcium and phosphate to fluoride tooth varnish provided no additional remineralizing benefit in primary teeth compared to fluoride alone.

Keywords: calcium phosphate, fluoride varnish, microhardness, primary teeth

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INTRODUCTION

Fluoride is an important adjunct in the prevention of the dental caries (Rao and Malhotra, 2011). The use of topically applied fluoride has been researched as a means to reduce the risk of dental caries

(Hawkins *et al*, 2003).Tooth varnish is easier to apply and reduces the risk of overingestion of fluoride (Hawkins *et al*, 2003).

Fluoride, calcium and phosphate ions are important for tooth remineralization (Garcia-Godoy and Hicks, 2008). Fluoride tooth varnish with calcium and phosphate is commercially available. Tricalcium phosphate (TCP) and amorphous calcium phosphate (ACP) may enhance remineralization effect (Alamoudi *et al* 2003; Zhao *et al*, 2011).

There are few studies evaluating the efficacy of the addition of calcium and phosphate to fluoride to enhance remineralization of primary teeth. Therefore, we aimed to compare the efficacy of plain fluoride tooth varnish with a commercial ACP fluoride varnish, a commercial TCP fluoride varnish and TCP fluoride made by Mahidol University, Thailand on remineralization of primary enamel caries as measured by tooth surface microhardness.

MATERIALS AND METHODS

Specimen preparation

A total of 50 sound human extracted primary incisors were used for our study. The radicular part of each tooth was removed. The specimens were then embedded in self-cured acrylic resin with the labial surface leveled on top, lying flat and parallel to the horizontal plane. The specimens were then polished using silicon carbide sandpaper using progressively higher grit levels (400, 600, 1,200, 2,000 and 2,500 grit) to obtain a flat, smooth surface. Each prepared specimen was then kept in deionized water at room temperature until used. The baseline surface microhardness of the sound enamel of each tooth was measured on the labial surface using a Vickers indenter (FM-700e Type D, Future-tech, Tokyo, Japan) with 100 grams of force for 15 seconds (Rirattanapong *et al*, 2015). Four measurements for each specimen were obtained at each part of the study and the average of those readings was used for our calculations. This study was approved by The Ethics Committee of Mahidol University, Thailand (MU-DT/PY-1RE 2014/DT076).

Demineralizing and remineralizing solutions preparation

For this study, we prepared 2 demineralizing solutions and 1 remineralizing solution. Demineralizing solution 1 (D1) consisted of 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, 0.5 M acetic acid adjusted to a pH of 4.4 with 1M KOH. Demineralizing solution 2 (D2) consisted of the same components as D1 but the pH was adjusted to 4.7 with 1M KOH. The remineralizing solution (R) consisted of 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, 0.15 M KCl adjusted to a pH of 7.0 with 1M KOH (Thaveesangpanich *et al*, 2005). The demineralizing and remineralizing solutions were freshly prepared for each cycle and kept in the separated containers for each group throughout the study.

Artificial caries lesions formation

Each primary tooth specimen was immersed in 3 ml D1 and incubated at 37°C (Sheldon Manufacturing, model 1545, Cornelius, OR) for 4 days (Thaveesangpanich *et al*, 2005). The specimen was then rinsed with 15 ml deionized water and wiped dry with a piece of tissue paper. All specimens were processed in the same manner and then immersed in artificial saliva as modified from Amaechi *et al* (1999). The surface microhardness was measured with a Vickers indenter.

Test groups

After artificial caries formation, the 50 specimens were then randomly divided into five groups of 10 teeth each. Group

Table1
Microhardness values at baseline, before pH-cycling and after pH-cycling.

Group	Treatment	Condition (Mean±SD; VHN)		
		Baseline	Before pH-cycling	After pH-cycling
A	Control (No treatment)	313.46±26.47[a]	134.32±19.90[b]	118.61±17.40[c]
B	Duraphat® (5%NaF)	325.76±34.90[a]	133.12±16.39[b]	192.51±20.95[d]
C	Clinpro™ (5%NaF+TCP)	320.26±26.28[a]	133.27±22.43[b]	173.24±23.87[d]
D	Enamel Pro® (5%NaF+ACP)	313.44±27.26[a]	143.92±15.22[b]	188.11±26.68[d]
E	TCP-fluoride varnish (5%NaF+TCP)	306.31±35.20[a]	127.46±19.99[b]	202.12±33.38[d]

Different letters indicate statistically significant difference ($p<0.05$). SD, standard deviation; VHN, vicker hardness number.

A (control): no treatment; Group B: 5% NaF (Duraphat®) varnish; Group C: 5% NaF enhanced with TCP (Clinpro™ White) varnish; Group D: 5% NaF enhanced with ACP (Enamel Pro®) varnish; Group E: 5% NaF enhanced with TCP (Mahidol) varnish.

The fluoride varnish products were applied to the respective teeth by group according to the manufacturer's instructions and then the teeth were stored for 24 hours in a moist environment after which the varnish was removed by brushing and rinsing with deionized water. The teeth samples were then subjected to pH-cycling for 7 days.

pH-cycling

The teeth samples were then exposed to pH-cycling for 7 days in order to mimic the oral environment. Each cycle consisted of 3 hours of demineralization with D2 solution twice daily with two hours of remineralization with R solution in between and placed in R solution overnight (16 hours) at 37ºC in an incubator shaker (Series 25 Incubator Shaker®, Champaign, IL) (150 rpm) (ten Cate and Duijsters, 1982).This process was repeated daily for 7 days. After pH-cycling, the surface microhardness was measured with a Vickers indenter.

Statistical analysis

The one-way analysis of variance (ANOVA) and Tukey's multiple comparison tests were used to compare the surface microhardness values at baseline and before and after pH-cycling and compare the mean surface microhardness among groups (SPSS, version 20.0 for Windows; IBM, Armonk, NY). Significance was set at $p<0.05$.

RESULTS

The mean and standard deviations of surface microhardness values measured at the different time intervals during the experiment are shown in the Table 1. The mean baseline of surface microhardness value [±standard deviation (SD)] was 315.85±29.80 VHN. The mean baseline microhardness values did not differ significantly by study group ($p=0.665$).

Before pH-cycling, the mean surface microhardness value (±SD) was 134.42±18.96 VHN; this did not differ significantly by study group ($p=0.425$).

After pH-cycling, the mean microhardness value for all treatment groups were significantly ($p=0.000$) greater than the microhardness value of the control

group. However, there was no significant difference in surface microhardness among the study groups ($p>0.05$). The varnish with NaF only was not significantly different in remineralizing efficacy from the NaF varnish with calcium and phosphate.

DISCUSSION

In our study, the baseline enamel surface microhardness was 315.85±29.80 VHN similar to 328.45±26.56 VHN reported by Rirattanapong *et al* (2015). Before pH-cycling, the mean of microhardness in our study was 134.42±18.96 VHN similar to a previous study by Rirattanapong *et al* (2015) (129.48±30.75 VHN).

After pH-cycling, there was no significant difference among the treatment groups. In our study, none of the varnishes resulted in remineralization of the carious lesions similar to a study by Santos *et al* (2009). This could be due to the severity of the demineralization caused by the pH-cycling in the primary teeth we used for our study. Compared to permanent teeth, the enamel of primary teeth is thinner and has a lower mineral content but does have a higher organic content and more imperfections in the hydroxyapatite crystals, making the transfer of fluoride ions across the crystals less pronounced; these variations in structure may influence caries remineralization (Buzalaf *et al*, 2010).

In our study, we found no significant difference in remineralization among the various fluoride varnishes tested, similar to a study by Rirattanapong *et al* (2014). These findings suggest no additional benefit to adding calcium or phosphate to the fluoride varnish.

Nalbantgil *et al* (2013) found 5% NaF enhanced with ACP (Enamel Pro®) and 5% NaF (Duraflor®) were not significantly different in the inhibiting and preventing demineralization of enamel as tested by tooth microhardness.

Our results are in contrast to those of Alamoudi *et al* (2013) who studied the effect of adding TCP to 5% NaF varnish; they used a 10-day pH-cycling model and found 5% NaF with TCP (Clinpro™) had a significantly greater effect on microhardness than 5% NaF (Durashield®) alone. The difference between our results and theirs could be due to differences in pH-cycling model, pH level and the varnishes used in the study.

In our study, there was no added benefit with enhancing 5% NaF with ACP or TCP over 5% NaF alone for tooth remineralization in primary teeth. In conclusion, all the remineralizing agents were effective in preventing further demineralization but there was no additional benefit to adding calcium or phosphate; therefore, cost is the only factor that should effect the decision as to which tooth varnish to use.

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