Effects of Glass lonomer Sealant on Occlusal Surface to the Changes of Proximal Enamel Lesion

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Objective: To compare the changes of proximal enamel lesion depth between applying fluoride varnish and using GIC as a sealant on occlusal surface, both with exposure to a fluoridated toothpaste:

Methods: Two enamel lesions size 2 mm in diameter were created at the proximal surface of 72 permanent molars and randomly separated into 2 groups. All teeth were cut mesio-distally to separate each tooth to control and treatment halves. Treatment halves of first group were applied glass ionomer cement as a sealant on occlusal surface and treatment halves of another group were applied fluoride varnish (FV) on occlusal surface. All specimens were pH-cycled and brushed twice daily with fluoridated toothpaste for 14 days except treatment halves in FV group which left unbrushed in the first 24 hr. Lesion depth of all specimens were compared under polarized light microscope and measured with Image-Pro Plus $^{\circledR}$. Results-The mean lesion depth of both treatment halves (GIC and F-Varnish) were significantly less than their control halves. Percentage of lesion reduction in GIC group (26.55%) was significantly higher than fluoride varnish group (18.30%).

Conclusions: With fluoridated toothpaste exposure, Glass ionomer cement as an occlusal sealant should be a better approach to reduce the enamel lesion depth at proximal surface on the same tooth when compared with applying fluoride varnish.

Keywords: Glass ionomer cement, Occlusal sealand, Proximal enamel lesion, Lesion depth, Non-invasive technique

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INTRODUCTION

Dental caries is a multifactorial disease that most affected children in Thailand [1]. In 2012, Thai national dental health survey found the incidence of dental caries in 12 years old children was 52.3 percent. Total decayed, missing, filled teeth score (DMFT score) was 1.3 teeth/ person [1]. Dental caries lesions have various stages and can be found in every surfaces of tooth. In primary dentition, the broad contact area between first and second molars contributes to a high proportion of proximal caries. In young permanent dentition, the permanent first molar is the most caries-susceptible tooth and the mesial surface is the majority of proximal lesions [2].

An earliest sign of caries development is visualized as chalky white spots or lines. Progression of enamel caries lesions can be effectively arrested if the conditions at tooth surface are changed to those less favorable to the cariogenic bacteria. For the enamel caries lesions, non-operative interceptive treatments such as oral hygiene instruction, fluoride application and sealant are preferred [3].

GIC has been claimed to have suitable properties of sealant material under uncontrolled moisture because of its hydrophilic property. [4] Additional beneficial properties such as fluoride releasing, rapid setting and good biocompatibility make GIC to be an interesting sealant material. A new generation of GIC material, Fuji VIITM (GC Co., Japan) has been introduced as a high-level fluoride releasing material with suitable for promoting remineralization to adjacent lesion [5,6]. Recharging fluoride ions back to restoration occurs during topical fluoride application and brushing with a fluoridated toothpaste [7,8]. However, no studies have yet reported the effect of GIC (Fuji VIITM) to proximal enamel lesion on the same tooth. The outcomes of this research will enable the clinical treatment option for proximal enamel lesion in permanent molar teeth. The ultimate goal is to be able to reduce proximal enamel lesion depth with fluoridated toothpaste exposure by using GIC (Fuji VIITM) on the occlusal surface.

Materials and Methods

1. Sample selection and preparation of tooth surface

Sample size was calculated according to the study in 1999 which study effectiveness of GIC for remineralization proximal enamel lesion in permanent molar [8]. The research protocol and informed consent form were reviewed and approved by the Faculty of Dentistry/Faculty of Pharmacy, Mahidol University, Institutional Review Board. Seventy-two extracted third molars with no enamel and dentin caries, no evidence of cracks or hypoplasia were selected for this study. The apical of the root was sealed with melted sticky wax. Two pieces of tapes, size 2 mm in diameter, were applied to either side of the sound proximal enamel surfaces at the middle 1/3 of the crown. The entire crown of tooth was covered with 2 layers of acid resistant nail varnish. When the second layer was dry, two tapes were removed to reveal the windows of sound enamel sizes 2 mm.

2. Lesion formation and tooth separation

All teeth were suspended in an artificial caries solution for 14 days as described by Featherstone et al [9]. to produce incipient caries lesion of 100-150 µm deep. This solution is composed of 75 mmol/L acetic acid, 2.0 mmol/L calcium and 2.0 mmol/L phosphate adjusted to pH 4.3, 37°C. Seventy-two teeth were randomly assigned using computer program into two groups, fluoride varnish (FV) and GIC, thirty-six teeth per group. Each tooth was then cut mesio-distally into 2-halves using a hard tissue microtome with slow speed diamond saw under copious water spray. Each pair of tooth was randomly used as the control and the test specimen.

3. Intervention for artificial enamel **Resion**

All control specimens were placed into a pH-cycling process without any intervention. All test specimens were applied either with different two interventions. FV Group (Fluoride varnish (Duraphat \mathcal{B}) group): The varnish 0.05 g was applied on occlusal surface of each test specimen. Then they were placed into a pHcycling process. Samples in FV group were left unbrushed for 24 hr. in this process. (Glass ionomer sealant (Fuji VII™) sealant group): Fuji VII™ was mixed according to the manufacturer's instructions and placed on the occlusal surface of tooth. All of them were cured with halogen light source for 40 seconds to accelerate the setting process (according to the manufacturer's instructions). Then they were placed into a pH-cycling process.

4. pH-cvcling process

The process imitated the changes in pH of the oral environment for 14 days. Each

cycling was kept at 37° C involved 3 hours of demineralization (2.2 mmol/L CaCl₂ and NaH₂PO₄, 0.05 mol/L acetic acid, with pH adjusted to 4.7) twice daily and 2 hours of remineralization (1.5) mmol/L CaCl₂, 0.9 mmol/L NaH₂PO₄, 0.15 mol/L KCI with pH adjusted to 7.0 with 1 mol/L KOH) between the periods of demineralization [10]. All specimens in both groups were brushed with pea-sized portion of fluoride toothpaste (0.32 g.) for 3 minutes twice daily (before and after the 16-hour remineralization period) and then rinsed with deionized water.

5. Thin section preparation

After completion of 14-day pH cycle, all specimens were cut longitudinally through the middle of lesion using a slow speed diamond saw under copious water spray and grounded with wet 800-2500 grit silicon carbide paper. The thickness of each thin section were finely prepared to be around 100-150 microns and measured by electronic digital caliper.

6. Polarizing light microscope measurement

All specimens were placed in water and examined at 10x magnification under a polarizing light microscope and photographs were taken with a digital camera. The pictures of lesion depth were measured in micron, by using a computerized calculation method with Image-Pro Plus. The deepest point of the lesion and 150 microns to the left and right were marked. The line from each mark that perpendicular to the outer surface was measured as the lesion depth. Each specimen requires these three depths for calculating the mean of the lesion depth.

7. Statistic analysis

The mean and standard deviation of lesion depth in each group was tested for normal distribution (using Kolmogorov-smirnov test). A paired t-test was used to test the difference between the mean of the lesion depth of the control and the treatment in FV groups. A Wilcoxon test was used to test the difference between the mean lesion depth of the control and the treatment in GIC groups. A t-test was used to test the difference between the mean lesion depths of two treatment groups. A Mann-Whitney U test was used to test the difference

between the percentage changes of two groups. P-value equal to or less than 0.05 determines the significant level for all statistic tests.

Results

The mean \pm SD of lesion depth in each group was tested for normal distribution using Kolmogorov-smirnov test. The results showed that control specimens in GIC group was not normal distribution (p-value = 0.044). The mean ± SD of lesion depth of both groups were shown in Table 1.

Different letters indicated statistically significant difference ($p < 0.05$)

The results showed that the lesion depth \pm SD of control specimens in each group was not statistically significant different. The treatment and control in each group was compared and found that the mean lesion depth of the treated groups were statistically significant reduced. The lesion depth in GIC group and FV group decreased 26.55% and 18.30% respectively when compared with their control. The percentage changes between GIC group and FV group were statistically significant different, as shown in Table 1.

Discussion

For treatment of proximal enamel lesion, fluoride has been used to enhance remineralization for a long time. Fluoride varnish is a professionally applied fluoride with highly concentrated fluoride product (22,600 ppm). Among the fluoride varnish products. Duraphat $^{\circledR}$ was chosen because it had high percentage of caries reduction [11], release fluoride better than Duraflor[®][12], more fluoride concentration than Fluor-Protector[®] [13] and more enamel fluoride uptake [14]. This study showed mean lesion depth reduction by Duraphat $^{\circledR}$ was 18.30%. This result was supported by other previous studies that showed overall enamel lesion depth reduction following fluoride varnish applications around lesion ranging approximately from 18 to 70 % when compared with untreated controls [15]. Many studies shown that fluoride varnish application with daily use of dental floss can reverse proximal enamel lesion [16-18] but long term prognosis is still questionable because the patient compliance is strongly needed.

GIC becomes one of the important dental materials for high caries risk patients. The main properties are releasing fluoride ions for uptake by enamel and dentin, able to absorb fluoride ions from its surroundings and prolong released when the level of fluoride in the environment drops [19]. Previous studies found that GIC releases as much as 50 ppm fluoride, early after initial chemical setting, then maintains the concentration between 0.2-4 ppm for months [20, 21]. Some studies found that GIC exposure to standard brushing with a fluoridated toothpaste can prolong fluoride release [22]. In this study, all sample were brushed twice daily with fluoridated toothpaste similar to another study in 1999 which found that addition fluoridated toothpaste overwhelmingly increases the ability of fluoridated materials to inhibit demineralization and promote remineralization [8].

Many in vitro studies showed that placing GIC adjacent to proximal enamel lesions can regress enamel lesion significantly [5,8]. GIC released fluoride rapidly in the first day and gradually decreased for 8 weeks. Enamel at 10-200 microns depth will uptake fluoride ion from GIC which was increasing with release rate [23] and was retained for 6 months long. One in vivo study using Fuji VII applied directly to the proximal enamel lesion. The result showed that lesion progression was slower than control but restoration was completely loss in 6 months follow-up [24]. For the purpose of better retention, this study used Fuji VII as a sealant on occlusal surface.

Fuji VII was claimed to release fluoride higher than previous GIC. The result from this study showed that using Fuji VII as a sealant on occlusal surface can regress caries challenge at proximal lesion for 26.55% and promote more remineralization when compared with Duraphat $^{\circledR}$. Even though the fluoride released from Fuji VII was very small when compare with Duraphat $^{\textcircled{\textrm{R}}}.$ but it is sufficient to promote remineralization because only a small concentration $(0.1$ ppm.) of fluoride in dental plaque is required to inhibit enamel demineralization and facilitate remineralization. Moreover, a major advantage of Fuji VII is the ability to recharge fluoride so that long-term slowly fluoride released into oral cavity is found.

Four samples from GIC group were excluded, due to enamel fracture at the outer surface that may make the lesion depth measurement inaccurate. The cutting technique using hard tissue microtome with slow speed diamond saw was similar to other studies, the fracture was due to human effort.

The low progression of proximal enamel lesion from GIC in this in vitro study may be largely because the pH-cycling was operate in closed environment. In oral cavity, fluoride from GIC may be more difficult to reach the proximal lesion because a tooth is surrounded by saliva that is washed away by salivary flow rate. The saliva fluoride concentration in oral cavity will be less than that in the artificial saliva in this in vitro study. The distance from occlusal surface to proximal lesion may affect the concentration of fluoride. One study found that fluoride from GIC can release to 8.5 mm away from cavity

with the same fluoride concentration as releasing from 1 mm. in six months follow-up $[25]$. This study found that fluoride ions from occusal surface may reach proximal lesion and with the effect of exposure of fluoridated toothpaste, the enamel lesion regressed effectively.

For the sealant properties, one in vitro study indicated that the adhesion could be improved by conditioning the enamel surface before application of cement [26]. Because the setting reaction of glass ionomer sealant is quick, the ability of the sealant to penetrate into fissures and its adhesive strength may decrease if the instructions are not followed properly. Increase in the proportion of powder results in a more viscous cement which also sets faster, thus reducing the ability of the cement to flow readily and to adhere to the surface [27]. This study follows the manufacturer guideline in using GIC as a sealant and found no sealant loss through the experiment.

Conclusions

Clinically, common treatments for proximal enamel lesion are applying professional topical fluoride, diet advice and oral health motivation. Compliance from the patients is needed for these methods. Therefore, sometimes the clinician may find that enamel lesions progresses and develops to be cavitated lesion. The sealant application is a non-invasive treatment procedure that requires minimal chair time and also need less compliance. For these reasons and based on the results from this study, using Fuji VII as a sealant in deep pit and fissure teeth may be a choice to regress proximal enamel lesion and

other seen initial lesion e.g. buccal surface in those patients with high caries risk and irregular dental visit. However, for more confident in using this technique, the further clinical studies should be tested.

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