Effects of nano calcium carbonate and siwak toothpaste on demineralized enamel surface roughness

M Detara, S Triaminingsih* and B Irawan

Department of Dental Materials, Faculty of Dentistry, Universitas Indonesia, Jakarta 10430, Indonesia

*E-mail: ami_permana@yahoo.com

Abstract. The aim of this study was to analyze the effect of nano calcium carbonate and Siwak toothpaste on demineralized enamel surface roughness. Human tooth specimens immersed in 0.3% citric acid at pH 3.25 were brushed with nano calcium carbonate and Siwak toothpaste for 2, 4, and 6 weeks. Enamel surface roughness was tested using a Surface Roughness Tester with supporting scanning electron microscopy and energy dispersive spectroscopy assessments. It was found the application of nano calcium carbonate and Siwak toothpaste for 2, 4, and 6 weeks decreased the surface roughness of the demineralized enamel (p < 0.05). Brushing teeth with nano calcium carbonate and Siwak toothpaste should decrease the surface roughness of demineralized enamel.

1. Introduction

The enamel is the outermost part of the tooth surface in continuous contact with saliva and food. One of the biggest components in enamel is calcium hydroxyapatite crystal. Enamel is the strongest calcified tissue, but it is nonvital and can be lost forever to damage because the body cannot replace it [1]. Continuous demineralization processes cause the loss of minerals from the hydroxyapatite structure of the tooth enamel. Demineralization occurs when hydroxyapatite reacts with hydrogen ions at acidic pH ≤ 5.5. Factors causing demineralization include conditions with acidic saliva and plaque, the low buffering ability of saliva, and decreased saliva flow [2]. Demineralization affects the morphology, and the enamel loses its structure causing its surface to become rougher [3]. One of the early signs of demineralization is the appearance of a white spot. White spots are chalky white early lesions on the enamel surface where translucency has been lost. These lesions are reversible through remineralization.

External factors can trigger remineralization, like mouthwash, CPP-ACP, and toothpaste. Toothpaste contains considerable amounts of remineralization agents, one of which is calcium. Usually, the calcium contained in toothpaste is calcium carbonate and calcium phosphate, and toothpaste containing nano calcium carbonate is now available. It can prevent caries because it is proven to remineralize early caries lesions. Nano calcium carbonate has good retention to enamel surface because of its tiny particle size which ranges from tens to hundreds of nanometers. This
particle size also accelerates the dissolution of Ca\(^{2+}\) ions in nano calcium carbonate, therefore, increasing the concentration of Ca\(^{2+}\) ions and the saliva pH [4]. Another kind of toothpaste, namely herbal toothpaste, namely Siwak toothpaste. Siwak toothpaste is made from *Salvadora persica*, which contains natural fluoride [5]. Fluoride is one of the agents that can trigger remineralization. It reacts with free Ca\(^{2+}\) and HPO\(_4\)\(^{2-}\) ions and forms fluor apatite crystals, which can replace lost hydroxyapatite crystals, thus preventing demineralization [2]. The remineralization process can also reduce enamel surface roughness [3].

Enamel surface roughness is one of the predisposing factors for bacteria to adhere to teeth and stain them [3]. Also, the use of a toothbrush can also cause changes in enamel surface roughness [6]. This study was designed to determine the difference in efficacy of remineralization agents contained in nano calcium carbonate and herbal (Siwak) toothpaste in relation to caries prevention.

2. Materials and Methods
The specimens used were human third molars with caries but crack-free surfaces. A total of 27 tooth crown specimens were embedded in an acrylic resin, sanded with 2000 grid SiC paper, and polished with 1 µm alumina. The specimens were randomly divided into three brushing groups with 9 specimens each, one group was brushed using distilled water only, one group was brushed using nano calcium carbonate toothpaste (Nano Systema), and the other group was brushed using siwak toothpaste.

The baseline enamel surface roughness was measured using a Surface Roughness Tester (Mitutoyo SJ 301). Measurements were performed thrice on each specimen, and the average value was taken. After surface roughness measurement, each specimen was immersed in demineralization solution, 0.3% citric acid at pH 3.25, for 3 minutes on an orbital shaker set at 70 rpm [7]. Afterward, each specimen was cleaned using an ultrasonic cleaner for 5 minutes. The enamel surface roughness was then measured again.

After measurement of demineralized enamel surface roughness, brushing was performed using the materials according to each group with distilled water only, with Nano Systema toothpaste, or with Siwak toothpaste. The teeth were brushed using a Pierrot electric toothbrush with a load of 150 g [8]. The toothpaste solution was made from 3 g of toothpaste dissolved in 3 ml of distilled water. Brushing was done for 14 minutes, which is assumed as equivalent to 2 weeks brushing, and it was repeated to obtain brushing durations equivalent to 4 and 6 weeks [9]. After brushing every 14 minutes, the enamel surface roughness was measured in the same way as the baseline measurement and after demineralized measurements.

Scanning Electron Microscope (SEM) was used to observe the surface morphology and Energy Dispersive Spectroscopy (EDS) for elemental analysis to observe the surfaces’ components. Five specimens were used to obtain representative specimens before treatment, after demineralization, and after brushing with distilled water, Nano Systema toothpaste, or Siwak toothpaste equivalent to 6 weeks of brushing.

The results of enamel surface roughness were analyzed using the Saphiro–Wilk and Levene statistic tests to determine data distribution and homogeneity. The results showed the data distribution to be abnormal but homogenous; therefore, Kruskal–Wallis, Mann–Whitney, and Wilcoxon tests were used for statistical analyses.

3. Results
Measurement results of enamel surface roughness after demineralization and brushing with distilled water, Nano systema, and Siwak toothpaste are shown in table 1.
Table 1. Mean value of enamel surface roughness of each group

| Brushing Material | Surface roughness value (Ra, µm) | Baseline | Demineralization | 2 weeks | 4 weeks | 6 weeks |
|-------------------|----------------------------------|---------|------------------|---------|---------|---------|
|                   | Ra ± SD                          | Ra ± SD | Ra ± SD          | Ra ± SD | Ra ± SD | Ra ± SD |
| Distilled water   | 0.046 ± 0.004                    | 0.110 ± 0.009 | 0.091 ± 0.009 | 0.083 ± 0.010 | 0.076 ± 0.010 |
| Nano Systema Toothpaste | 0.045 ± 0.004                  | 0.113 ± 0.010 | 0.068 ± 0.009 | 0.061 ± 0.008 | 0.063 ± 0.011 |
| Siwak Toothpaste  | 0.050 ± 0.005                    | 0.113 ± 0.012 | 0.079 ± 0.006 | 0.067 ± 0.007 | 0.064 ± 0.007 |

Table 1 describes the differences in enamel surface roughness after immersion in demineralization solution and brushing for durations equivalent to 2, 4, and 6 weeks. The enamel surface roughness of all specimens was increased after demineralization to a very similar extent. After brushing for durations equivalent to 2 and 4 weeks, the enamel roughness decreased progressively in all groups, with it being lowest in the Nano Systema toothpaste group. After brushing for durations equivalent to 6 weeks, the enamel surface roughness increased again in the Nano Systema group but decreased further in the distilled water and Siwak toothpaste groups. The lowest surface roughness at the end of the brushing equivalent to 6 weeks was for the Nano Systema group, followed closely by the Siwak toothpaste group.

The mean significance value of baseline enamel roughness was $p > 0.05$. The significance values of enamel roughness after demineralization had $p > 0.05$; therefore, the mean value differences of enamel surface roughness for all three groups after demineralization were insignificant. The significance value of enamel roughness means after brushing for a period equivalent to 2 weeks had a $p < 0.05$. A significant difference was found in surface roughness mean values after brushing for a period equivalent to 2 weeks. Similarly, surface roughness mean values after brushing for a duration equivalent to 4 and 6 weeks also showed significance values of $p < 0.05$.

The Mann–Whitney test was performed following Kruskal–Wallis to determine the significance of enamel surface roughness mean values among the groups. The enamel surface roughness mean value difference before treatment and after demineralization between the specimens of the distilled water group and those of the Nano Systema group was insignificant ($p > 0.05$). The results were different on brushing for a duration equivalent to 2, 4, and 6 weeks, which showed significant differences ($p < 0.05$). Similar results were obtained on the comparison between specimens of the distilled water group and those of the Siwak group. Finally, the enamel surface roughness mean value comparisons between specimens of the Nano Systema group and those of the Siwak group showed significant differences on brushing for a period equivalent to 2 weeks, but all the other values showed insignificant differences ($p > 0.05$).

The subsequent statistical test was the Wilcoxon test to determine the significance between treatments on each of the brushing groups. The comparisons between enamel surface roughness mean values at baseline and after demineralization showed significant differences ($p < 0.05$) on all groups. The same results were seen upon comparisons between the enamel surface roughness mean values after demineralization and after brushing for duration equivalent to either 2, 4, or 6 weeks ($p < 0.05$). The same result was also obtained on the comparison between enamel surface roughness mean value after brushing for durations equivalent to 2 and 4 weeks. Likewise, the comparison between enamel surface roughness mean value before treatment and after brushing for a duration equivalent to 6 weeks showed significant differences ($p < 0.05$) in all groups. However, the difference in enamel surface roughness means values between brushing for duration equivalent to 4 weeks and 6 weeks was only significant ($p < 0.05$) on the distilled water group. The Nano Systema group and Siwak group showed the insignificant difference in enamel surface roughness mean values between brushing for durations equivalent to 4 and 6 weeks.
Representative images of SEM observation results on enamel surface before, after demineralization, and after brushing for durations equivalent to 6 weeks are shown in Figure 1. The results of the elemental analysis of enamel surface using EDS are depicted in Table 2.

Figure 1. SEM images of tooth enamel surfaces (2000x). a. Before demineralization. b. After demineralization. c. After brushing for a duration equivalent to 6 weeks using distilled water. d. After brushing for a duration equivalent to 6 weeks using Nano Systema toothpaste. e. After brushing for a period equivalent to 6 weeks using Siwak toothpaste.

Table 2. Elemental analysis using EDS on tooth enamel surface before treatment, after demineralization, and after brushing with distilled water, Nano Systema, or Siwak for a period equivalent to 6 weeks (% weight).

| Elements on tooth enamel surface (% weight) | Treatment |
|-------------------------------------------|-----------|
|                                           | Baseline  | Demineralization | Distilled Water | Brushing with Nano Systema Toothpaste | Brushing with Siwak Toothpaste |
| C                                         | 6.44      | 15.33            | 18.53           | 11.57                                | 20.78                           |
| O                                         | 43.16     | 38.80            | 38.02           | 40.43                                | 35.33                           |
| F                                         | 0         | 0                | 0.17            | 0                                    | 0.58                            |
| Na                                        | 0         | 0.74             | 0.48            | 0.44                                 | 0.50                            |
| Mg                                        | 0.28      | 0                | 0.14            | 0.44                                 | 0.09                            |
| P                                         | 17.94     | 16.42            | 15.12           | 16.63                                | 14.91                           |
| Cl                                        | 0         | 0                | 0.37            | 0                                    | 0.08                            |
| K                                         | 0.23      | 0.12             | 0.16            | 0.30                                 | 0.18                            |
| Ca                                        | 31.94     | 28.59            | 26.98           | 30.19                                | 27.64                           |
Table 2 shows a decrease in calcium on the enamel surface before treatment and after demineralization from 31.94 (% weight) to 28.59 (% weight). The same happened to phosphate from 17.94 (% weight) to 16.42 (% weight) after demineralization. Fluoride at 0.17 (% weight) was found in the group brushed with only distilled water for a period equivalent to 6 weeks. An increase in calcium on the enamel surface from 28.59 (% weight) to 30.19 (% weight) was seen after brushing with Nano Systema toothpaste for a period equivalent to 6 weeks. The same occurred with phosphate, which increased from 16.42 (% weight) to 16.63 (% weight). As much as 0.58 (% weight) fluoride was seen after brushing with Siwak toothpaste for a period equivalent to 6 weeks on the enamel surface. However, no fluoride was found on the baseline, after demineralization, or after brushing with Nano Systema toothpaste.

4. Discussion
In this study, the demineralization process was stimulated using 0.3% citric acid solution at a pH 3.25 to resemble an early enamel lesion. A study conducted by Attin, et al. stated that citric acid solution has a high potential for causing demineralization because it increases calcium in the tooth enamel surface [10]. According to Zhou, et al., the enamel immersed in citric acid solution increases its surface roughness [7]. The increase in roughness after demineralization can be caused by the dissolution of hydroxyapatite. In this study, enamel surface roughness increased significantly after immersion in 0.3% citric acid solution at 3.25 pH. This result is supported by the SEM image after demineralization, which showed clearer enamel prisms and based on the elemental analysis using EDS, which showed a decrease in calcium and phosphate on the enamel surface. If a demineralization process is left to continue, an extensive lesion on enamel will form and cause irreversible enamel loss [2]. To prevent such outcomes, a remineralization process is needed to replace the lost hydroxyapatite structures. Remineralization occurs when there are sufficient calcium and phosphate ions in the saliva to repair the dissolved hydroxyapatite structure. Several agents can help the saliva in triggering remineralization, one of which is toothpaste.

For this study, it compared brushing with distilled water, Nano System toothpaste, and Siwak toothpaste. The results showed that brushing with only distilled water for a period equivalent to 6 weeks can significantly decrease the enamel surface. This is important because it shows that the use of a toothbrush affects the enamel surface roughness. A decrease in enamel surface roughness after brushing with only distilled water may be due to pressure during brushing. According to Wiegand, et al. (2007), the bigger the load used during tooth brushing, the higher the demineralization degree [8]. According to our results, the demineralized enamel smoothened after brushing with only distilled water. This may be due to the weakened structure of the demineralized enamel; therefore, the load from tooth brushing can abrade the sides of enamel prisms, thus smoothing the enamel surface. Therefore, the roughness decrease observed was not due to a remineralization process. This result is supported by SEM images showing unclear enamel prism borders. Moreover, calcium and phosphate increases on the enamel surface were not evident from the EDS analysis of enamel brushed with only distilled water.

The remineralization process on demineralized enamel may occur when the pH of the saliva is in a neutral state (when the saliva’s buffering ability is stable) and when there are enough Ca2+ and PO4-3 ions in the saliva. Factors that can help remineralization are saliva, mouthwash, topical fluoride, CPP-ACP, and toothpaste [2]. Brushing with toothpaste can aid saliva to trigger remineralization. According to Nakashima, et al., toothpaste containing nano calcium carbonate can remineralize early lesions of enamel because nano calcium carbonate has good retention on enamel surface as it forms colloid particles followed by Ca2+ deposits [4]. The minuscule particle sizes accelerate the dissolution of Ca2+ ions on nano calcium carbonate, thus increasing the concentration of Ca2+ ions and causing remineralization [4]. In this study, brushing with Nano Systema toothpaste which contains nano
calcium carbonate produced a significant decrease in roughness after brushing for a period equivalent to 6 weeks. This result was supported by the SEM image of enamel surface brushed with Nano Systema toothpaste, which showed white images on the enamel surface, and by the EDS elemental analysis showing increases in calcium and phosphate, on the enamel surface. Brushing with Siwak toothpaste also provides fluoride. Fluoride can trigger remineralization because it creates fluoro apatite to replace lost hydroxyapatite crystals [2]. Our results showed a significant decrease in enamel surface roughness after brushing with Siwak toothpaste for a period equivalent to 6 weeks. This is supported by the SEM images showing white colored spots on the enamel surface and by the EDS elemental analysis showing increased fluoride content after brushing with Siwak toothpaste.

According to Heshmat, et al., remineralization can decrease enamel surface roughness [3]. This study showed significant enamel surface roughness decrease after brushing for durations equivalent to 2, 4, and 6 weeks using Nano Systema and Siwak toothpaste. This proved that the remineralization process occurred after brushing with Nano Systema and Siwak toothpaste, which is supported by SEM images and EDS elemental analyses. However, the enamel surface roughness decreases after brushing with Nano Systema and Siwak toothpaste were not only affected by toothpaste but also affected by the pressure applied to the toothbrush. We described enamel surface roughness decreases after brushing with distilled water alone. This finding agrees with those of Wiegand et al. which stated that the amount of tooth brushing loads affects the enamel surface roughness [8]. However, the changes in enamel surface roughness seen with the Nano Systema and Siwak toothpaste were caused by remineralization, as proven by our EDS results. Based on statistical tests, the comparison between enamel surface roughness mean values between the Nano Systema and Siwak groups at 2 weeks brushing showed a significant difference. This may be due to the nano calcium carbonate content in Nano Systema toothpaste; presumably, Ca2+ ions were dissolved faster, and Ca2+ concentration on the enamel surface increased compared with the content with the Siwak toothpaste [4]. Even though a significant decrease in surface roughness was evident, the statistical analysis results between enamel surface roughness at baseline and after brushing for a period equivalent to 6 weeks using the Nano Systema and Siwak toothpaste still showed a significant difference. This proves that brushing for a period equivalent to 6 weeks is not enough to return the enamel surface roughness to the same state as before the demineralization. But even though the enamel surface roughness after brushing did not completely recover, the roughness value was still under 0.5 µm (a roughness which can be felt by the tongue) [11]. In this study, the enamel surface roughness after brushing for a period equivalent to 6 weeks showed a roughness value under 0.2 µm. A study conducted by Tantanuch showed that bacteria could adhere to enamel surfaces when the roughness is over 0.2 µm [12]. Therefore, further studies are needed with more than 6 weeks of brushing.

5. Conclusion
In conclusion, brushing with toothpaste containing nano calcium carbonate and Siwak can decrease the surface roughness of demineralized enamel. Also, the nano calcium carbonate toothpaste can trigger remineralization faster because its smaller particle size causes it to dissolve more easily.

6. References
[1] Balogh M B and Fehrenbach M J 2006 Dental Embryology, Histology and Anatomy (USA: Elsevier) p 403
[2] Mount G J and W R Hume 2005 Preservation and Restoration of Tooth Structure (Queensland: Kowledge Books and Software) pp 22–41
[3] Hesmat H, Ganjkar M H, Jaberi S and Fard M J 2014 The effect of remin pro and MI paste plus on bleached enamel surface roughness J. Dent. Tehran Univ. Med. Sci. 11 131–6
[4] Nakashima S, Yoshie M, Sano H, and Bahar A 2009 Effect of a test dentifrice containing nano-sized calcium carbonate on remineralization of enamel lesions in vitro J. Oral Sci. 1 69–77
[5] Ezoddini-Ardakani F 2010 Efficacy of Miswak (Salvadora persica) in preventing dental caries Health 2 499–503
[6] Tellefsen G, Liljeborg A, Johannsen A and Johannsen G 2011 The role of the toothbrush in the abrasion process Int. J. Dent. Hyg 9 284–90
[7] Zhou C, Zhang D, Bai Y and Li S 2014 Casein phosphopeptide-amorphous calcium phosphate remineralization of primary teeth early enamel lesions J. Dent 42 21–9
[8] Wiegand A, Kowing L and Attin T 2007 Impact of brushing force on abrasion of abrasion of acid-softened and sound enamel Arch. Oral Biol. 52 1043–7
[9] Jayakumar A, Padmini H, Haritha A and Reddy K P 2010 Role of dentifrice in plaque removal: a clinical trial Indian J. Dent. Res. 21 213–7
[10] Attin T, Weiss K, Becker K, Buchalla W and Wiegand A 2005 Impact of modified acidic soft drinks on enamel erosion Oral Dis. 11 7–12
[11] Jones C S, Billington R W and Pearson G J 2004 The in vivo perception of roughness of restorations Br. Dent. J. 196 42–5
[12] Thananthuch S and Patanapiradeje V 2009 Effect of Thai wine of surface roughness and corrosion of various tooth-coloured filling materials J. Dent. Assoc. Thai. 59 100–8