EFFECTS OF FLUORIDE DENTIFRICE ON REMINERALIZATION OF DEMINERALIZED PRIMARY ENAMEL

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Abstract. This study was performed to compare the remineralizing effects of various concentrations of fluoride containing dentifrices against artificial demineralization of primary enamel. One hundred twenty primary incisors were partly covered with a nail varnish, leaving a 1x1 mm window, then placed in demineralizing solution for 96 hours to produce artificial carious lesions 60-100 μm in depth. They were assigned to 8 groups (A to H; *n*=15). Groups A-D were exposed to a half pea-sized portion of dentifrice (0.16 g) and groups E-H were exposed to a pea-sized portion of dentifrice (0.32 g), both groups with fluoride concentrations of 0, 250, 500 and 1,000 ppm. The pH-cycling method was carried out for 7 days, then the teeth were cut through the lesions and examined under a polarized light microscope; photographs were taken and analyzed. Lesion depth was measured using a computerized method using the Image-Pro® Plus Program. The results were analyzed using the One way ANOVA and LSD tests. The mean lesion depth in the 2 non-fluoridated control groups (A and E) were significantly deeper than in the fluoridated groups. There were no differences found between the half pea-sized and pea-sized dentifrice.

Key words: demineralization, dentrifice, fluoride, remineralization

INTRODUCTION

In the oral cavity there is a delicate balance of de-/remineralization of the enamel surface. The interruption of this balance results in dental caries; fluoride is a protective factor against dental caries (O'Mullane, 1994; Zero, 1999). Fluoridation of water, toothpaste, mouthrinse, gel and varnish have been used to enhance remineralization and reduce demineralization (O'Mullane, 1994; Shellis and Duckworth, 1994; Ten Cate and van Loveren, 1999). Toothpaste with fluoride was introduced into industrialized countries in the late 1960s and is now the most common vehicle delivering fluoride to the oral cavity (Twetman *et al*, 2004).

However, fluoride toothpaste may cause enamel fluorosis (Spencer and Do, 2008; Bronckers *et al*, 2009). The swallow reflex of children <6 years old, and especially in children <3 years old, is unpredictable (Siew Tan and Razak, 2005;

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Dincer, 2008). One way of reducing the amount of fluoride ingested is to reduce the level of fluoride in toothpaste to either 500 or 250 ppm (Ammari *et al*, 2003). Toothpaste with low fluoride concentrations (250, 500 and 550 ppm) have been marketed to reduce fluoride ingestion by young children in order to minimize the risk of dental fluorosis, but the effectiveness of this toothpaste is controversy (Bloch-Zupan, 2001).

A study of bovine enamel using pH cycling models showed with application of fluoride solutions at 275, 550 and 1,100 ppm four times a day, treatment groups had significantly greater percentages of recovery in surface microhardness and recovery of mineral loss compared to a placebo group without fluoride (Vieira *et al*, 2005). Damoto investigated the effects of various fluoride concentrations (0, 1, 250, 500, 1,000, 1,750 and 2,500 ppm) on enamel de/remineralization, over 5 weeks in an *in vitro* pH-cycling study of premolars. They found those treated with fluoride at 0 ppm and 1 ppm had demineralization. Remineralization was significantly greater in the 500 ppm fluoride group than the 250 ppm fluoride group. However, higher fluoride concentrations (1,000, 1,750 and 2,500 ppm) did not result in greater remineralization (Damato *et al*, 1990).

Another clinical trial found toothpaste with a fluoride concentration of 550 ppm had similar anti-caries efficacy to toothpaste with a fluoride concentration of 1,055 ppm (Winter *et al*, 1989).

There have been few studies of de/remineralization of carious lesions of enamel in primary teeth. Thaveesangpanich *et al* (2005a) studied two *in vitro* 7-day pH-cycling models. They found artificially caused caries of primary anterior teeth treated with a pea-sized quantity of toothpaste containing fluoride had a 20% increase in depth and area with the 7-day pH-cycling versus a 50% progression in depth and area in teeth treated with non-fluoride toothpaste, but the difference was not statistically significant. In another study by Thaveesangpanich *et al* (2005b), they found a pea-sized portion of toothpaste containing fluoride at 500 ppm slowed down demineralization progression better than a half pea-sized portion, the difference was significant.

The effectiveness of a low quantity of dentifrice needs to be studied further. Therefore, we investigated the effect of different quantities of dentifrice at different concentrations of fluoride on de/remineralization of the enamel of primary teeth.

MATERIALS AND METHODS

Sample selection

One hundred twenty primary incisors were collected from tooth extraction and naturally exfoliation. Teeth with sound enamel were selected to be use in this study.

Dentifrices used

All dentifrices were formulated and compounded by one of the authors. The dentifrices had the same ingredients, but the fluoride concentrations varied (0, 250, 500, 1000 ppm). The dentifrices were all prepared as slurry solutions by mixing with 30 ml deionized water and stirred with a magnetic stirrer (Carstir® model Cerastir, USA) at 150 rpm for 30 minutes.

Demineralizing and remineralizing solutions

The demineralizing and remineralizing solutions were prepared according to Thaveesangpanich *et al* (2005a). Demineralizing solution 1 (D1) consisted of 2.2 mM

$CaCl_2$, 2.2 mM NaH_2PO_4, 0.05 M acetic acid, with a pH adjusted to 4.4 with 1 M KOH. Demineralizing solution 2 (D2) contained the same components as D1, but the pH was adjusted to 4.7 with 1 M KOH. The remineralizing solution (R) was comprised of 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, 0.15 M KCl and adjusted to a pH of 7.0 with 1 M KOH. Demineralizing and remineralizing solutions were freshly prepared for each cycle and kept in separate plastic containers.

Specimen preparation

All samples were cleaned of soft tissue debris using a slurry of fluoride free pumice with a rubber cup. Primary incisors with hypoplasia or white spot lesions were excluded from the study. The teeth were kept in normal saline solution. The selected teeth were blot-dried with tissue paper and coated with acid resistant nail varnish (Revlon, USA), leaving a window of approximately 1x1 mm on the buccal surface. The root apices were sealed with sticky wax. Then, the teeth were immersed in deionized water until use.

Lesion formation

Each tooth was immersed in 3 ml of demineralizing solution 1 and incubated at 37ºC (Sheldon manufacturing, model 1545, Oregon, USA) for 96 hours to produce carious lesions of 60-100 µm deep (Thaveesangpanich *et al*, 2005a). Each tooth was then rinsed with 15 ml deionized water and wiped carefully with tissue paper. All teeth were processed in the same manner.

Test groups

One hundred twenty teeth were pooled and randomly assigned to eight groups, 15 sections per group. A half pea-sized portion of dentifrice (0.16 g) (Thaveesangpanich *et al*, 2005b) was used in groups A–D and a pea-sized portion of dentifrice (0.32 g) (Thaveesangpanich *et al*, 2005b) was used in groups E–H. The teeth were then placed in a self-cured acrylic block and immersed in deionized water until use. All groups were processed in the same manner.

pH-cycling process

The experimental process imitated the changes in pH of the oral environment for 7 days. All sections were subjected to the pH cycling procedure. Each cycle involved three hours of demineralization twice daily with two hours of remineralization in between. One-minute dentifrice slurry treatments were given before the first demineralizing cycle and both before and after the second demineralizing cycle. Then, all sections were placed in remineralizing solution overnight at 37ºC in a controlled environment incubator shaker (Series 25 Incubator Shaker®, USA) (150 rpm).

Thin section preparation

After completion of the 7-day pH cycle, each tooth was removed from the block. All teeth were cut longitudinal through the lesion using a slow speed diamond saw under copious water spray (Accutom–50, Streuers, Denmark) to create a thin section (approximately 400 µm thick). Then all thin sections were ground with wet 800 grit silicon carbide paper. The thickness of each thin section was measured by electronic digital caliper (Mitutoyo® model CD-6C, Japan). Sections with a thickness of 100 -150 µm were used.

Polarising light microscopy measurements

All sections were placed in water and examined at 10x magnification under a polarizing light microscope (Nikon® model eclipse E 400 pol, Japan) and photographs were taken with a digital camera (Nikon Coolpix 990, Japan). The pictures were then analyzed using a computerized calculation method with Image-Pro®Plus

(Media Cybernetics, MD, USA).

Intra-examination reliability

Twenty-four specimens (20% of all specimens) were randomly selected and re-examined by the same examiner under the same conditions using the same equipment. The intra-examination reliability was tested using the Pearson's correlation coefficient.

Statistical analysis

Means and standard deviations of lesion depth were calculated for each group. The Kolmogorov-Smirnov test (K-S test) was used to test the distribution of the data. The analysis of variance (ANOVA) and least significant difference (LSD) method were used to test differences in mean lesion depth among groups. A significant level of 0.05 was used for all statistical tests.

RESULTS

Results from duplicate examination showed the intra-examination reliability of the percentage of lesion depth as tested by the Pearson's correlation coefficient was 0.988, which shows good reliability.

The mean lesion depths are shown in Table 1. The mean lesion depth in the non-fluoride containing dentifrice group was higher than in the fluoride dentifrice group. The results of the mean lesion depths in the fluoridated dentifrice groups were significantly different from the control groups (Fig 1). However, no signifi-

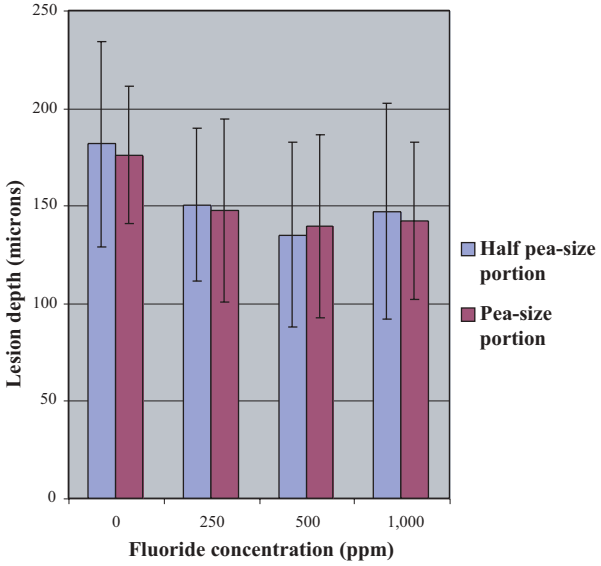

Fig 1–Graph showing mean lesion depth of all groups in primary teeth enamel after treatment.

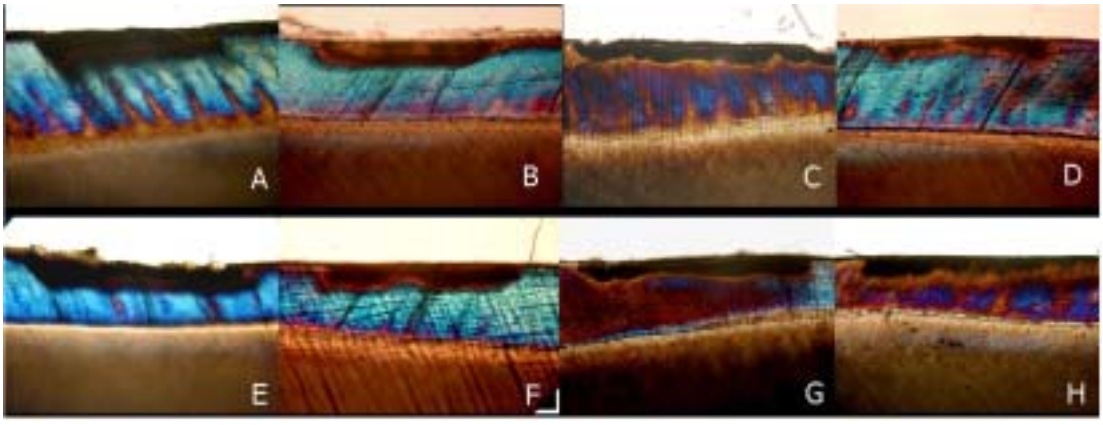

Fig 2–Polarized light photomicrograph of enamel lesion after treatment in Groups A-H.

Table 1
Mean (standard deviation) lesion depth in each group (n=15).

Fluoride concentration (ppm)	Dentifrice portion size		
	Half pea-sized	Pea-sized	Total
0	181.32 (52.61)	175.90 (35.46)	178.61 (44.17)[a]
250	150.38 (39.42)	147.50 (46.95)	148.94 (42.62)
500	134.89 (47.31)	139.35 (46.98)	137.12 (46.38)
1,000	146.85 (55.29)	141.93 (40.33)	144.39 (47.62)
Total	153.36 (50.79)	151.17 (44.13)	

[a]p = 0.05

cant differences existed among the fluoridated dentifrice groups.

There was no statistically significant difference in lesion depth between the half pea-sized and pea-sized portion groups.

When the mean lesion depths were analyzed with the interaction model (fluoride concentration *vs* dentifrice portion size), no statistically significant differences among the groups were seen (Fig 2).

DISCUSSION

The mean lesion depth in the non-fluoride containing dentifrice group was higher than in the fluoride containing dentifrice groups (p<0.05) because the fluoride-containing dentifrice was more effective at preventing progression of dental caries. Frequent, use of low fluoride concentration products which promote low and constant salivary fluoride levels have been accepted as an efficient way to prevent dental caries (Oliveby *et al*, 1987). Meta-analysis of 70 trials on the effectiveness of fluoride dentifrice compared to placebo for the prevention of dental caries in children, found clear evidence the use of fluoride dentifrice has a caries inhibiting effect on permanent dentition (Twetman *et al*, 2004). The review provided little information regarding the effect of fluoride toothpaste on caries incidence in primary dentition. An *in situ* study by Dijkman *et al* (1990) showed brushing with fluoride-free dentifrice treated samples did not result in a change in lesion depth, but using fluoride toothpaste at 1,250 ppm decreased lesion depth by about 35%.

This study compared the effect of various concentrations of fluoride dentifrice on the depth of enamel lesions in primary teeth. The results showed no significant difference existed in the depth of enamel lesions among fluoridated dentifrice groups. This observation is in agreement with a study by Winter *et al* (1989) which concluded that experimental toothpaste with fluoride at 550 ppm had a similar anticaries effect as toothpaste containing fluoride at 1,055 ppm. A study of bovine enamel (Vieira, 2005) using pH cycling revealed application of various concentrations of fluoride (275, 550 and 1,100 ppm) four times a day, gave better surface microhardness recovery and recovery of mineral loss compared to placebo (0 ppm), but there were no significant differences among the various concentrations of fluoride. De Kloet *et al* (1986) compared the differences in remineralization and fluoride uptake between dentifrices containing fluoride at 300 and 1,000 ppm on bovine enamel and found no statistically signifi-

cant difference between the two groups, in regard to fluoride uptake or susceptibility of the enamel to demineralization. However, Damoto *et al* (1990) concluded there was a linear fluoride dose effect when subjects used dentifrices containing fluoride at 0, 250 and 1,000 ppm. The differences in results may be due to the use of a different abrasive system and the type of fluoride used (Mellberg, 1991).

Most studies of dentifrice retention found young children frequently swallow sizable amounts of toothpaste while tooth brushing (Ripa, 1991; Levy *et al*, 1995). Inadvertent ingestion of toothpaste containing fluoride significantly increases fluoride intake by 2 to 6 year olds (Erdal and Buchanan, 2005). Children under 8 years of age should consume no more than 0.10 milligram fluoride per kilogram (mgF/kg) body weight to avoid an undesirable degree of fluorosis (Levy *et al*, 1995). Some studies recommend brushing should not commence until age two. A pea-sized (0.25-0.3g) amount of toothpaste on the brush is more than adequate to clean a young children's teeth. This amount is often exceeded, especially in children less than 4 years of age; they should use only a "smear layer" of low fluoride concentration toothpaste (Rock, 1994; Browne *et al*, 2005). Our study found no significant difference in lesion depth between half-pea sized and pea-sized groups at the same concentration of fluoride. The results of this study correspond to those by Thaveesangpanich *et al* (2005a,b) which found no significant differences between pea-sized and half pea-sized portions of toothpaste containing sodium fluoride at 500 ppm on artificially induced enamel caries on primary teeth. A small amount of fluoride toothpaste is preferable to reduce the risk of fluorosis. This suggests using only a half-pea sized portion of fluoride containing dentifrice is as effective in preventing dental caries as a pea sized portion of dentifrice containing fluoride.

In our study, we used the same pH-cycling model on primary teeth as that used by Thaveesangpanich *et al* (2005a), who found a pea-sized portion of toothpaste containing fluoride at 500 ppm significantly slowed down demineralization better than a half pea-sized portion. However, the first study by Thaveesangpanich *et al* (2005a) had contrasting results from the second study (Thaveesangpanich *et al*, 2005b). Our study had contrasting result from the first study by Thaveesangpanich *et al* (2005a), which may be due to a different type of fluoride used (Stookey *et al*, 1993). Study by Thaveesangpanich *et al* (2005a) used monofluorophostphate with a silica abrasive system, but our study used sodium fluoride with carboxymethylcellulose as a stabilizer. A different method was also used in determination of lesion depth, which may have contributed to these different results. Further studies are needed to clarify these differences.

In conclusion, fluoride containing dentifrice at 250, 500 and 1,000 ppm gave significantly greater remineralization than non-fluoride containing dentifrice. The lesion depths in the half pea-sized and pea-sized groups were similar and there were no differences in lesion depth among the various fluoride concentrations.

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