

A 3-MONTH STUDY OF FLUORIDE RELEASE FROM DIFFERENT CALCIUM PHOSPHATE FLUORIDE VARNISHES ON PRIMARY TEETH

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Abstract. A range of dental varnishes containing several calcium and phosphate compounds in addition to fluoride to promote remineralization have recently been commercialized. However, the fluoride varnish in the presence of calcium and phosphate can react to form relative poorly soluble phases and its bioavailability. Most of previous studies have focused on fluoride release over a short period. The purpose of this *in vitro* study was to evaluate the fluoride release from different fluoride varnishes on primary teeth during 3 months. Twenty-five sound primary incisors were randomly divided into five groups: Group A-control group (no treatment), Group B - 5% sodium fluoride varnish (5%NaF)(Duraphat[®]), Group C-5% sodium fluoride plus tricalcium phosphate varnish (5%NaF+TCP)(Clinpro[™] White), Group D- 5% sodium fluoride plus amorphous calcium phosphate varnish (5%NaF+ACP)(Enamel Pro[®]), and Group E-5% sodium fluoride plus tricalcium phosphate varnish (5%NaF+TCP)(Mahidol). The samples were then immersed in artificial saliva at room temperature until used. The concentration of fluoride released was measured with F-ion-specific electrode at 2, 4, 8, 12, 24, and 48 hours and then weekly for three months. To analyze the results, we used the one-way ANOVA and Tukey's multiple comparison tests at a 95% level of confidence. Group E had the greatest initial fluoride release within the first 24 hours and Group B had the lowest initial release fluoride of the treatment groups. Group B had a slower rate of decline in fluoride release over time than the other treatment groups. By 3 months, the varnishes with the highest to the lowest release of fluoride were Group B=C>E>D>A. All the treatment samples released more fluoride than the control group. Duraphat[®](5%NaF) and Clinpro[™](5%NaF+TCP) had the highest release of fluoride at 3-month evaluation. The TCP fluoride varnish released more fluoride than the ACP fluoride varnish by 3 months.

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

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INTRODUCTION

Dental caries are currently the most prevalent chronic disease afflicting children in Thailand (Petersen *et al*, 2015). The dental caries process is a continuum involving many cycles of demineraliza-

tion and remineralization (Featherstone, 2008). The current concept in dental treatment focuses on prevention and early treatment of caries. The basic principle is remineralization of early carious lesions. A biological therapeutic approach is preferable to traditional surgery for early carious lesions. A key element of the biological approach is application of remineralizing agents to the tooth structure (Rao and Malhotra, 2011). Fluoride is an important adjunct to prevention of the dental caries (Rao and Malhotra, 2011). The use of topically applied fluoride has been widely researched as a means to reduce the risk of dental caries (Miller *et al*, 2012). Fluoride varnish is preferred because it is easy to apply and reduces the risk of over ingestion of fluoride (Hawkins *et al*, 2003).

The availability of calcium, phosphate and fluoride is important to provide an environment ideal for remineralization (Garcia-Godoy and Hicks, 2008). In the past, the use of calcium and phosphate ions for remineralization has not been successful (Reynolds, 2008). However, recently calcium phosphate-based remineralization systems have been developed and are sold commercially; the manufacturers claim the type of calcium phosphate affects its bioavailability and remineralization (Reynolds, 2008). Many manufacturers have modified fluoride varnishes, adding calcium and phosphate ions in order to improve remineralization.

Previous studies have found fluoride varnishes initially release a large amount of fluoride but this gradually decreases over time (Shen and Autio-Gold, 2002; Ritwik *et al*, 2012). It is thought the resin additives affect the amount and rate of fluoride released (Shen and Autio-Gold, 2002).

Caries prevention requires certain concentrations of fluoride over long periods (Castillo and Milgrom, 2004). Levertt *et al* (1993a,b) found prolonged salivary fluoride levels also prevented caries. Maintaining low levels of fluoride over long periods can inhibit demineralization and promote remineralization (ten Cate, 1999). Fluoride varnishes were developed to provide prolonged the contact between fluoride and tooth enamel. However, most studies have focused on fluoride release over a short time period (Ritwik *et al*, 2012; Comar *et al*, 2014).

The purpose of this *in vitro* study was to compare fluoride release from amorphous calcium phosphate (ACP) fluoride varnish and tricalcium phosphate (TCP) fluoride varnish over a 3-month period.

MATERIALS AND METHODS

Specimen preparation

This study was approved by The Ethics Committee of Mahidol University. Twenty-five sound human primary incisors were collected and stored in normal saline solution at room temperature until use. The root apices of each tooth were covered with sticky wax, blotted-dry with a piece of tissue paper and coated with acid resistant nail varnish (Revlon, Miami, FL) in two layers, leaving one square window 5x5 mm on an intact labial surface. Each treatment tooth was painted with 30 mg of respective fluoride varnish (Castillo *et al*, 2001). The teeth were divided into 5 equal groups: Group A-control group, no treatment; Group B-5% sodium fluoride varnish (5%NaF) (Duraphat®); Group C-5% sodium fluoride varnish plus tricalcium phosphate (5%NaF+TCP) varnish (Clinpro™ White); Group D-5% sodium fluoride plus amor-

Table 1
Study fluoride varnishes.

Active ingredients	Trade mark	Manufacturer
5% sodium fluoride	Duraphat® varnish	Colgate Oral Pharmaceuticals New York, NY Lot 1C 37/50(NC) Exp 12/2016
5% sodium fluoride plus tricalcium phosphate	Clinpro™ White varnish	Premier, MDSS GmbH Schiffgraben 41 30175 Lot N501853 Exp 07/2015
5% sodium fluoride plus amorphous calcium phosphate	Enamel Pro® varnish	OMNI Preventive Care, A 3M ESPE Company, West Palm Beach, FL Lot 44870 Exp 02/2016
5% sodium fluoride plus tricalcium phosphate	Mahidol varnish	Faculty of Dentistry, Mahidol University, Thailand (Fresh preparation)

phous calcium phosphate (5%NaF+ACP) varnish (Enamel Pro®); Group E-5% sodium fluoride varnish plus tricalcium phosphate (5%NaF+TCP) varnish (Mahidol). The details of each of the varnishes are shown in the Table 1.

Fluoride release analysis

Each tooth was immersed in 60 ml artificial saliva at room temperature and placed on a laboratory shaker to simulate mouth conditions (Amaechi *et al*, 1999).

Fluoride release was analyzed using a fluoride ion-sensitive electrode (Orion 96-09; Thermo Electron, Waltham, MA). Each time the fluoride level was checked, the original 60 ml of artificial saliva was swirled around and 3 ml of it was removed to measure fluoride release. The 3 ml sample of artificial saliva was then mixed with 0.3 ml of total ionic strength adjusting buffer (TISAB III) and the fluoride level was measured. The fluoride concentration for each sample

was determined 3 times and the average was used for analysis. The fluoride ion concentration was determined at 2, 4, 8, 12, 24, and 48 hours and then weekly for three months.

Statistical analysis

One-way analysis of variance (ANOVA) and Tukey's multiple comparison were used to test for differences in the mean fluoride concentration among the study groups (SPSS, version 20.0 for Windows; IBM, Armonk, NY). Significance was set at $p < 0.05$.

RESULTS

Our study groups differed by fluoride concentration ($p < 0.05$) (Table 2). A graph of the fluoride concentration overtime is shown in Fig 1. All tested varnishes released detectable fluoride. Mahidol (5%NaF+TCP) varnish had the greatest fluoride release at 24 hours. There was no detectable fluoride in the control group.

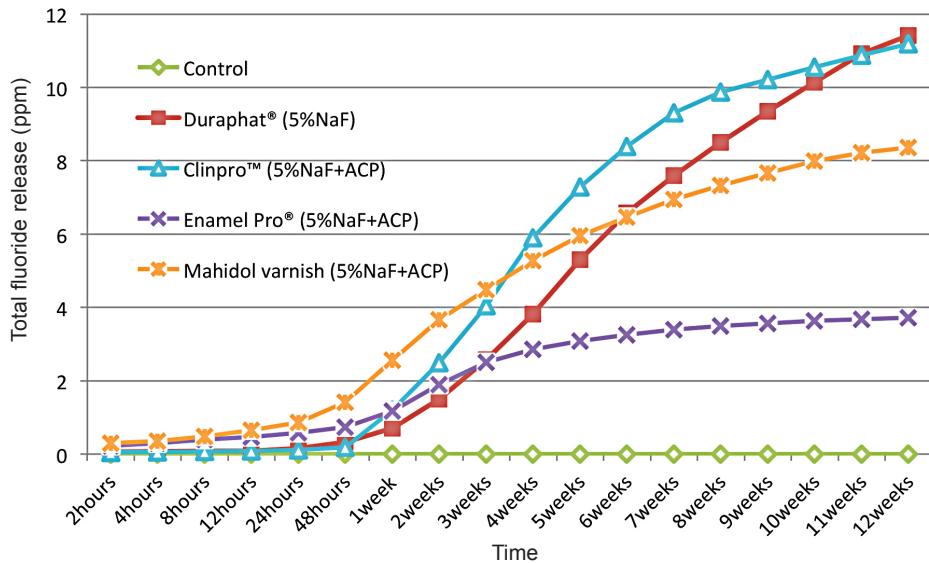


Fig 1–Average fluoride release (ppm) during 3 months evaluation.

The cumulative concentrations of fluoride released by 3 months, for the study groups ranging from the highest to the lowest were: Group B=C>E>D>A. The fluoride concentration released by all the treatment groups were significantly greater than the control group by 3 months. Duraphat® (5%NaF) and Clinpro™ (5%NaF+TCP) had the highest release of fluoride at 3-month evaluation. Clinpro™ White and Mahidol (5%NaF+TCP) varnish released more fluoride than Enamel pro® (5%NaF+ACP) varnish by 3 months.

The products with the greatest release rate of fluoride by week were: Mahidol (5%NaF+TCP) varnish during the first week, Enamel pro® (5%NaF+ACP) varnish during the second week, Clinpro™ (5%NaF+TCP) varnish during the fourth week and Duraphat® (5%NaF) varnish during the fifth week. Duraphat® (5%NaF) had a lower initial fluoride release than the other treatment groups but it had less of a decline in fluoride release than the

other treatment groups.

DISCUSSION

The fluoride release rates in our studied treatment groups continued for the 3 months of this study, similar to study by Castillo *et al* (2001, 2004). In our study, all the treatment groups released at least 0.03 ppm fluoride in the saliva, similar to a study by Garcia-Godoy and Hicks (2008).

The fluoride released by the studied treatment varnishes in our study varied by type of varnish, suggesting the resin carriers and additives can affect fluoride release (Shen and Autio-Gold, 2002; Jablonowski *et al*, 2012). In our study Enamel Pro® (5%NaF+ACP) released more fluoride than Clinpro™ (5%NaF+TCP) and Duraphat® (5%NaF) in the first 24 hours, similar to the findings of Cochrane *et al* (2014).

Jablonowski *et al* (2012) found Enamel Pro®(5%NaF+ACP) released significantly

Table 2
Average fluoride release (ppm).

	Group A Control	Group B Duraphat® 5%NaF	Group C Clinpro™ 5%NaF+TCP	Group D Enamel Pro® 5%NaF+ACP	Group E Mahidol varnish 5%NaF+TCP
2 hours	0	0.06 ±0.02 ^a	0.05± 0.01 ^a	0.22±0.03 ^b	0.30±0.08 ^b
4 hours	0	0.07±0.01 ^a	0.06±0.0 ^a	0.30±0.04 ^b	0.36±0.07 ^b
8 hours	0	0.08±0.01 ^a	0.07±0.01 ^a	0.39±0.01 ^b	0.48±0.07 ^b
12 hours	0	0.09±0.02 ^a	0.09±0.02 ^a	0.47±0.06 ^b	0.65±0.09 ^b
24 hours	0	0.16±0.07 ^a	0.12±0.03 ^a	0.58±0.07	0.87±0.13
48 hours	0	0.33±0.08 ^a	0.18±0.06 ^a	0.74±0.04 ^b	1.41±0.41 ^b
1 week	0	0.71±0.28 ^a	1.20±0.16 ^a	1.17±0.18 ^a	2.56±0.25
2 weeks	0	1.50±0.47 ^a	2.49±0.07 ^{a,b}	1.90±0.28 ^a	3.67±0.71 ^b
3 weeks	0	2.58±0.61 ^a	4.04±0.25 ^b	2.50±0.22 ^a	4.49±0.22 ^b
4 weeks	0	3.82±0.50 ^a	5.90±0.57 ^b	2.86±0.41 ^a	5.28±0.59 ^b
5 weeks	0	5.30±0.88 ^a	7.28±0.18 ^b	3.09±0.45	5.95±0.74 ^{a,b}
6 weeks	0	6.58±0.22 ^a	8.39±0.50	3.26±0.35	6.46±0.59 ^a
7 weeks	0	7.60±0.19 ^{a,b}	9.31±0.96 ^a	3.40±0.36	6.95±0.43 ^b
8 weeks	0	8.50±0.27 ^a	9.87±0.67 ^a	3.49±0.35	7.33±0.23
9 weeks	0	9.35±0.39	10.21±0.31	3.56±0.31	7.66±31
10 weeks	0	10.14±0.59 ^a	10.55±0.47 ^a	3.64±0.30	7.99±0.41
11 week	0	10.92±0.33 ^a	10.88±0.30 ^a	3.68±0.27	8.22±0.40
12 weeks	0	11.42±0.67 ^a	11.19±0.38 ^a	3.72±0.27	8.36±0.41

The same letters in the same row indicate no significant difference ($p \geq 0.05$).

more than Duraphat®(5%NaF); they postulated this was due to the fact ACP has a noncrystalline character and no systematic structure, making it more soluble and active than other crystalline calcium phosphates. ACP dissolves quickly and provides fast apatite reprecipitation for a demineralized lesion (Ulkur *et al*, 2014). Moreover, Milburn *et al* (2015) reported the fluoride release rate depends on the varnish viscosity. Enamel Pro®(5%NaF+ACP) was less viscous than the other varnishes, which could have resulted in greater fluoride release initially but more rapid depletion. Duraphat®(5%NaF) was more viscous than the other products and exhibited a more sustained and release over time, similar to studies by Castillo *et al*

(2001) and Jablonoski *et al* (2012).

The varnish released by Mahidol (5%NaF+TCP) had the greatest release rate of fluoride during the first 24 hours but less than Clinpro™(5%NaF+TCP) and Duraphat® (5%NaF) at 3-month evaluation. This could be due to the lower viscosity of the Mahidol varnish(5%NaF+TCP).

In our study, there were no difference in fluoride release rates by 3 months between Duraphat®(5%NaF) and Clinpro™ (5%NaF+TCP), but even though previous studies have shown a synergistic effect between calcium phosphate and fluoride (Cochrane *et al*, 2014), we did not find this in our study. This could be due to differences in type of specimens, type of buffer solution, different temperatures, and

duration of study (Jablonoski *et al*, 2012).

This *in vitro* study measured the level of fluoride release. It would appear the more fluoride a product release, the more fluoride available for the enamel to absorb. However, we did not study the fluoride uptake by enamel. The dynamics of human saliva on fluoride release was not evaluated. Fluoride varnishes have been developed to promote the formation of intraoral fluoride reservoirs due to the formation of calcium fluoride (CaF₂), 'calcium fluoride-like' or biologically/bacterially bound calcium fluoride (CaF₂) (Rošin-Grget *et al*, 2013). The formation of these reservoirs is limited by fluoride ion and calcium ion availability (Vogel, 2011). In our study, calcium release rate was not measured. Further studies are needed to investigate fluoride uptake and calcium and inorganic phosphate ion release from a variety of calcium and fluoride containing varnishes both with *in vitro* and *in vivo* trials. From our study, the addition of calcium and phosphate compounds to fluoride varnish did not affect the fluoride release. Duraphat[®] (5%NaF) and Clinpro[™] (5%NaF+TCP) had the highest release of fluoride at 3-month evaluation. Clinpro[™] (5%NaF+TCP) and Mahidol(5%NaF+TCP) varnish released more fluoride than Enamel pro[®] (5%NaF+ACP) fluoride varnish by 3 months.

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