Effect of Dentifrices on Early Enamel Lesion in Primary Teeth: *in vitro study*

ผลของยาสีฟันต่อรอยโรคฟันผุระยะแรกบนผิวเคลือบฟันน้ำนม: การศึกษาในห้องปฏิบัติการ

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ABSTRACT

Fluoride is the most effective agent for caries prevention. In Thailand, there are many types of dentifrice that contain various concentrations of fluoride with additional ingredients, such as xylitol. The aim of this study was to evaluate the effect of fluoridated dentifrices with and without xylitol by micro-CT : 1000 ppm MFP (Colgate® Original), 500 ppm NaF (Kodomo Lion® Gel), and 500 ppm NaF with xylitol (Kodomo Lion® Xylitol Plus) on early enamel lesion of primary teeth *in vitro*. Forty primary tooth samples were prepared and produce artificial carious lesions 150-200 μm deep, then randomly divided into four groups (artificial saliva as control group), and finally underwent 7-day pH cycling at 37˚C. Micro-CT was used to evaluate mineral density before and after pH cycling. Fluoridated dentifrice 500 ppm NaF with xylitol tended to inhibit further demineralization better than 500 ppm NaF alone but less than 1000 ppm MFP. In conclusion all tested dentifrices showed no significant effect by micro-CT on early enamel lesion of primary teeth under condition of 7-day pH cycling model.

บทคัดย่อ

ฟลูออไรด์เป็นสารที่ได้รับการยอมรับว่ามีส่วนช่วยในการป้องกันฟันผุ ปัจจุบันในประเทศไทยมียาสีฟันที่มีความเข้มข้นของฟลูออไรด์แตกต่างกัน บางชนิดได้เพิ่มสารชนิดอื่น เช่น ไซลิทอล เพื่อหวังเพิ่มประสิทธิภาพของยาสีฟัน ฟันน้ำนม 40 ซี่ถูกเตรียมและทำให้เกิดรอยโรคฟันผุระยะแรกที่มีความลึก 150-200 ไมโครเมตร หลังจากนั้นนำไปผ่านการเลียนแบบสภาวะการเปลี่ยนแปลงสภวะความเป็นกรดด่างในช่องปากเป็นเวลา 7 วัน ยาสีฟันผสมฟลูออไรด์ 1000 ppm MFP (ยาสีฟันคอลเกตออริจินัล) 500 ppm NaF (ยาสีฟันโคโดโมไลออนเจล) และ 500 ppm NaF ผสมไซลิทอล (ยาสีฟันโคโดโมไลออนไซลิทอลพลัส) เป็นกลุ่มทดลอง และน้ำลายเทียมเป็นกลุ่มควบคุม ใช้เครื่องเอกซเรย์คอมพิวเตอร์ระดับไมโครเมตรประเมินการเปลี่ยนแปลงของความหนาแน่นแร่ธาตุก่อนและหลังทดลอง พบว่ายาสีฟันฟลูออไรด์ 500 ppm NaF ผสมไซลิทอลมีแนวโน้มยับยั้งการดำเนินของรอยโรคฟันผุระยะแรกได้ดีกว่ายาสีฟันที่มีแต่ฟลูออไรด์ 500 ppm NaF เพียงอย่างเดียวแต่น้อยกว่ายาสีฟันฟลูออไรด์ 1000 ppm MFP อย่างไรก็ดียาสีฟันทุกชนิดที่ใช้ในการทดลอง ไม่มีผลต่อรอยโรคฟันผุระยะแรกอย่างมีนัยสำคัญทางสถิติ เมื่อวัดค่าด้วยเครื่องเอกซเรย์คอมพิวเตอร์ระดับไมโครเมตรภายใต้สภาวะที่กำหนด

Key Words: Dentifrice, Fluoride, Micro-computed tomography (micro-CT)

คำสำคัญ: ยาสีฟัน ฟลูออไรด์ เครื่องเอกซเรย์ระดับไมโครเมตร

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Introduction

Dental caries is the major cause of tooth pain and tooth lost in Thai children. (Bureau of Dental Health, 2013) The clinical outcome of a dental carious lesion is determined by the dynamic balance between demineralization and remineralization. When consuming sugar substance, plaque bacteria on tooth enamel will produce acid by fermentation. Enamel crystals will dissolve from the subsurface enamel when the pH of the oral cavity is below 5.5. But if there are supersaturation of Ca^{2+}, PO_4^{3-} and OH^- in saliva and oral fluid, remineralization can occur. (Ole Fejerskov & Edwina Kidd, 2008) Early enamel lesions which that have a shining hard surface can progress if no preventive steps are taken. However, when the tooth structure becomes cavitated, a dental restoration is needed to restore the function, integrity, and morphology of the missing tooth structure. Dental treatment has a high cost and can be complicated, especially in children who are anxious or display limited cooperation. The successful dental treatment of children may require basic behavior management, advanced behavior management, or pharmacological behavior management, such as treatment under sedation or general anesthesia.

Enamel crystals differ from pure hydroxyapatite because they can contain other inorganic ions such as carbonate ions or fluoride ions. The inclusion of fluoride ions results in fluorhydroxyapatite which usually precipitate on surface of enamel under dental biofilm. Fluorhydroxyapatite is more resistant to demineralization than hydroxyapatite. (Buzalaf et al., 2011) Fluoride ions are mainly introduced to oral cavity via topical application. Systematic reviews indicated that brushing with a fluoridated dentifrice significantly reduced the incidence of dental caries. (Marinho et al., 2003; Walsh et al., 2010) However, excessive use of a fluoridated dentifrice by children less than 2 years of age is a risk factor for dental fluorosis in the permanent dentition, so young children are recommended to use fluoridated dentifrice with less than 1000 ppm fluoride. (Wong et al., 2010) Although the use of a low concentration of fluoridated dentifrice may carry a lower risk of fluorosis, this must be balanced against the consequent reduction in its cariostatic effect.

Xylitol is a polyol (sugar alcohol), and has been used for many years as a non-acidogenic sweetener in numerous products. A major advantage of xylitol is that it cannot be fermented by plaque bacteria. Xylitol-containing products including chewing gum, mouth rinses, and solutions have been shown to reduce the incidence of dental caries and enamel demineralization, and to promote the remineralization of artificially demineralized enamel. (Rekola, 1986; Smits & Arens, 1988; Scheinin et al., 1993; Mäkinen et al., 1995; Miake et al., 2003) A study has suggested that including xylitol into a fluoridated dentifrice might be more effective in enhancing remineralization than fluoride alone. (Sano et al., 2007) In Thailand, there are many dentifrices for children that contain xylitol in addition to 500 ppm fluoride. But their effectiveness in the remineralization of early carious primary tooth lesions is still unresolved.

There are many methods for evaluating the effectiveness of a treatment on remineralization, such as the surface microhardness test, cross-sectioned microhardness test, polarized light microscopy, and transverse microradiography. However, these methods require time-consuming preparation of serial sections and the tooth samples are destroyed. Micro-computed tomography (micro-CT) is a form of CT scanning that

was mainly developed for laboratory purposes to analyze structure or mineral density of bone or tooth samples. (Anderson et al.,1996; Davis & Wong, 1996; Dowker et al., 2003) The major advantages of micro-CT are that it is a non-invasive and non-destructive procedure and that it can provide high quality analytical data and internal visualization in three dimensions making it suitable for longitudinal studies. Currently, commercial micro-CT devices are used in dental research for qualitative analysis and three-dimensional evaluation of the mineral content of enamel and dentin, dental materials, dental treatment procedures, and dental products. (Dowker et al., 2003; Liu et al., 2012) It has been found that using micro-CT images is a more reliable method for making linear measurements compared to direct measurements. (Kim et al., 2007)

Objectives of the study

To evaluate the effect of 1000 ppm fluoridated dentifrice, 500 ppm fluoridated dentifrice and 500 ppm fluoridated dentifrice with xylitol on early enamel lesion of primary teeth *in vitro*.

Methodology

The protocol for this study was approved by the Ethics Committee of the Faculty of Dentistry, Chulalongkorn University, Bangkok (number 73/2556). Forty sound extracted or naturally exfoliated primary incisors were selected from a pool of teeth stored in 0.1% thymol from a number of dental hospitals in Thailand. A hard tissue microtome (1000, BUEHLER, USA) was used to reduce the teeth leaving an approximately 1x1 mm wide labial surface of enamel. The surfaces of the tooth sample, except the labial enamel, were coated with acid-resistant nail varnish (Revlon®, New York, USA). The tooth samples were mounted with sticky wax into a custom made resin block that fit the micro-CT holder. (Fig 1)

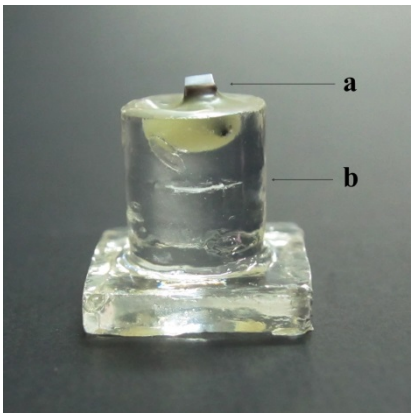

Fig 1 Custom made resin block for tooth sample (a) tooth sample showing 1x1 mm labial enamel surface at the top of the sample, (b) reference line

The tooth samples were immersed in demineralizing solution containing 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, 0.05 M acetic acid, and 1 M KOH (pH = 4.5) at 37˚C for 48 hours to produce artificial dental carious lesions approximately 150-200 µm deep. (Rirattanapong et al., 2010) The tooth samples were randomly assigned to four experimental groups (n=10). The experimental groups were 1000 ppm monofluorophosphate (MFP): Colgate® Original, 500 ppm sodium fluoride (NaF): Kodomo Lion® Gel, 500 ppm NaF with xylitol: Kodomo Lion® Xylitol Plus, and artificial saliva as control.

The dentifrice slurry was prepared by thoroughly mixing a 3-to-1 ratio of deionized water and dentifrice (by weight) followed by centrifugation at 4000 rpm for 20 minutes. Fresh dentifrice slurries were prepared and stored in separate containers designated for each experimental group. The tooth samples were subjected to pH cycling for 7 days at 37˚C. Each cycle consisted of a sequence of a

one-minute dentifrice slurry treatment, 3 hours of demineralization. 2 hours of remineralization, one-minute dentifrice slurry treatment, 3 hours of demineralization, one-minute dentifrice slurry treatment, and finally placed in remineralizing solution overnight. The demineralizing solution used in the demineralization portions of the pH cycle was the same as that used to develop the artificial lesions with the exception of pH (4.7), and the remineralizing solution contained 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, 0.15 M KCl, and 1 M KOH (pH = 7.0).

A micro-CT scanner (μCT 35, Scanco, Switzerland) was used to assess the mineral content of the tooth samples after artificial carious lesion induction and after pH cycling (pre-test and post-test values). The x-ray source was set at 70kVp and 114 μA. The integration time was 400 milliseconds. The samples were scanned 1 mm deep from the top of the enamel at 1024x1024 pixels resolution. Each reference of each tooth sample was used at every evaluation point to obtain the same area of interest. (Fig 2) To distinguish enamel from dentin, a visually determined threshold of 1500 mgHA/cm^3 was applied to all tooth samples. To measure the mineral content of the samples, the linear attenuation coefficients were converted to mineral concentration values assuming that the component absorbing the x-ray was calcium hydroxyapatite. A hydroxyapatite phantom was used to check the calibration of the system. Five different densities were used (0, 100, 200, 400, and 800 mgHA/cm^3), and their attenuation coefficients, density values, and standard deviations were checked weekly to ensure the stability of the x-ray tube.

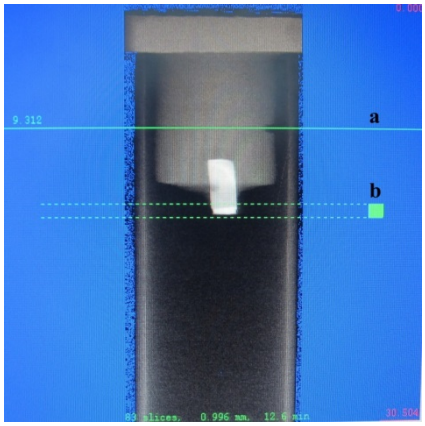

Fig 2 Custom made resin block with tooth sample in micro-CT holder before scanning. (a) Reference line, (b) 1 mm area of interest

The data collected from micro-CT scanner were the mineral density (MD) values from surface of the enamel to 200 μm deep at pre-test and post-test. Percentage of mineral density change was calculated from the formula:

$$\% \text{ MD change} = \frac{MD_{post\text{-}test} - MD_{pre\text{-}test}}{MD_{pre\text{-}test}} \times 100$$

The mean and standard deviation of MD was calculated for each group. The Kolmogorov-Smirnov test (one-sample K-S test) was used to analyze the distribution of the data. Differences between pre-test and post-test values were compared using the paired t-test. The ANOVA test was used to analyze the differences in percentage of MD change between the groups. A significant level of 0.05 was used for all statistical tests.

Table 1 Mean mineral density (MD) and percentage of mineral density (MD) change

No.	Dentifrice	F^- source	Mean MD ($mgHA/cm^3$)		% MD change
			Pre-test	Post-test	
1	Colgate® Original	1000 ppm MFP	1937.9171 ±81.2580	1933.2756 ±49.0688	-0.1576 ±2.5559
2	Kodomo Lion® Gel	500 ppm NaF	1969.6019 ±43.2492	1949.8961 ±52.5024	-1.0014 ±1.4242
3	Kodomo Lion® Xylitol Plus	500 ppm NaF + xylitol	1927.6298 ±36.6621	1919.0346 ±33.9261	-0.4378 ±1.1393
4	Artificial saliva	None	1928.8513 ±53.8374	1912.3122 ±63.0661	-0.6178 ±1.7108

Results

The mineral density was measured at the same area of each tooth sample before and after pH cycling (pre-test and post-test). The means and standard deviations of all experimental groups are shown in Table 1. There were no significant differences found between the post-test and pre-test MD values in each group ($p>0.05$). There were also no statistically significant differences in percentage of mineral density change between the experimental groups ($p=0.66$).

Discussion and Conclusions

In this present study, all tooth samples showed no significantly different in early enamel lesion behavior from each other before pH cycling. There were also no significantly different of MD of each tested dentifrices after pH cycling. The less minus percentage of MD change, the more tested dentifrice had demineralizing inhibition effect on early enamel lesion. Although all tested dentifrices did not had significantly different of percentage of mineral density change, the 1000 ppm MFP dentifrice tended to inhibit further demineralization better than 500 ppm NaF with xylitol, 500 ppm NaF without xylitol, respectively.

The use of 7-day pH cycling model for primary tooth had been proved appropriated in using to imitate daily oral environment of human. (Thaveesangpanich et al., 2005a) According to studies that use this method, some found only significantly different of less increase of lesion depth after pH cycling. (Yimcharoen et al., 2011; Thaveesangpanich et al., 2005b) On the other hand, some studies found significant reduction of lesion depth after pH cycling. (Itthagarun et al., 2007; Ekambaram et al., 2011) In this study, we found out that fluoridated dentifrices with and without xylitol didn't have significantly remineralized effect on early enamel lesion of primary teeth by analysis of mineral density change from micro-CT. This might be due to different method of evaluation. Although micro-CT might be able to use substitute to microradiograph, polarization light microscope or transverse microradiography. (Lo et al., 2011) But the data analyzed by micro-CT images are generated from the reconstruction of the linear attenuation coefficient within an object from the attenuation measurements of an X-ray beam passing through the sample at different viewing angles.

Differences in linear attenuation coefficient among different tissues are responsible for X-ray image contrast, which allows quantitative analyses to be made.

Although the major advantages of micro-CT are that it is a non-invasive and non-destructive procedure and that it can provide high quality analytical data and internal visualization in three dimensions making it suitable for longitudinal studies, but the disadvantage is creation of fixed position on the tooth samples for same scanning area in every period of time. In this study, we create a reference line for every tooth sample on custom made resin block which can make sure that scanning and analyzing the same area of interest.

Studies of preventive effect of fluoride in clinical trial participants usually were in mixed or permanent dentition (Marinho et al., 2003; Walsh et al., 2010) and in a dose response effect (Cain et al., 1994; Lagerweij & ten Cate, 2002; Negri & Cury, 2002) But the conclusion in primary dentition is still unclear. Although this study showed no statistically different in percentage of MD change in early enamel lesion of primary teeth, but dentifrice with higher fluoride concentration (1000 ppm MFP) seemed to retard caries progression more than lower fluoride concentration (500 ppm NaF with and without xylitol).

The effectiveness of using fluoridated dentifrice (MFP or NaF) was showed in clinical studies that could reduce caries increment. (Stephen et al., 1994; Fan et al., 2008) But *in vitro* study, 1000 ppm MFP only a minor inhibitory effect on lesion formation of dental enamel and had the least deposited of fluoride ions and phosphate ions on enamel surface, which was not statistically significant different to 30 ppm solution of NaF. (Toda & Featherstone; 2008) This might be due to sodium MFP can be able to release fluoride ions only when hydrolyzed by plaque enzyme. (Pearce & Dibdin, 1995; Pearce & Dibdin, 2003; Tzanavaras & Themelis, 2001) In this study, 1000 ppm MFP dentifrice (Colgate® Original) didn't show significantly change of mineral density after pH cycling may be because of this pH cycling model consists of only an inorganic solution so the enzyme system required for MFP hydrolysis was absent.

Although, there was no significant difference between experimental groups, but there was found that artificial saliva using in this study had effectiveness more than the 500 ppm NaF without xylitol. Artificial saliva using in this study according to the formulation used by Amaechi. (Amaechi et al., 1999) The artificial saliva contained potassium chloride, magnesium chloride, calcium chloride, dipotassium hydrogen phosphate and potassium dihydrogen phosphate, which may help remineralize enamel. Panich and Poolthong (2009) also found the increase of enamel hardness surface when using artificial saliva in their study. (Panich & Poolthong, 2009)

In conclusion, all tested dentifrices showed no significantly effect on early enamel lesion of primary teeth by micro-CT under condition of 7-day pH cycling model.

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