การเปรียบเทียบความแข็งผิวของเคลือบฟันน้ำนมภายหลังการใช้ฟลูออไรด์วานิชสองชนิด

Comparison of Surface Microhardness of Enamel after Fluoride Varnish Application on Primary Teeth

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บทคัดย่อ

ฟลูออไรด์วานิชถูกนำมาใช้ในการส่งเสริมกระบวนการคืนกลับของแร่ธาตุอย่างแพร่หลายมาเป็นเวลาหลายปี ซึ่งเมื่อไม่นานมานี้ก็ได้มีการเติมแคลเซียมและฟอสเฟตไอออนเข้ามาในฟลูออไรด์วานิช เพื่อเพิ่มกระบวนการการคืนกลับของแร่ธาตุ มีวัตถุประสงค์เพื่อประเมินผลของ โซเดียมฟลูออไรด์วานิชความเข้มข้นร้อยละ 5 ที่มีส่วนผสมของอะมอร์ฟัสแคลเซียมฟอสเฟต (Enamel Pro®) เปรียบเทียบกับ โซเดียมฟลูออไรด์วานิชความเข้มข้นร้อยละ 5 (Duraphat®) โดยวัดค่าความแข็งผิวของรอยผุบนฟันน้ำนม ดำเนินการโดย ฟันหน้าน้ำนมที่ไม่มีรอยผุจำนวน 27 ซี่ จะถูกสุ่มแบ่งออกเป็น 3 กลุ่ม (เอ บี ซี) กลุ่มละ 9 ซี่ ทุกกลุ่มถูกนำไปเข้ากระบวนการที่ทำให้เกิดการสูญเสียแร่ธาตุ และนำไปวัดค่าความแข็งผิวเริ่มต้นด้วยเครื่องทดสอบวิกเกอร์ จากนั้น กลุ่มเอจะนำไปแช่ในน้ำกลั่น (กลุ่มควบคุม), กลุ่มบีทาด้วยโซเดียมฟลูออไรด์วานิชความเข้มข้นร้อยละ 5 (Duraphat®) และกลุ่มซีทาด้วยโซเดียมฟลูออไรด์วานิชความเข้มข้นร้อยละ5 ที่มีส่วนผสมของอะมอร์ฟัสแคลเซียมฟอสเฟต (Enamel Pro®) และนำไปผ่านกระบวนการจำลองการเปลี่ยนแปลงสภาวะในช่องปากเป็นเวลานาน 7 วัน จากนั้นจะถูกนำไปวัดค่าความแข็งผิวอีกครั้ง เพื่อเปรียบเทียบกับค่า

ความแข็งผิวเริ่มต้น ผลการทดลอง ความแตกต่างของค่าเฉลี่ยความแข็งผิวระหว่างกลุ่ม วิเคราะห์ผลทางสถิติด้วย เอสพีเอสเอส แพร์ที-เทส และ วันเวย์อะโนว่า ที่ค่าความเชื่อมั่นร้อยละ 95 พบว่าค่าเฉลี่ยความแข็งผิวของกลุ่มบีและซีมีความแตกต่าง อย่างมีนัยสำคัญทางสถิติเมื่อเทียบกับกลุ่มควบคุม (กลุ่มเอ = 248.49 ± 31.56 กลุ่มบี = 379.97±101.48 กลุ่มซี = 1065.82±259.27) และเมื่อเปรียบเทียบระหว่างฟลูออไรด์วานิชทั้งสองชนิด พบว่ากลุ่มที่ทาด้วย Enamel Pro® มีค่าความแข็งผิวที่สูงกว่ากลุ่มที่ทาด้วย Duraphat® จึงสรุปได้ว่าการเติมอะมอร์ฟัสแคลเซียมฟอสเฟตเข้าไปในฟลูออไรด์วานิชความเข้มข้นร้อยละ 5 ช่วยเพิ่มประสิทธิภาพในการคืนกลับของแร่ธาตุ ในการทดลองในฟันน้ำนมในห้องปฏิบัติการ

คำสำคัญ: *ความแข็งผิวของเคลือบฟัน ฟลูออไรด์วานิช ฟันน้ำนม*

Abstract

The success of using fluoride varnish in promoting remineralization has been widely known for many years. Recently, the addition of calcium and phosphate ions into fluoride varnish has been developed to enhance the remineralization process. This study aimed to evaluate the effects of using 5% sodium fluoride (NaF) varnish with ACP (Enamel Pro®) compared with conventional 5% NaF varnish (Duraphat®) on the surface microhardness of caries-like lesions on primary teeth enamel. Twenty-seven extracted healthy primary anterior teeth were randomly divided into three groups (A, B and C ; n = 9). Group A teeth were exposed to distilled water (control group), group B were applied with 5% NaF varnish (Duraphat®) and group C were treated with 5% NaF varnish with ACP (Enamel Pro®). A Vicker microhardness number (VHN) was measured as baseline measurements. All groups were subjected to demineralization process before the treatment. After seven days of pH cycling, VNH was measured again to compare with the initial measurements. Differences in mean microhardness number between groups were analyzed using the paired t-test and one way ANOVA at a 95% level of confidence. From the results, the mean microhardness of Group B and C were significantly different from control group (Group A = 248.49± 31.56, Group B = 379.97±101.48 and Group C = 1065.82±259.27). Comparisons made between 2 fluoride varnish groups showed that the mean VNH of 5% NaF varnish with ACP group was greater than that of 5% NaF varnish group. In conclusion, the changes of the surface microhardness of 5% NaF varnish with ACP was significantly higher than that of the 5% NaF varnish.

Keywords : *Surface microhardness Fluoride varnish Primary teeth*

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1. Introduction

Dental caries is a multifactorial disease affecting most people in industrialized countries and developing countries all over the world. The 7th National Dental Health Survey in 2012 regarding the caries status in Thai children found that children at 3

year of age have 51.7 percent of dental caries, while the children aged 5-6 years have caries up to 78.5 percent indicating a high prevalence of dental caries in Thai children (Dental Health division, 2012). The microorganisms in the oral cavity produce organic acids, including lactic, formic, acetic and propionic acid after carbohydrate consumption. These acids diffuse into the enamel, dentine, or cementum, partially dissolving the mineral crystals and consequently render the enamel to a porous form a status called demineralization. Early lesion is initially appeared as a white spot lesion. Consequently, the cavitation would occur if the process continues. The dental caries process is a dynamic process that requires repeated episodes of prolonged exposure to acidic condition consistently below the critical pH for enamel dissolution (pH 5.5) called demineralization and intervening period of return to the resting pH of plaque (pH 7.0) called remineralization (Featherstone, 1999).

At this initial stage, the progression of dental caries can be reversed by the remineralization process. Remineralization is a process in which minerals are returned to the molecular structure of the tooth itself. Calcium and phosphate in the saliva and plaque permit the recovery of some lost mineral content by the enamel. In order for this process to take place, the surface of the tooth needs to be clean combined with sufficient flow of saliva and adequate amount of calcium. (Hicks et.al., 2004)

Treatment of early dental caries by remineralization has the potential to significantly advance non-invasive clinical management of the disease. Several mechanisms are available for aided remineralization. The most well-known is the delivery of topical fluoride, which has been proven to be a highly effective measure for prevention of caries.

Currently, there are 2 widely used types of professional topical fluorides; fluoride gel and fluoride varnish. Fluoride varnish seems to be suitable for promoting remineralization in these lesions especially in small children because it can be applied directly at the lesion which can provide topical effect of fluoride to form CaF_2 in the deeper layer of enamel surface. CaF_2 will slowly release fluoride ions at the junction of plaque and tooth surface. It also can inhibit metabolism of the oral microorganisms by penetrating into the bacterial cells and inhibit their growth. Calcium fluoride deposit in the pores of the lesion is more effective in inhibiting demineralization than fluoroapatite. (Featherstone, 2008)

Fluoride varnish is a highly concentrated form of fluoride which is applied to the tooth's surface, by a dentist, dental hygienist or other health care professional, as a type of topical fluoride therapy. It is able to stay in contact with the tooth surface for several hours and can be used to help prevent decay on the demineralized tooth surface and to treat dentin hypersensitivity. (Eugenio, 2000)

Nowadays, it is widely known that calcium and phosphate ions are the primary constituents of tooth mineral. In order for the remineralization process to take place; adequate amount of these ions are required, however saliva provide only small amount of them. More recently, this has led to introduction of new materials containing calcium and phosphate ions. Addition of calcium and phosphate

ions into fluoride varnishes has been developed to supplement the amounts of these ions in saliva and enhance remineralization by fluoride. (Christos, 2007)

The purpose of this in vitro study is to compare the effect of Duraphat® (5% sodium Fluoride) and Enamel Pro® varnish (5%w/v sodium fluoride with amorphous calcium phosphate).

2. Objectives

To compare changes in the surface microhardness after application of two types of fluoride varnishes, Duraphat® and Enamel Pro® on the enamel of primary teeth after caries-like lesion simulation.

3. Materials and Methods

3.1 Sample selection

Twenty seven human primary incisors were collected from extraction or naturally exfoliation. Teeth with sound enamel were selected to be used in this study.

$$N_0 = \frac{Z_{\alpha/2}^2 \sigma^2}{d^2} = \frac{(2.33^2)(6.12^2)}{5^2} = 8.15$$

confidence interval 99%,

$Z_{\alpha/2} = 2.33$ $\sigma = 6.12$ $d = 5$

Exclusion criteria were cracks, enamel hypoplasia, dental fluorosis, decay and tetracycline teeth. (ethic no.09/2557)

3.2 Preparation of specimens

Sound extracted or naturally exfoliated human primary incisors teeth were stored in 0.1% thymol solution at room temperature. All teeth were cleaned of soft tissue debris and polished with fine pumice to remove organic contaminant then kept in normal saline.

All teeth were blotted-dried with tissue paper then each specimen was embedded in cylinder tube (2 cm. diameters, 2 cm. height) by using self-cured acrylic and allowed to cure at room temperature for 1 hour. The exposed surfaces of the teeth were ground and polished using 800, 1,000, 2,000 and 4,000-grit silicon carbide abrasive papers lubricated with water to produce flat surfaces and then polished with silica powder. Finally, all specimens were cleaned in ultrasonically for 4 minutes.The baseline of surface microhardness (SMH) of each tooth was measured using a microhardness tester with a Vicker diamond indenter under a 100 gram load for 15 seconds. The microhardness numbers (VHN) of four indentations at each border was obtained. The mean values calculated were considered as the mean base line micro hardness (SMH) of each specimen.

After SMH measurements, thirty specimens were used in this study. The specimens were immersed in deionized water until being used.

Materials and Instruments

3.2.1 Fluoride varnishes used in this study

3.2.1.1 Duraphat® (5% sodium fluoride)

3.2.1.2 Enamel Pro® Varnish (5% sodium fluoride with amorphous calcium phosphate)

3.2.2 Demineralizing and remineralizing solutions

3.2.2.1 Demineralizing solution 1 (D1) comprised of 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, 0.05 M CH_3COOH and pH was adjusted to 4.4 with 1.0 M KOH

3.2.2.2 Demineralizing solution 2 (D2) comprised of 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, 0.05 M CH_3COOH and pH was adjusted to 4.7 with 1.0 M KOH

3.2.2.3 Remineralizing solution (R) comprised of 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, 0.15 M KCl and the pH was adjusted to 7.0 with 1.0 KOH

3.2.3 Incubator (MEMMERT model 600)

3.2.4 Vicker microhardness tester (FM-ARS 9000, Future-Tech, Tokyo, Japan)

3.2.3 Grouping

The specimens were pooled and randomly assigned into three groups; groups A B and C comprising of nine specimens in each group. The fluoride varnish was applied before pH-cycling process as followed:

3.2.3.1 Group A (Control group): no surface treatment was performed.

3.2.3.2 Group B 5% sodium fluoride (Duraphat®) applied as a thin layer of varnish. It allowed to be absorbed for 20 seconds and then air dried

3.2.3.3 Group C: 5% sodium fluoride applied with amorphous calcium phosphate (Enamel Pro® Varnish) as a thin layer of varnish was applied. It allowed to be absorbed for 20 seconds and then air dried

3.3 Formation of caries-like lesion

Each specimen was immersed in the demineralizing solution (D1) and incubated at 37°C for 3 hours to produce artificial demineralized lesions. The specimen was rinsed with deionized water and wiped off with tissue paper. Subsequently the surface microhardness of the specimens was measured again to be considered as demineralized enamel surface microhardness.

3.4 Varnish application and surface microhardness

3.4.1 pH-cycling process

The experimental process aimed to replicate the pH changes occurring in the oral environment for 7 days. All specimens were subjected to pH-cycling process as shown in Table 1. The fluoride varnish was applied in day 1 before the start of pH-cycling process. (Buzalaf et. al. 2010)

Table 1 Showed pH-cycling process

Time period	Procedures
3 hrs.	Immersed in demineralizaing solution (D2) 36 ml.
	Rinsed with deionized water 50 ml.
2 hrs.	Dried with tissue paper.
	Immersed in remineralizing solution (R) 36 ml.
3 hrs.	Rinsed with deionized water 50 ml.
	Dried with tissue paper.
	Immersed in demineralizing solution
Overnight	(D2) 36 ml.
(about 16 hrs. at 37 °C in	Rinsed with deionized water 50 ml.
controlled environment	Dried with tissue paper.
shaker, 150 rpm)	Immersed in remineralizing solution
	(R) 36 ml.
	Rinsed with deionized water 50 ml.
	Dried with tissue paper.

3.4.2 Surface microhardness measurement

Seven days after the completion of pH cycling process, post treatment surface microhardness of all specimens were obtained.

Table 2 Mean (VHN) of surface microhardness in each group (N=9)

Group	Condition (Mean VHN±SD) (N=9)		
	Baseline	After Demineralized	After Remineralized
Control		134.48 ± 38.10	248.49 ± 31.56
Duraphat®	326.27 ± 14.98	171.66 ± 46.25	379.96 ± 101.48
Enamel Pro®		178.20 ± 23.23	1065.82 ± 259.27

3.5 Data analysis

The following equation was used to calculate the percentage surface microhardness recovery (%SMHR)

$$\%SMHR = \frac{SMH\ post\ treatment - SMH\ pre\ treatment}{SMH\ baseline - SMH\ pre\ treatment} \times 100$$

Statistical analysis

Mean and standard deviations for baseline surface microhardness, pre-treatment, post-treatment and percentage surface microhardness recovery were calculated for each group. Data was analyzed with SPSS version 13.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Surface microhardness of baseline,pre-treatment, post-treatment and percentage surface microhardness recovery were tested for normal distribution using the K-S test

Differences between baseline, pre-treatment, post-treatment within the same group were compared using Paired-Samples T-Test. The percentage surface microhardness recovery of the different groups was compared using the one-way ANOVA test, and Tukey post-hoc test was used to determine the significance of intergroup variations.

4. Results

The mean surface microhardness was 326.27±14.98 VHN. The K-S test revealed that all data were in normal distribution. Then undergone pH-cycling for 7 days. The mean surface microhardness values after demineralized with demineralizing solution (D1) were shown in table 2. These values showed statistically significant.

The mean percentage surface microhardness recovery (%SMHR) in the Enamel Pro® group was greater than Duraphat® group while the control group had the lowest %SMHR as shown in Table 3. The one-way ANOVA found these differences to be significant (P<0.05).

Table 3 Mean percentage surface microhardness recovery (%SMHR) in each group (N=9)

Group	Percentage surface microhardness recovery (%SMHR)
Control	55.86 ± 18.41
Duraphat®	137.58 ± 69.35
Enamel Pro®	600.04 ± 190.71

5. Discussion

The purpose of this study was to compare the remineralization effects of two types of fluoride varnishes on primary incisors. As early carious lesion frequently found at incisors teeth of young children. We used the surface microhardness parameter to verify the remineralization effects of fluoride varnish on artificial carious lesion. Surface microhardness has been used as a reliable indicator of the efficacy of fluoride. It is considered as an effective measure of the overall impact of the mineralization on the tooth.(Hosoya, 2000)

In this study, we follow the manufacturer's direction for application time of fluoride varnish 20 seconds.

From this study the mean value of enamel surface microhardness of primary teeth before the experiment was 326.27±14.98 VHN, which is similar to other studies.(Rirattanapong, 2011) After specimens were treated with fluoride varnish, all specimens were subjected to pH cycling procedure. These experimental process imitated the change of pH in oral environment for 7 days. The result showed that significantly higher surface microhardness for the specimens that were treated with fluoride varnish whether with or without Amorphous calcium phosphate (ACP). The specimens that were treated with Enamel Pro® varnish also showed higher surface microhardness than specimens that were treated with Duraphat®. This could suggested that fluoride varnishes whether with or without ACP had significantly higher remineralizaion effects than an untreated teeth and ACP may enhance the remineralizing effect. After applied fluoride varnish CaF_2 was deposit on the enamel surface. CaF_2 acts as a pH-controlled reservoir of fluoride that was found in saliva and tooth surface. The amount of CaF_2 will increase on demineralized enamel surface. The rate-controlling factor appears to be phosphate, which controls the dissolution rate of CaF_2 at high pH. Increasing fluoride concentration by prolonging the exposure time of topical fluoride or using a fluoride solution with low pH can increase CaF_2 formation. CaF_2 formed at low pH contains less internal phosphate which has been shown to be less soluble. Nevertheless, CaF_2 that deposit on the enamel surface also slowly releases fluoride to interfere with demineralization events and increases the amount of fluorapatite on enamel, which has been shown to enhance its resistance to demineralization (Schemehorn et.al., 1999).

From the results as shown in table 3, specimens which treated with Enamel Pro® varnish also have higher percentage surface microhardness recovery than ones treated with Duraphat®. This could suggest that even though there was CaF_2 on the enamel surface in both group but ACP might impact the surface microhardness. Amorphous calcium phosphate (ACP) is the initial solid phase that precipitates from a highly supersaturated calcium phosphate solution, and can convert readily to stable crystalline phases such as octacalcium phosphate or apatite products. Therefore, adding amorphous calcium phosphate might act as reservoir of calcium and phosphate ions that readily to precipitate into

apatite. There is a sound rationale for the addition of calcium ions to fluoride containing varnishes in an attempt to produce an increased retention of fluoride and calcium ions in the oral environment. Additionally, the remineralization potential of saliva is also calcium limited so additional calcium and phosphate ions from sources such as varnish may lead to improved remineralization of early lesions. (Boskey, 1997) Hence, a number of manufacturers have modified fluoride varnishes to include calcium and inorganic phosphate ions in an attempt to further improve efficacy.

6. Conclusion

This study has revealed that ACP acts as a safe and novel carrier for calcium, phosphate and fluoride ions to promote enamel remineralization. The calcium phosphate-based remineralization technologies are promising adjunctive treatments to topical fluoride therapy in the non-invasive management of early caries lesions. The results of this study shows that the surface microhardness of 5% sodium fluoride varnish with ACP is significantly higher than that of 5% NaF varnish.

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