

# The remineralization quality by fluoridated dentifrice on artificial incipient caries lesion

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**Objectives:** To evaluate the surface hardness, fluoride content, and topography of artificial incipient caries remineralized using fluoride dentifrice.

**Materials and Methods:** Human enamel specimens (n = 100) were randomly assigned to 4 groups (n = 25): artificial saliva, 1,100-ppm, 1,450-ppm, and 5,000-ppm fluoride dentifrice (FD). Artificial incipient caries samples were immersed in fluoride dentifrice slurry twice for 2 min during a 10-day pH-cycling. The percentage of surface hardness recovery (%SMHR) was calculated. Topography was evaluated using scanning electron microscopy and fluoride content was analyzed by energy dispersive x-ray spectroscopy. The differences in the %SMHR and fluoride content were compared at the 95% confidence level.

**Results:** The significantly highest %SMHR and the highest fluoride content was found in the 5,000-ppm FD group. There was no significant difference in %SMHR or fluoride content between the 1,100-ppm and 1,450-ppm FD groups. The SEM image of the 5,000-ppm FD group specimens demonstrated a uniformed layer of densely packed calcium fluoride globules, while those of the 1,100-ppm and 1,450-ppm FD specimens were loosely packed.

**Conclusions:** The daily use of 5,000 ppm FD results in a homogenous scatter and densely pack of calcium fluoride globules while the regular use of 1,100 ppm and 1,450 fluoride dentifrice precipitates in a loosely cluster of calcium fluoride agglomerates. The difference in the pattern and amount of calcium fluoride may influence surface hardness.

**Keywords:** calcium fluoride, fluoride dentifrice, incipient caries, remineralization, surface hardness

**How to cite:** How to cite: Manosubsak N, Sukarawan W, Sriarj W. The remineralization quality by fluoridated dentifrice on artificial incipient caries lesion. M Dent J 2019; 39: 53-62.

## Introduction

Dental caries is caused by an imbalance between demineralization and remineralization. A white spot lesion is the earliest clinical sign of dental caries. Demineralization is a dynamic process, which can be reversed, or arrested. Demineralization can be interrupted at any stage of caries progression however, it is preferable to halt demineralization at the initial stage [1].

Detecting an initial caries lesion and evaluating its activity provides the opportunity to halt or reverse the demineralization and eliminate the need for a

restoration [2]. Fluoride is one of the most widely used caries prevention agents [3]. The maximum therapeutic effect of fluoride for cavity prevention is found with topical application on the enamel surface, suggesting that free fluoride ions in the solution surrounding the enamel crystals have an essential role [4,5].

When the fluoride concentration is increased to the range from 100 to 10,000 ppm due to the exposure to topical fluoride such as dentifrices, the formation of calcium fluoride (CaF<sub>2</sub>) takes place [6]. There are two steps in forming calcium fluoride. First, a slight dissolution of enamel surface

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Received : 11 February 2019

Accepted : 23 April 2019

results in the release of calcium ions [7]. The source of calcium may be either the enamel itself, remaining saliva, plaque fluid or calculus [8]. Second, calcium ions react with fluoride that is applied, thereby forming calcium fluoride globules [7]. These compounds precipitate not only on sound enamel surfaces but also on enamel carious lesions. This precipitated surface layer prevents further acid solubility of mineral in the deeper layer and more resistant to acid attack [9]. Therefore, the deposition of calcium fluoride in early carious lesions is expected to be an important mechanism in remineralization of caries.

The home-use of fluoride dentifrice (FD) is the principal method for preventing caries. FD significantly prevents caries at fluoride concentrations  $\geq 1,000$ -ppm [10]. Moreover, a study demonstrated that the caries preventive effect increased as the fluoride concentration increased [10]. It has been proposed that 1,500-ppm FD has a greater anti-caries effect compared with 1,000-ppm FD [11,12]. However, FD containing  $\geq 1,500$ -ppm is not widely available. Higher concentration fluoride dentifrices, including 5,000-ppm fluoride dentifrice, have been introduced for caries prevention in adolescents with high caries risk [13], adults and elderly, especially for preventing root caries [14,15].

Several in vitro studies were conducted to study the remineralization effect of 1,000-ppm FD on enamel evaluated by surface hardness [16, 17]. The application of 1,000-ppm FD significantly increased enamel surface hardness [18-21]. Moreover, dentifrice containing 1,100- and 1,450-ppm fluoride demonstrated enamel protection against an erosive challenge in vitro [22, 23]. The remineralization measured by mineral uptake in lesions after using 1,500-ppm FD was higher compared with 500-ppm FD [24]. Another study claimed that 5,000-ppm FD significantly enhanced mineral uptake in enamel lesions in bovine teeth compared with 1,500-ppm FD [25].

Most studies have quantified the remineralization effectiveness, e.g. percentage or depth of remineralization, of home-use topical fluoride agents [26-30]. However, the remineralization quality such as the surface hardness and topography of the remineralized surface are rarely investigated and disparate results. The objectives of this study were to evaluate the remineralization of incipient lesions after using 1,100-, 1,450-ppm or 5,000-ppm FD as shown by microhardness, scanning electron microscopy, and energy dispersive x-ray spectroscopy.

## Materials and methods

### Sample preparation

The study protocol was approved by the Human Ethics Committee, Faculty of Dentistry, Chulalongkorn University (HREC-DCU 2017-032). One hundred human permanent premolar teeth extracted for orthodontic reasons were used in this study. The buccal and lingual surfaces the teeth were examined visually using an explorer and stereomicroscope (10X magnification). Teeth that were free of caries, restorations, hypoplasia, cracks, white spot lesion, and other enamel defects were included in the study. The selected teeth were ground flat and polished using 1,000, 2,000, and 4,000 grit abrasive paper to expose fresh enamel and remove the fluoride rich layer that may interfere with demineralization during pH cycling. A slow speed cutting machine (ISOMET1000TM, Buehler, USA) was used to section the teeth into a  $2 \times 5 \times 3$  mm<sup>3</sup> blocks. Eighty samples were embedded in resin blocks. The surfaces of each sample were painted with red acid-resistant nail varnish except for a  $2 \times 5$  mm<sup>2</sup> window on the enamel surface. The baseline surface hardness of each sample was measured using a Microhardness tester machine (FM-810, FUTURE-TECH, Japan). Samples with microhardness ranging from 300 KHN[31]. Sample size was determined using the G\*Power 3.1.9.2

at a power of 0.8 and confidence interval, type-I error of 0.05. Samples were pooled and randomly assigned to 4 groups (n=20): artificial saliva, 1,100-ppm FD, (Colgate total<sup>®</sup>, Colgate-Palmolive, Thailand), 1,450-ppm FD, (Colgate total<sup>®</sup>, Colgate-Palmolive, England) and 5,000-ppm brush-on Gel, (Colgate PreviDent<sup>®</sup>, Colgate-Palmolive, Thailand).

### Artificial caries formation

The samples were immersed in demineralization solution for 4 days to create the initial caries lesions (150-200  $\mu\text{m}$  deep) [26]. The demineralization solution contained 2.2 mM  $\text{CaCl}_2$ , 2.2 mM  $\text{NaH}_2\text{PO}_4$ , and 0.05 M acetic acid at pH 4.4 adjusted using 1M KOH 3 ml as previously described [32]. The samples were stored at 37°C in an incubator shaker. The solution was changed every 24 hours [33]. After demineralization, each sample was rinsed in deionized water for 20 seconds and dried with tissue paper.

### Fluoride treatment and pH-cycling

The samples in group B, C and D were immersed in FD slurry 3 ml for 2 minutes. The dentifrice slurry was prepared by thoroughly mixing deionized water and dentifrice at a 3:1 ratio (by weight) [26]. The treated samples were stored separate containers in artificial saliva for 24 hours. The artificial saliva was prepared by mixing methyl-p-hydroxybenzoate 2.0 g, sodium carboxymethyl cellulose 10.0 g, KCl 0.625 g,  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  0.059 g,  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  0.166 g,  $\text{K}_2\text{HPO}_4$  0.804 g, and  $\text{KH}_2\text{PO}_4$  0.326 g in 1000 ml water, pH 7 [34]. The samples were subsequently subjected to 10 days pH cycling. Each cycle consisted of 3 hours demineralization, 2 hours remineralization, 3 hours demineralization, and 15 hours remineralization [6]. The demineralized

solution contained 2.2 mM  $\text{CaCl}_2$ , 2.2 mM  $\text{NaH}_2\text{PO}_4$ , and 0.05 M acetic acid, pH 4.7 adjusted using 1M KOH. The remineralized solution consisted of 1.5 mM  $\text{CaCl}_2$ , 0.9 mM  $\text{NaH}_2\text{PO}_4$ , and 0.15 M KCl, pH 7 adjusted using 1M KOH [32]. To exclude the potential influence of fluoride from other sources on the process of remineralization, both demineralized and remineralized solution contained no fluoride. The solutions were freshly prepared for each cycle. The samples in group B, C and D were immersed in this slurry 3 ml per sample after each demineralization session for 2 minutes. Each sample was thoroughly rinsed with deionized water for 20 seconds before being immersed in the remineralized solution. The time employed in rinsing the samples and changing the solution did not exceed 1 hour. The demineralization and remineralization cycles were continuously performed for 10 days.

### Surface hardness measurement

Surface hardness was determined using a microhardness tester machine. A Knoop diamond indenter was utilized. Before every indentation, an image of the surface was recorded to ensure that it was flat, clean, and free of damage. Five indentations were performed on each sample including sound enamel, demineralized enamel and fluoride treated enamel surface after 10 days pH cycling. The maximum indentation load was 50 g for 10 seconds [16,19]. The distance between the indentations was at least 100  $\mu\text{m}$ .

The mean values of the five measurements at baseline, after demineralization, and after pH cycling) were compared and the percentage surface hardness recovery was calculated as follows [16]:

$$\text{The percentage surface hardness recovery} = \frac{(\text{Hardness after pH cycling} - \text{Hardness after demineralization}) \times 100}{\text{Baseline hardness} - \text{Hardness after demineralization}}$$

## Scanning electron microscope (SEM) and energy dispersive spectroscopy (EDS)

Scanning electron microscope was utilized to examine the morphology of the enamel surface in each group and energy dispersive spectroscopy was used to detect the presence of fluoride ions on the sample surface. After pH-cycling, 5 samples from each group were placed on a carbon sheet and mounted on aluminum stubs for EDS analysis. EDS analysis was performed at an acceleration voltage of 15kV [35]. The resulting X-ray was processed identify the elements and each element's concentration. For each sample, ten 150  $\mu\text{m}$  x 150  $\mu\text{m}$  areas were randomly selected [36]. The elemental concentration of fluoride was calculated from the total elemental composition of the enamel surface and expressed as mean weight percent (wt%). After EDS analysis, 3 samples were then air dried, sputtered coated with gold and attached to aluminum stubs. The surface of the sample was scanned by SEM at 15,000X and 60,000X magnification and the most representative image of the enamel surface was captured. The size of globules was measured.

## Statistical analysis

The SPSS statistic 22 program was used for statistical analysis. The Kruskal-wallis and Mann-Whitney tests were performed to analyze the difference in the percentage of surface hardness recovery after 10 days pH cycling between the 4 groups at a 95% confidence level. The difference in the fluoride composition between the 4 groups was analyzed by one-way ANOVA and Games-

Howell test at a 95% confidence level.

## Results

### Surface hardness

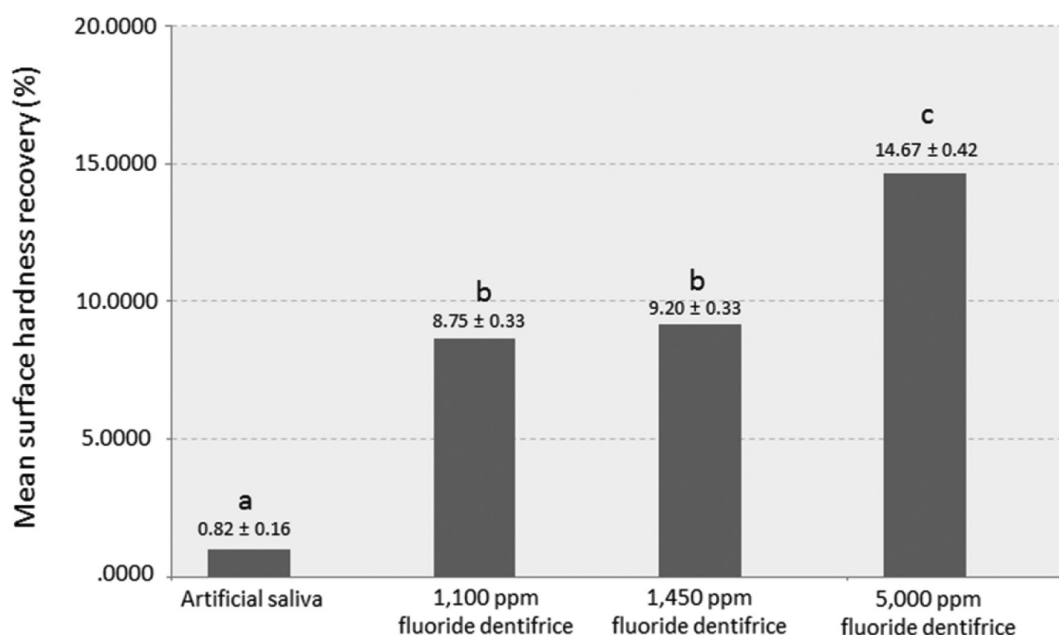
We determined the mean surface hardness at baseline, after artificial caries lesion formation, and after the pH-cycling, and the percentage surface hardness recovery (Table 1).

The groups had similar mean surface hardness at baseline and after artificial caries lesion formation ( $p=0.992$ ,  $p=0.927$ ). The FD treatment groups demonstrated a significant concentration-dependent increase (91.67–108.80 KHN) in enamel surface hardness ( $p < 0.001$ ) after pH cycling compared with the artificial saliva control group ( $71.27 \pm 2.56$  KHN). Moreover, the surface hardness of the 5,000-ppm FD group was significantly higher compared with the other FD groups. The percentage surface hardness recovery results revealed a similar pattern (Table 1, Fig. 1). The highest percentage surface hardness recovery ( $14.67 \pm 0.42\%$ ) was found in the 5,000-ppm FD group, which was significantly different compared with the other FD groups ( $p < 0.001$ ). Although the 1,100-ppm FD group demonstrated the lowest percentage surface hardness recovery ( $8.75 \pm 0.33\%$ ) among the FD groups, it was not significantly different from that of the 1450-ppm FD group ( $9.20 \pm 0.33\%$ ) ( $p=0.234$ ). In contrast, the control group had the significantly lowest percentage surface hardness recovery ( $0.82 \pm 0.16\%$ ) ( $p < 0.001$ ).

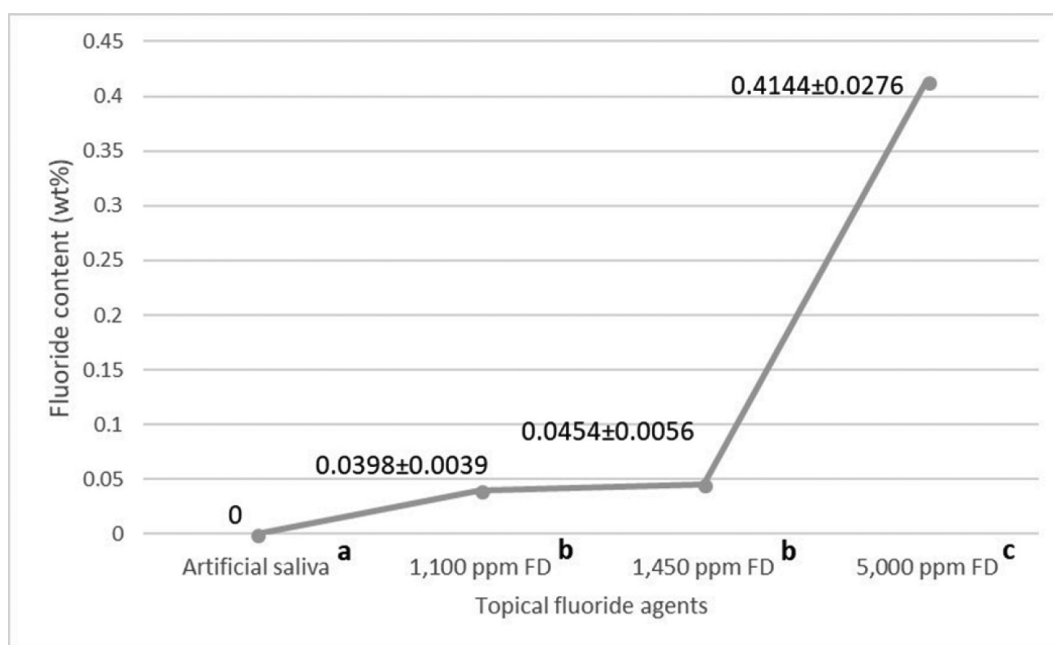
**Table 1** The mean surface hardness of the groups at baseline, after artificial caries lesion formation, after the pH-cycling, and the percentage surface hardness recovery

Treatment	Surface hardness (KHN)			Percentage surface hardness recovery (%)
	Baseline (KHN)	After demineralization (KHN)	After pH-cycling (KHN)	
Artificial saliva	$330.16 \pm 5.06^a$	$69.15 \pm 2.62^a$	$71.27 \pm 2.56^a$	$0.82 \pm 0.16^a$
1,100 ppm FD	$330.36 \pm 4.57^a$	$68.74 \pm 2.99^a$	$91.67 \pm 2.28^b$	$8.75 \pm 0.33^b$
1,450 ppm FD	$329.32 \pm 3.90^a$	$68.18 \pm 1.90^a$	$92.22 \pm 1.33^b$	$9.20 \pm 0.33^b$
5,000 ppm FD	$330.06 \pm 3.99^a$	$70.49 \pm 2.21^a$	$108.80 \pm 1.77^c$	$14.67 \pm 0.42^c$

Different superscript letters in the same column indicate significant differences between groups ( $p < 0.001$ ).



**Figure 1** The mean and standard deviation of percentage surface hardness recovery. Different letters indicate significant differences between groups ( $p < 0.001$ )



**Figure 2** The mean and standard deviation of the enamel surface fluoride content of the groups after 10 d pH-cycling. Different letters indicate significant differences between groups ( $p < 0.005$ ).

### Fluoride content of remineralized surface

The mean and standard deviation of the enamel surface fluoride content after 10 d pH-cycling was analyzed (Fig. 2). The FD groups demonstrated significantly increased enamel surface fluoride content compared with the control group ( $p < 0.005$ ). The 5,000-ppm FD

group showed the highest fluoride content ( $0.4144 \pm 0.0276\%$ ) followed by the 1,450-ppm FD ( $0.0454 \pm 0.0056\%$ ) and 1,100-ppm FD ( $0.0398 \pm 0.0039\%$ ). The 5,000-ppm FD group had a significantly higher fluoride content compared with the 1,450-ppm and 1,100-ppm FD ( $p < 0.005$ ). However, no significant difference in fluoride

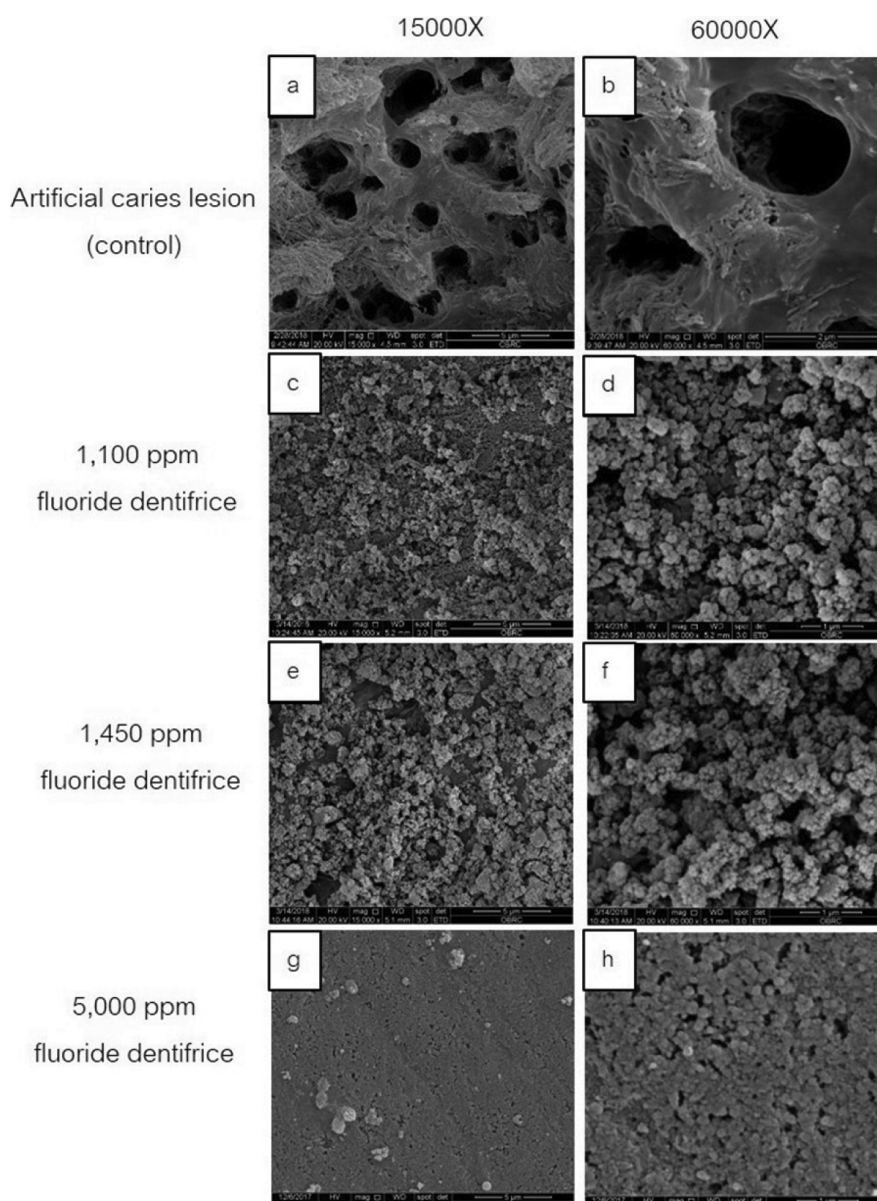


content was observed between the 1,450-ppm and 1,100-ppm FD groups ( $p=0.844$ ). No fluoride was detected on the control group enamel surface.

### The SEM images of remineralized surface

We used SEM to observe the enamel surface morphology of each group. The control samples demonstrated a porous appearance to confirm our protocol in artificial caries formation (Fig. 3a, 3b). The SEM images of the enamel surface of the 1,100-ppm (Fig. 3c, 3d) and 1,450-ppm FD (Fig. 3e, 3f) group samples revealed loosely packed clusters of spherical fluoride globules.

The individual globules ranged from 20-100 nm in diameter. The remineralized surface morphology of the 1,100-ppm and 1,450-ppm FD group samples was similar. The fluoride globules formed on the surface of the 5,000-ppm FD group samples (Fig. 3g, 3h) showed a distinctive pattern compared with those formed on the 1,100-ppm and 1,450-ppm FD samples. The 5,000-ppm FD group samples had a uniform layer of densely packed 60-100 nm in diameter calcium fluoride globules. The spherical globules on the 5,000-ppm FD samples were less varied in size compared with those in the 1,100-ppm and 1,450-ppm FD group.



**Figure 3** Representative scanning electron microscope images of the enamel samples.

## Discussion

In the present study, the effect of 1,100-, 1,450- and 5,000-ppm FD on the remineralization of incipient enamel caries lesions after pH cycling based on surface hardness and SEM and EDS analysis were investigated. We found a dose-dependent increase in surface hardness recovery. The SEM images demonstrated a similar dose-dependent increase in globules on the initial caries surface that EDS identified as calcium fluoride.

Each FD treatment significantly increased the early caries lesion surface hardness compared with the control group. The surface hardness of the 5,000-ppm FD group was the significantly highest among the FD treated group, while that of the 1,110-ppm and 1,450-ppm FD groups were not significantly different. Surface hardness measurement is one of the several methods used to measure the remineralization effect. Surface hardness is a mechanical property of material which represents the ability of materials to withstand elastic deformation, plastic deformation as well as destruction [37]. The surface hardness measurement is widely used in the study of remineralized effect of topical fluoride agents [16, 20, 21].

Surface hardness indicates the degree of mineralization of tooth surface which directly depended upon the mineral content. The increase in microhardness of initial caries lesion results from the precipitation of calcium fluoride which creates a barrier on the tooth surface [38]. The surface layer contains higher amount of fluoride in the form of calcium fluoride making it more resistant to caries and increase the surface hardness. When the fluoride concentration is increased in the range from 100- to 10,000-ppm by the use of topical fluoride agents such as dentifrices. These calcium fluoride globules can deposit on the surface creating the layer on the initial caries lesions [6]. Topical fluoride agents play an important role in

remineralization owing to the formation of calcium fluoride on the surface of initial caries [39].

The findings of our study imply that the use of FD results in the deposition of a protective layer of calcium fluoride on surface of initial caries lesion and this layer increase surface hardness. Our surface hardness recovery results correspond with the morphological images of calcium fluoride globules and the amount fluoride in the different FD groups. Furthermore, this study determined a potential mechanism by which different fluoride concentration in dentifrice affect the physical properties of enamel. The use of a higher fluoride concentration resulted in higher fluoride level availability on the treated surface and a higher calcium fluoride globules formation which improved the surface hardness of initial caries lesion.

FD has been used as a primary intervention for the prevention of caries in all ages. Varied fluoride concentration is available ranged from a low concentration like 500-ppm to a high concentration such as 5,000-ppm FD. The standard fluoridated dentifrice containing 1,000-ppm fluoride concentration has been subjected in considerable in vitro, in situ and even a clinical trial [20, 40, 41]. According to Cochrane review, the caries preventive effects increased with the higher fluoride concentration. The caries preventive effect was significant only for fluoride concentrations of 1,000-ppm and above [10]. Center for Disease Control (CDC) stated that dentifrice containing 1,500-ppm fluoride has been reported to be slightly more efficacious in reducing dental caries in U.S. and European studies. This fluoridated dentifrice with this concentration has been marketed in the United States, but it is not widely used in all countries, including Thailand. These formulations might benefit children over 6 years old living in the non-fluoridated area considered to be at high risk for dental caries [42,43]. However, the risk of fluorosis should be considered when FD is used in children.

Another factor to consider in caries prevention is the frequency of tooth brushing. The study showed that tooth brushing less than two times a day significantly caused an increment of caries lesions when compared with two times a day and more tooth brushing [44]. We found that there was no significant difference in surface hardness after treatment with 1,100-ppm or 1,450-ppm FD which was in accordance with some studies [22, 23]. However, several studies found a significant difference [11, 12, 24]. This can be implied that the use of 1,000-ppm FD at least twice a day may be adequate in caries prevention.

The use of daily brushing with 1,100-ppm and 1,450-ppm FD is a low concentration and high frequency approach that results in a slow F penetration in to the deeper subsurface caries lesion and has been a basic method for preventing caries for decades [45]. However, the typical method may not enough for the patient with active caries. The use of 5,000-ppm FD surprisingly produced a protective layer on the initial caries lesion. Consequently, 5,000-ppm FD should be considered as one of the alternatives for adolescents with high caries risk [13], adults and elderly, especially for preventing root caries [14,15]. The use of this FD recovered the surface hardness of the initial lesion to a certain extent. If the application of FD continues, the hardness may be recovered more, and reach the baseline surface hardness. The recovered surface from the use of these topical fluoride agents and a protective layer may provide a protection against erosive challenges. These FDs should be considered for the further study.

## Conclusions

Each fluoride dentifrice reacts with the initial caries surface differently, producing its own pattern of calcium fluoride globules formation. After 10 days pH-cycling, the daily use of 5,000-ppm FD resulted in homogenous and densely packed calcium fluoride

globules, while the use of 1,100-ppm and 1,450-FD resulted in the precipitation of loosely clustered calcium fluoride globules. The remineralization as evidenced by surface hardness increased fluoride concentration-dependently. The daily use of 1,000-ppm FD may be adequate for preventing caries. A higher fluoride concentration dentifrice such as 5,000-ppm FD should be considered an alternative for adolescents with high caries risk, adults and elderly, especially for preventing root caries.

## Acknowledgements

The authors are grateful to Dr. Chutima Trairatvorakul and Dr. Kevin Tompkins for reviewing the manuscript, Dr. Soranun Chantarangsu for her help in statistical analysis, Dr. Kanet Wongravee and Dr. Supeera Nootchanat for their assistance in the EDS analysis. Funding for this research was provided by grant from the Faculty of Dentistry, Chulalongkorn University.

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