The Efficacy of Non-fluoridated Toothpastes on Artificial Enamel Caries in Primary Teeth: An In Vitro Study

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Aims: The aim of this study was to evaluate the remineralizing effect among various non-fluoridated toothpastes on artificial caries in primary teeth. 

Materials and Methods: Fifty sound primary incisor teeth were embedded in self-curing acrylic resin and immersed in demineralizing solution for 4 days forming artificial caries. All teeth were divided into five groups (10 teeth/group): Group I deionized water (control); Group II 1000 ppm F (Kodomo®); Group III non-fluoridated toothpaste containing calcium glycerophosphate and calcium lactate (Dokbuaku®); Group IV casein phosphopeptides-amorphous calcium phosphate (CPP-ACP) paste (GC Tooth Mousse®), and Group V non-fluoridated toothpaste containing nanohydroxyapatite (NHA) (Apagard®). The specimens were subjected to pH-cycling and immersed in toothpaste slurry twice for 7 days. Baseline, before, and after pH-cycling surface microhardness (SMH) values were measured by Vickers hardness numbers, and the percentage recovery of SMH (%SMHR) was calculated. Data were analyzed by one-way analysis of variance and Tukey’s multiple comparison at 95% confidence intervals. 

Results: The SMH values of test groups were significantly higher than those of the control group (p = 0.00). The %SMHR was −5.72±7.03% in the control group, Group II was significantly higher than Group III but there were no significant differences among Groups II, IV, and V (p > 0.05). Conclusion: Non-fluoridated toothpastes containing CPP-ACP or NHA for young children had efficacy in remineralizing effect on primary teeth comparable with 1000 ppm fluoridated toothpaste.

Keywords: Calcium phosphate-based agent, microhardness, non-fluoridated toothpaste, primary teeth, remineralization

INTRODUCTION

Dental caries is one of the considerable oral health problem affecting children’s living quality as it was a result of continuing imbalance of remineralization and demineralization in oral cavity. Fluoride has been widely used for decades as the remineralizing agent to enhance remineralization, which is the main strategy for caries prevention and reduction of initial caries. It is recommended that brushing teeth with 1000 ppm fluoride concentration toothpaste should be used twice daily as a primary preventive care in children. [1]

Nevertheless, lots of evidence have confirmed that using high fluoride concentration for long duration and without parental supervision of tooth brushing increased risk of fluoride toxicity, especially fluorosis in children under 6 years of age.[2,3] Nowadays, there are many alternative non-fluoridated remineralizing toothpastes that have been developed and launched onto the market to overcome the dental fluorosis. A calcium phosphate-based agent such as...
casein phosphopeptides-amorphous calcium phosphate (CPP-ACP) has been most studied and clinically proven for its anti-caries characteristic. As calcium and phosphate ions release in low pH condition, they can inhibit demineralization and favor the remineralization of the initial lesion by precipitation of the released ions, similar to calcium glycerophosphate (CaGP) and calcium lactate (CL), but the study of the latter two is still limited. Recently, nanohydroxyapatite (NHA) has been introduced as a calcium phosphate-based agent with potential in remineralizing effect as it consists of morphology similar to the apatite crystal in human enamel but with higher efficacy than the previous hydroxyapatite due to its smaller size as nanoparticles.

There are lots of alternative non-fluoridated toothpastes that now are available in the market. However, there is still some controversy among parents when choosing toothpaste for their young children, which can provide the most carries preventive effect while minimizing risk of fluorosis as much as possible.

To our knowledge, there is limited investigation that compares the efficacy in remineralizing effect of those available non-fluoridated to fluoridated toothpaste in primary teeth. Thus, this in vitro study is aimed to evaluate the efficacy of remineralizing effect through surface microhardness (SMH) between various non-fluoridated toothpastes and fluoridated toothpastes on artificial caries in primary teeth. We hypothesized that there is no difference of remineralizing effect between various non-fluoridated toothpastes and fluoridated toothpastes in primary teeth.

Materials and Methods

The sample size was based on the study of Rirattanapong et al., which evaluated the remineralizing effect of various fluoride varnishes through SMH technique in primary teeth. The sample size calculation in this study was under the one-way analysis of variance (ANOVA) and Tukey’s multiple comparison test at a 95% level of confidence; a sample size of 10 teeth per group was used. This study was approved by The Ethics Committee of Mahidol University, Thailand (MU-DT/PY-IRE 2020/DT020).

Fifty coronal parts of sound primary incisors were used in this study. The labial surface of the specimen was embedded on the top of self-cured acrylic resin, parallel to the horizontal plane. Then, they were polished by sandpaper with 1000, 1500, 2000, and 2500 grits, respectively, to obtain a flat and smooth surface. The prepared specimens were kept in deionized water at room temperature until used.

The baseline SMH was measured from the labial surface of sound enamel of each specimen using a Vickers indenter (FM-700e Type D, Future-tech, Tokyo, Japan) with 100 g of force for 15 s as Vickers hardness numbers (VHNs). There were four measurements from each specimen at each part of the study: baseline, before, and after pH-cycling procedure. The average of those readings was used and calculated as the percentage recovery of SMH (%SMHR) = 100 × (SMH after pH-cycling − SMH before pH-cycling) / (SMH baseline − SMH before pH-cycling).

There were two demineralizing solutions and one remineralizing solution that were prepared and used in this study. Demineralizing solution 1 (D1) consisted of 2.2 mM CaCl₂, 2.2 mM NaH₂PO₄, 0.05 M acetic acid adjusted to a pH of 4.4 with 1 M KOH. Demineralizing solution 2 (D2) consisted of the same components as D1, but the pH was adjusted to 4.7. The remineralizing solution (R) consisted of 1.5 mM CaCl₂, 0.9 mM NaH₂PO₄, 0.15 M KCl, and adjusted to a pH of 7.0 with 1 M KOH. The demineralizing and remineralizing solutions were freshly prepared for each cycle of pH-cycling process and kept separately in the containers for each group throughout the study.

The specimen was immersed in D1 at 37°C in an incubator (Sheldon Manufacturing, Model 1545, Cornelius, OR, USA) for 4 days to produce 60–100 µm depth of carious lesion. It was then rinsed with 15 mL deionized water and dried with a piece of tissue paper. All specimens were kept in artificial saliva containing 0.65 g/L KCl, 0.058 g/L MgCl₂, 0.165 g/L of CaCl₂, K₂[HPO₄]₂, and KH₂[PO₄]₂, 2 g/L NaCO₂CH₃ cellulose, and deionized water to make 1 L as modified from the technique of Amaechi et al. until used.

All specimens will be pooled and randomly divided into five groups as follows: Group I deionized water (control); Group II 0.22% sodium fluoride (NaF) or 1000 ppm F toothpaste (Kodomo® Toothpaste Ultra Shield Formula, Lion Corporation, Bangkok, Thailand); Group III non-fluoridated toothpaste containing CaGP and CL (Dokbuaku® Kids, Twinlotus Co., Ltd, Bangkok, Thailand); Group IV CPP-ACP paste (GC Tooth Mousse®, GC International Tokyo, Japan); and Group V non-fluoridated toothpaste containing NHA (Apagard® Apakid’s, Sangi Co., Ltd, Tokyo, Japan).

The toothpaste slurry will be prepared in a 1:3 ratio of toothpaste:deionized water. All toothpaste slurries were freshly prepared and were stirred by a magnetic stirrer for 20 min before use.

The pH-cycling process was aimed to simulate the pH changes that occurred in the oral cavity with brushing tooth twice daily for 7 days. All specimens will be subjected to pH-cycling that consisted of 3-h
demineralization in D2 solution twice daily with 2-hour remineralization in R solution at the middle and immersed overnight in R solution (16 h) at 37°C in an incubator. The specimens were immersed for 2 min in toothpaste slurry twice daily, before and after the overnight remineralization.

**Statistical analysis**

The one-way analysis of variance (ANOVA) was used to compare the SMH values at baseline, before, and after pH-cycling. Tukey’s multiple comparison tests were used to test the difference of mean SMH and percentage recovery of SMH among groups. The data analysis was set at 0.05 for significant level in all tests.

**Results**

From a total of 50 specimens of primary teeth randomly divided into five groups, the mean and standard deviations of SMH values at baseline, before/after pH-cycling, and the percentage recovery of SMH (%SMHR) among groups were shown in Table 1. The mean SMH value ± standard deviation (SD) at baseline and before pH-cycling was 332.14±23.02 and 115.56±19.15 VHN, respectively. There were no statistically significant differences of SMH value between all groups at baseline and before pH-cycling (p > 0.05).

After pH-cycling, the mean SMH values of test groups were significantly higher than those of the control group (p = 0.00), and Group II showed the highest SMH values among test groups. However, there were no statistically significant differences among them (p > 0.05).

The percentage recovery of SMH (%SMHR) could not be obtained from the control group, and it also decreased by 5.72±7.03% from baseline, whereas the highest %SMHR was found in Group II which was significantly higher than Group III (p < 0.05). Nevertheless, the comparison between groups showed no statistically significant differences in %SMHR among Groups II, IV, and V (p > 0.05), as shown in Table 2.

**Discussion**

In this study, the pH-cycling process was used to imitate the demineralization and remineralization that occur in oral cavity involving caries formation and anti-caries effect.[15] A 7-day pH-cycling was chosen because it was found to be the most appropriate period for the study in primary teeth as the enamel lesions could appear in 7 days and become dentin lesions afterwards.[16] SMH has been widely used in determining the change of mineralization in enamel carious lesions as the SMH values vary in line with the remaining calcium contents.[17,18] In fact, there were many factors that could affect the interpretation of SMH values such as sample preparation, indenter position, and the selective area. Nevertheless, as we had controlled these factors, it was found that the differences of SMH values between control and all test groups at baseline and before pH-cycling was not significantly different (p > 0.05). Moreover, the baseline and before pH-cycling SMH values (332.14±23.02 and 115.56±19.15 VHN, respectively) were similar to previous studies that performed in primary teeth.[8,19]

The present study showed that the efficacy in remineralizing effect of CPP-ACP, NHA, and fluoridated toothpastes was similar in primary teeth as there was no statistical difference of %SMHR on artificial caries after 7 days of pH-cycling. This result was consistent with previous studies that showed remineralizing effect of non-fluoridated agent; calcium phosphate-based agent was comparable to fluoride.[20,21] This might be due to the fact that fluorides do require calcium and phosphate from saliva to enhance and take part in the remineralization process, thus the efficacy of remineralizing effect of CPP-ACP, NHA, and calcium phosphate-based agent possibly be equal to fluoride.

CPP-ACP has been laboratory and clinically proved for its efficacy in significant reduction of initial carious lesions. The mechanism of CPP-ACP was that it could stabilize calcium and phosphate ions, releasing large amount of those ions as a buffering process in low pH condition and resulting in remineralization. Previous

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**Table 1: SMH at baseline, before/after pH-cycling, and %SMHR**

| Groups          | SMH (mean±SD; VHN) | %SMHR (mean±SD) |
|-----------------|--------------------|-----------------|
|                 | Baseline | Before pH-cycling | After pH-cycling |
| I Control       | 340.120±21.80*   | 115.186±20.95†  | 103.337±21.67‡ |
| II Kodomo® (0.22% NaF) | 323.350±19.17*   | 116.216±20.11†  | 194.665±25.58‖ |
| III Dokbuaku® (CaGP + CL) | 330.040±23.99*   | 116.068±22.92†  | 156.888±44.62§ |
| IV GC Tooth Mousse™ (CPP-ACP) | 325.462±23.26*  | 113.903±16.97†  | 160.304±38.83§ |
| V Apagard® Apakid’s (nHA)   | 335.735±26.24*   | 116.419±18.33†  | 163.176±32.97§ |

One-way ANOVA and Tukey’s multiple comparison test. Different symbols indicate statistically significant difference (p < 0.05). SD = standard deviation; VHN = Vickers hardness numbers.
Conversely, some study found that CPP-ACP had inferior efficacy than 500 ppm F toothpaste.\(^{[23]}\) This might be due to the fact that they used very small amount of CPP-ACP slurry compared with that used by the authors. Thus there was insufficient amount of calcium and phosphate to enhance remineralization and also they used different methods to evaluate the remineralizing effect which was polarized light microscopy instead of SMH as in our study.

Recently, NHA has been increasingly studied as its structure was the most similar to that of human enamel. NHA was claimed for higher efficacy in remineralizing effect than HA due to its smaller size as nanoparticles which improved hydrophilic and wetting characteristics and the ability to bind tightly as a thin layer on the tooth surface, obtaining higher remineralizing effect and surface hardness when compared with the earlier hydroxyapatite.\(^{[23,24]}\) The previous study showed that the optimal concentration of NHA that was able to remineralize initial carious lesions was 10\%,\(^{[25]}\) which also had remineralizing effect comparable to 900 ppm F toothpaste.\(^{[26]}\) Although the result of our study was similar to the previous one, we could not assure that which concentration of NHA had comparable efficacy to 1,000 ppm F toothpaste because the NHA toothpaste that we used in this study did not identify the concentration due to the trade secret, but the previous study showed that 1,000 ppm F toothpaste with 7\% NHA had superior efficacy than 1,000 ppm F toothpaste only.\(^{[27]}\)

In contrast to other calcium phosphate-based agents, it was found that non-fluoridated toothpaste containing CaGP and CL had significantly lower %SMHR than fluoride toothpaste. This might be due to the mechanism of anticariogenication of only CaGP and CL, which normally interacts with the tooth mineral and was insufficient to provide a significant remineralizing effect like when they were synergic with fluoride. As mentioned in the previous study, it was found that 0.25\% CaGP supplemented in low F toothpaste (500 ppm F) had similar remineralizing effect to high F toothpaste (1100 ppm F).\(^{[28,29]}\) However, we could not identify the concentration of CaGP that is used in this study as it was not mentioned on the ingredients label, and it might be less than the effective range of remineralization of 0.25–2\%.\(^{[10]}\) Moreover, although CL has been proved for its efficacy in enhancing remineralization, mostly those studies were performed in mouthwash and chewing gum that did not compare to fluoride use.\(^{[31,32]}\) From the result, it could imply that toothpaste containing CaGP and CL that now commercially available in the market for young children had less efficacy in remineralizing effect than 1,000 ppm F toothpaste.

The limitation according to this in vitro study was that the pH-cycling could not imitate the exact dynamic process of de-/remineralization in oral environments and there were other factors that could affect those process in real conditions such as the salivary contents and the absence of bacteria in both artificial saliva and caries.

In accordance with the result obtained from this study, it was found that toothpaste containing non-fluoridated agents, CPP-ACP and NHA, had efficacy in remineralizing effect on primary teeth comparable to 1000 ppm fluoridated toothpaste. Thus, non-fluoridated toothpaste containing CPP-ACP or NHA for children could be used alternatively in case of parents’ concern of fluoride dosage and toxicity, especially in young children under 6 years of age.

Further clinical studies are needed to confirm these findings and also to evaluate the effectiveness and efficiency of non-fluoridated toothpaste containing CPP-ACP or NHA in young children.

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### Conflicts of Interest
The authors declare that we have no conflict of interest.

### Authors Contributions
Not applicable.

### Ethical Policy and Institutional Review Board Statement
This study was approved by the Ethics Committee of Mahidol University, Thailand (MU-DT/PY-IRE 2020/DT020).

### Patient Declaration of Consent
Nil.

| Groups  | SMH  | %SMHR  |
|---------|------|--------|
| II - III | 0.10 | 0.03*  |
| II - IV | 0.20 | 0.14   |
| II - V  | 0.33 | 0.07   |
| III - IV| 1.00 | 0.97   |
| III - V | 1.00 | 1.00   |
| IV - V  | 1.00 | 1.00   |

Tukey’s multiple comparison test. *Statistically significant difference (p < 0.05)
**DATA AVAILABILITY STATEMENT**

The additional data of this study are available on request from Dr. Pornpailin Kasemkhun at p.kasemkhun@gmail.com.

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