Effects of Apacider® Mangostin Adhesive Pastes Combined with Fluoride Varnish on Remineralization Potential of Artificial Enamel Carious Lesions: In vitro Study

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Abstract

Calcium phosphate-based technologies show promising efficacy as adjunctive treatment for fluoride therapy in the management of early carious lesions. Apacider® Mangostin Adhesive Pastes (AMAP) have been introduced as new alternative for the prevention and management of initial lesions. This study aimed to examine the effects of fluoride varnish combined with AMAP on remineralization of artificial enamel carious lesions in comparison to fluoride varnish alone, and AMAP alone. Artificial carious lesions were created on the buccal surfaces of 60 extracted premolars. Specimens were randomly divided into 5 groups: (1) fluoride varnish application at first day in combination with AMAP once a day continuously for 10 days, (2) fluoride varnish application at first day only, (3) AMAP application once a day continuously for 10 days, (4) blank AMAP application once a day continuously for 10 days, and (5) no treatment. All samples were treated under pH cycling for 10 days. Remineralization effect was evaluated by the difference in surface microhardness before and after agent application. There was a significant change in the mean surface microhardness before and after application in all groups (p < 0.001 each). A significant decrease in microhardness was observed in the fluoride varnish combined with AMAP group and fluoride varnish alone group, while a significant increase was found in AMAP alone group. The extent of the change was statistically different between AMAP group and fluoride varnish combined with AMAP group (p < 0.001). Comparison between fluoride varnish combined with AMAP group and fluoride varnish group showed no statistical difference (p = 0.99). In conclusion, AMAP has highest remineralization potential on artificial carious enamel lesions among all comparison groups. The application of fluoride varnish in combination with AMAP had no remineralization effect on artificial enamel carious lesions.

Keywords: Artificial caries, Fluoride varnish, Apacider® Mangostin Adhesive Pastes, Remineralization, Surface hardness
Introduction

Non-cavitated carious lesions represent the initial carious lesions caused by loss of calcium and phosphate ions from dissolution of hydroxypatite crystal in the enamel structure. Initial carious lesions can be arrested and reversed by remineralization. New technologies for caries prevention and caries arrest have been developed in accordance with the minimally invasive intervention concept. The philosophy includes early diagnosis of dental caries, assessment of individual caries risk, remineralization of early carious lesions, minimally surgical intervention of cavitated lesions with adhesive dental materials and repair rather than replacement of faulty restorations. The aim of remineralization strategy on initial carious lesions is to revert the lesions or to stop the progression, to preserve remaining tooth structure, as well as to improve strength, function and aesthetics.

The effectiveness of fluoride on caries prevention was confirmed by many studies. It has also been proven to potentially arrest caries process. If fluoride ions are presented in demineralized enamel surface, it can be absorbed to destroy apatite crystals and attracted calcium and phosphate ions to build new fluorapatites. However, its ability to provide complete remineralization is limited by the availability of calcium and phosphate ions. Moreover, the high concentration of fluoride has the most effective remineralization effect on superficial surface of lesions. This is attributable to blocked crystal voids, which in turn reducing the penetration of ions to subsurface lesions resulting in incomplete remineralization. Recently, a range of novel calcium phosphate-based technologies have been developed for clinical application and show promising efficacy as adjunctive treatment for fluoride therapy in the management of early carious lesions.

Apacider® AW (Sangi Co. Ltd., Tokyo, Japan) is an inorganic antimicrobial agent, based on apatite containing silver and zinc metals. It enhances remineralizing activity from calcium-phosphate and antibacterial activity from silver ions. An in vitro study of the effects of Apacider® varnish on surface microhardness and remineralization of enamel was performed by Juntavee et al. in 2009. The study showed significantly increased enamel microhardness and increased remineralization of white spot lesions at a level similar to fluoride varnish and CPP-ACP. In 2015, Apacider® Mangostin Adhesive Pastes (AMAP) has been developed by Soda and colleagues as a new alternative agent for the prevention and management of initial lesions with Apacider® AW as remineralizing agent and α-mangostin as antibacterial agent. An in vitro study showed that AMAP application can provide acid resistance and enhance consistent mineral gain during acid attack on artificial carious lesions. Several previous studies have shown that remineralization of dental enamel could be enhanced by a sole application of AMAP or fluoride varnish. However, studies on synergistic remineralization effects of fluoride varnish application combined with AMAP on artificial enamel carious lesions have not been performed.

The objectives of this in vitro study were to...
evaluate the effects of fluoride varnish combined with AMAP (FV-AMAP) in comparison to fluoride varnish only (FV), and AMAP only, on remineralization of artificial enamel carious lesions. Research hypothesis has been proposed on whether the application of fluoride varnish combined with AMAP has the remineralization effect on artificial carious enamel lesions.

Materials and Methods

This in vitro study was conducted at the Faculty of Dentistry, Khon Kaen University. The study protocol was exempted from review by Khon Kaen University Ethics Committee in Human Research (HE592272).

1. Tooth specimen preparation

Sample size was calculated using the Piface program based on the study of Sodata, Sample size was calculated by. Using the formula for One-way ANOVA with a significance level of 5 %, power of 80 % and within-group standard deviation of 5.3 kgf/mm², a sample size of 10 specimens per group would be needed to detect a difference of 9.9 kgf/mm². Taking into account the possibly higher standard deviation in this study, 12 specimens were selected per group.

Sixty human lower premolars extracted for orthodontic treatment were collected. The tooth specimens were stored in 0.1 % thymol solution prior to the experiment. Calculus and soft tissue debris were removed using a 3/4 gracey curette. Specimens with carious lesions, crack, abrasion, enamel hypoplasia, fluorosis, tetracycline stain, or restorative material were excluded from this study. Tooth crown was separated from the root at cemento-enamel junction by using a tooth cutting machine (Mecatome T180, Brié-et-Angonnes, France) with a diamond-coated blade under water cooling. In order to prepare flat and smooth enamel surfaces, specimens were polished on the buccal surfaces with a polishing machine (Ecomet, Buehler, USA) using silicone carbide waterproof abrasive paper no. 1000, 1200, 1500, 2000 and 4000, respectively. Each specimen was coated with acid–resistant nail vanish (Revlon, New York, USA) except in a 4x4 mm² window area at the middle part of the buccal surface. The specimens were mounted in plastic blocks with buccal surface facing outward.

2. Artificial carious lesion formation

Sound tooth specimens were immersed in 5 mL of synthetic polymer gels, as described by Reynolds with minor modifications, for 12 hours at 37°C to induce artificial carious lesions on buccal enamel. The gel contained 20 g/L Carbopol™, 500 mg/L hydroxyapatite, 0.1 mol/L lactic acid, at pH 5.0.

3. Sample allocation and treatment protocol

Tooth specimens were randomly assigned into five groups (n = 12) as follows:

- **Group 1** Fluoride varnish (Duraphat®) application as recommended by the manufacturer on the first day in combination with AMAP for 3 minutes once daily continuously for 10 days (FV-AMAP)
- **Group 2** Fluoride varnish application as recommended by the manufacturer on the first day only (FV)
- **Group 3** AMAP application for 3 minutes once daily for 10 consecutive days (AMAP)
- **Group 4** Blank AMAP application for 3 minutes once daily for 10 consecutive days
- **Group 5** No treatment

The materials used and compositions are presented in Table 1.

4. Treatment of artificial carious lesions and pH cycling regimen

The solutions used in the pH-cycling model included: demineralizing solution, remineralizing solution and artificial saliva. Remineralizing and demineralizing solutions were prepared with analytical-grade chemicals and deionized water according to the method of Kumar et al. for use in pH cycling. The demineralizing solution contains 2.2 mM CaCl₂, 2.2 mM KH₂PO₄, 0.05 M acetic acid and adjusted to pH 4.4 with 1 M KOH. The composition of remineralizing solution was 1.5 mM CaCl₂, 0.9 mM NaH₂PO₄, 0.15 M KCl to pH 7.0. Artificial saliva was prepared with 0.650 g/L KCl, 0.058 g/L MgCl₂, 0.165 g/L CaCl₂,
0.804 g/L, 0.365 g/L \( \text{K}_2\text{HPO}_4 \), 2.00 g/L \( \text{C}_6\text{H}_5\text{COONa} \), 7.80 g/L \( \text{C}_8\text{H}_{15}\text{NaO}_8 \), and adjusted to pH 7.0. The pH-cycle solutions were freshly prepared daily. All tooth specimens were stored individually in 10 ml of solutions in scintillation vials. The specimens were subjected to pH-cycling system in a shaking water bath (Wisebath, Korea) for 10 cycles at 37°C. Each cycle was composed of demineralization for 3 hours twice a day with remineralization for 2 hours twice a day in between, and then the assigned agents were applied on the specimens by microbrushes. The specimens were left for 3 minutes after agent application, and then immersed in artificial saliva for 14 hours. The specimens were rinsed with deionized water after incubation with each solution. Demineralization and remineralization cycles are shown in Table 2.

Table 1 List of materials used in the study

<table>
<thead>
<tr>
<th>Materials</th>
<th>Composition</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoride varnish-Duraphat®</td>
<td>2.26 % sodium fluoride, ethanol, colophonium, mastix, shellac, wax, saccharine, flavor</td>
<td>Colgate-Palmovie, Hamburg, Germany</td>
</tr>
<tr>
<td>Apacider® Mangostin Adhesive Pastes</td>
<td>Apacider® AW, alpha-mangostin, fumed silica, Eudragit®, polyethylene glycol, 95 % ethyl alcohol, paraben</td>
<td>In patent of Khon Kaen University, Thailand</td>
</tr>
<tr>
<td>Blank Apacider® Mangostin Adhesive Pastes</td>
<td>Fumed silica, Eudragit®, polyethylene glycol, 95 % ethyl alcohol, paraben</td>
<td>In patent of Khon Kaen University, Thailand</td>
</tr>
</tbody>
</table>

Table 2 The pH-cycling regimen in the experiment

<table>
<thead>
<tr>
<th>Time</th>
<th>Duration</th>
<th>Experimental solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.00 am - 2.00 pm</td>
<td>3 hours</td>
<td>Demineralizing solution</td>
</tr>
<tr>
<td>2.00 pm - 4.00 pm</td>
<td>2 hours</td>
<td>Remineralizing solution</td>
</tr>
<tr>
<td>4.00 pm - 7.00 pm</td>
<td>3 hours</td>
<td>Demineralizing solution</td>
</tr>
<tr>
<td>7.00 pm - 9.00 pm</td>
<td>2 hours</td>
<td>Remineralizing solution</td>
</tr>
<tr>
<td>9.00 pm – 11.00 am</td>
<td>3 minute</td>
<td>Testing agents</td>
</tr>
<tr>
<td></td>
<td>14 hours</td>
<td>Submerged in artificial saliva</td>
</tr>
</tbody>
</table>

5. Assessment of surface microhardness (SMH)

The measurements of SMH were performed at baseline (before lesion formation), before agent application (after lesion formation) and after agent application using a microhardness tester (Future-Tech FM Corporation, Japan) with a Vickers diamond indenter giving force of 100 g for 5 seconds. The represented figure of indentation mark was shown on FT-ARS software version 1.15.13 (Future-Tech Corporation, Japan). SMH values were automatically calculated in this software. The average SMH was calculated and recorded as mean SMH before agent application (SMH1) and mean SMH after agent application (SMH2). Mean change in SMH (\( \Delta \text{SMH} \)) was calculated by the formula: \( \Delta \text{SMH} = \text{SMH1} - \text{SMH2} \). Surface microhardness testing and indentation mark on enamel surface were shown in Fig. 1.
Means ± standard deviations (SD) of SMH before and after agent application are shown in Table 3. Before lesion formation, mean baseline SMH was 398.9 ± 9.9 kgf/mm² with no statistically significant differences among the five groups (p = 0.58). After lesion formation and before the agent application, the mean SMH was 178.6 ± 9.9 kgf/mm² with no statistically significant differences among the five groups (p = 0.35). There was a significant difference in SMH before and after application in each group (p < 0.001). FV-AMAP and FV groups had a decrease in SMH after agent application. In contrast, AMAP application resulted in an increase in SMH. The extent of the change was significantly different between AMAP and FV-AMAP groups (p < 0.001). However, there was no significant difference between FV-AMAP and FV groups (p = 0.99). The mean change in SMH in each group is shown in Table 4.

![Indentation mark on enamel surface (d1 and d2 are the diagonal length of indentation in mm.)](image)

Table 3  Mean and standard deviation (mean, SD) of surface microhardness before (SMH1) and after (SMH2) agent application

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of specimen</th>
<th>SMH1 (kgf/mm²)</th>
<th>SMH2 (kgf/mm²)</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>FV-AMAP</td>
<td>12</td>
<td>174.5 ± 11.0</td>
<td>155.7 ± 12.4</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>FV</td>
<td>12</td>
<td>181.7 ± 7.7</td>
<td>161.6 ± 14.3</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>AMAP</td>
<td>12</td>
<td>180.1 ± 8.2</td>
<td>197.6 ± 11.3</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Blank AMAP</td>
<td>12</td>
<td>180.4 ± 10.4</td>
<td>89.3 ± 12.0</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>No treatment</td>
<td>12</td>
<td>176.4 ± 11.2</td>
<td>84.1 ± 13.5</td>
<td>&lt; 0.001*</td>
</tr>
</tbody>
</table>

*p Paired sample t-test, t(SMH1 and SMH2) are significant difference (p < 0.001).

FV-AMAP = Fluoride varnish combined with Apacider® Mangostin Adhesive Pastes; FV = Fluoride varnish; AMAP = Apacider® Mangostin Adhesive Pastes
Table 4 Mean change in surface microhardness (ΔSMH) in each group

<table>
<thead>
<tr>
<th>Group</th>
<th>ΔSMH (kgf/mm²)</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lower bound</td>
<td>Upper bound</td>
</tr>
<tr>
<td>FV-AMAP</td>
<td>-18.8 ± 6.3²</td>
<td>-22.8 -14.8</td>
</tr>
<tr>
<td>FV</td>
<td>-20.1 ± 8.5¹</td>
<td>-25.5 -14.7</td>
</tr>
<tr>
<td>AMAP</td>
<td>17.6 ± 6.4³</td>
<td>13.5 21.6</td>
</tr>
<tr>
<td>Blank AMAP</td>
<td>-91.1 ± 7.0²</td>
<td>-95.6 -86.7</td>
</tr>
<tr>
<td>No treatment</td>
<td>-92.3 ± 10.2¹</td>
<td>-98.8 -85.8</td>
</tr>
</tbody>
</table>

².³ Different superscript letters indicate statistically significant differences (One-way ANOVA, Tukey’s test, P < 0.05)

Discussion

To our knowledge, this study was the first in vitro study to evaluate the remineralization effect of fluoride varnish combined with AMAP using surface microhardness measurement. The microhardness test examines structural changes and the degree of mineralization of a substrate, especially after different treatments on dental enamel surfaces in unbalanced situations.²⁰,²¹ The Vickers microhardness test was chosen for our study due to its efficiency on the small round area and because it requires less enamel surface preparation.²¹ The SMH baseline (398.9 kgf/mm²) of this study corresponds to the average microhardness value of sound human enamel of 370-420 kgf/mm².³

Artificial enamel carious lesion can be prepared by various techniques for the remineralization assessment of therapeutic agents. In other studies, the lesions were prepared by lactate or acetate gel at pH 4.4-5.0, to simulate organic acid produced by cariogenic bacteria. However, to mimic in vivo carious lesion, there should be subsurface lesion with less demineralized surface layer.²³ Therefore, we used synthetic polymer gels, composed of polyacrylic acid (Carbopol™), lactic acid and hydroxyapatite, for artificial caries formation.¹⁸ The polyacrylic acid was supplemented as the main factor to preserve the surface layer and produce in vitro subsurface caries formation.²⁴ In the pH cycling model to simulate pH condition in an oral environment with dynamic mineral loss and gain, remineralizing agents were applied in the model for 10 days.²³ The application of fluoride varnish was designed to mimic clinical situation under the manufacturer’s recommendation; however, in FV-AMAP group the fluoride varnish was applied on the first day followed by AMAP application until the end of ten days of the experimental cycle.

The results of this study showed partial remineralization on artificial enamel carious lesion which was possibly due to calcium and phosphate ions deficiency.⁴ The deficiency of ions was attributed to the dissolution of hydroxyapatite crystal during artificial carious lesions formation, therefore, fluorapatites and calcium fluoride (CaF₂) synthesis was interrupted.²⁵ Partial remineralization was also found in FV-AMAP application. This may result from the hypemineralization of the surface layer physically blocks subsequent ingress of calcium and phosphate ions into subsurface layer of the lesion.²⁶ In contrast, AMAP application alone was able to provide calcium and phosphate ions, that simultaneously diffuse into subsurface lesion for molecular structure restoration.²⁷ Similarly, Sodata’s study in 2015 also observed homogeneous remineralization of artificial carious lesions after AMAP application.¹² Porosity and depth of artificial carious lesion play a critical role in mineral diffusion. Larger
porosities can induce higher mineral deposition, however, deeper lesions with longer distance can create difficulty for mineral ion absorption. The results of AMAP group can be explained that small molecular structure of calcium and phosphate ions could be absorbed into deeper enamel porosity and deposited significantly larger amount of mineral than fluoride varnish counterpart as reflected by the higher values of surface SMH.

The result from a previous study showed that fluoride varnish enhanced remineralization process of artificial carious lesions after agent application under the same pH cycling regimen as used by this study. However, with different chemical formula for artificial carious lesion formation used in this study, it was shown that surface SMH values after fluoride varnish application were not increased. In our study, the reduction of SMH values after lesion formation was higher than in the previous study, indicating that the lesions have more progressive dissolution of apatite crystals or higher calcium phosphate ion depletion.

Our study found that fluoride varnish can only provide net mineral gain at a very early carious enamel lesion and reveal less acid tolerance comparing to AMAP. The application of AMAP alone showed superior effects on surface SMH after pH cycling in deeper carious enamel lesion than fluoride varnish and fluoride varnish combined with AMAP.

Conclusion

The results suggest that ten days’ application of once daily-AMAP has the most effective remineralization potential on artificial carious enamel lesions when compared with other groups. The application of fluoride varnish application at first day in combination with AMAP once daily-continuously for 10 days has no significant remineralization effect on artificial carious enamel lesions.

Acknowledgement

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