Effects of miswak and nano calcium carbonate toothpastes on the hardness of demineralized human tooth surfaces

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Abstract. This study aimed to investigate and compare the effects of brushing with miswak and nano calcium carbonate toothpaste on surface hardness of human tooth surfaces that had been demineralized using citric acid. After brushing for fourteen or twenty-eight minutes, surface hardness was measured using a Knoop Hardness Tester and data were compared using Wilcoxon, Kruskal--Wallis and Mann-Whitney tests. These analyses showed that increase in surface hardness was greater after application of nano calcium carbonate toothpaste than after application of miswak toothpaste (p < 0.05).

1. Introduction
Enamel is a crystalline material comprising calcium hydroxyapatite and covered entire anatomical crowns of human teeth. Enamel lesions are formed after exposed to acid and are exacerbated by plaque retention on tooth surfaces. Lesions on tooth surface initially appear as white spots and develop into holes after that. These chalky white spots on tooth surfaces are not always considered caries, despite serving as an early indicator of the lesion formation. Early lesions formed due to demineralization of enamel surfaces, and contributing factors include plaque retention, acid exposure, and decreased salivary flow and buffering capacity [1,2].

Early lesions are reversible and can be recovered through remineralization, which restores the structure of enamel teeth and increases the hardness of enamels that have been demineralized [1,3]. Remineralization can occur under the condition of sufficient calcium, phosphate and fluoride concentrations, and increased flow and salivary buffer capacity. Saliva was previously identified as an important factor in remineralization, and Ca²⁺ and HPO₄²⁻ content likely replaced losses of ions during demineralization. HPO₄²⁻ also increases the buffering of saliva to ameliorate the conditions that cause initial lesions [4]. Although saliva also contains an average fluoride content of 0.03 ppm, in addition to calcium and phosphate and these constituents could be supplemented from mouthwash, toothpaste, and topical application of remineralization agents [5]. The main function of toothpaste is to prevent caries that occur due to demineralization and loss of calcium apatite which can be replaced from Ca²⁺ and fluoride compounds in toothpaste [4].

Toothpaste containing calcium phosphate and fluoride may trigger remineralization, and the formation of apatite minerals in lesion was shown to be affected by quantities of calcium and phosphate that enter the lesion [6]. Hydroxyapatite that is lost during demineralization can be replaced...
by fluoride, which settles on the lesions and forms fluoride apatites. However, the efficacy of fluoride to remineralization depends on the presence of calcium, because access and retention of fluoride in lesions of tooth enamel are dependent on calcium availability [6,7].

Modern toothpaste contains nano-sized calcium carbonate and fluoride this can be provided from herbal ingredients such as miswak, which is an extract of the *Salvadora persica* plant. Nano calcium carbonate releases calcium ions more quickly than calcium carbonate particles of larger sizes, as indicated by elevated levels of calcium and increased pH in sub-surface lesions after exposure to nano calcium carbonate for four weeks [6,8]. Although miswak toothpaste contains fluorine and calcium carbonate and can remineralize teeth within 21 days, nano-sized calcium carbonate may enhance the absorption of fluoride into the lesions [9].

Because no previous study compares remineralization following the use of nano calcium carbonate toothpaste and herbal toothpaste containing miswak, this present study aimed to determine the effectiveness of these toothpaste regarding of enamel hardness after remineralization.

2. Methods

A total of 24 third molar enamel specimens were mounted into molds using acrylic and were divided into three groups consisting of 8 specimens. Specimens were smoothed using silicone carbide paper of grades 800 and 2000 and were then polished using 1-μm alumina. Measurements of initial hardness were performed using a Knoop Hardness Tester (Zwick Roell-USA) with a load of 50 g for 15 s.

After initial measurements of hardness specimens were soaked in 25 mL of 0.3% citric acid solution (pH 3.25) for 3 min and enamel hardness was tested again after demineralization. Specimens were then brushed with distilled water (control), or with 3 mL of distilled water containing 3 g of nano calcium carbonate or miswak toothpaste. Brushing was conducted using Pierrot's electric toothbrush with a controlled brush pressure of 150 g. Brushing was conducted for 14 min (equivalent to 2 weeks) and 28 min (equivalent to 4 weeks) and enamel hardness was again measured as described above.

The resulting surface hardness values were tested normality and homogeneity using Levene's statistics. Subsequently, pairwise differences between treatment groups were identified using non-parametric Wilcoxon tests and multiple comparisons were made using Kruskal-Wallis tests. Finally, comparisons were made using Mann-Whitney tests and differences were considered significant when p<0.05.

3. Results

Changes in enamel hardness after demineralization and brushing for the equivalent of 2 and 4 weeks were expressed in hardness values.

Changes of hardness before and after immersion of the specimens in 0.3% citric acid solution and after brushing with distilled water, nano calcium carbonate toothpaste, or miswak toothpaste were determined using a Knoop Hardness Tester (Table 1). Enamel hardmesses decreased similarly after demineralization in all treatment groups.

| Groups                  | Initial Hardness | After Demineralization | Brushing equivalent to 2 weeks | Brushing equivalent to 4 weeks |
|-------------------------|------------------|------------------------|--------------------------------|--------------------------------|
| Distilled Water         | 361 ± 5.79       | 251 ± 3.27             | 253 ± 4.53                     | 254 ± 3.41                     |
| Nano-calcium carbonate  | 355 ± 9.59       | 246 ± 3.04             | 288 ± 3.82                     | 328 ± 2.46                     |
| toothpaste              |                  |                        |                                |                                |
| Miswak toothpaste       | 360 ± 5.30       | 251 ± 2.91             | 271 ± 2.58                     | 296 ± 1.86                     |

To identify significant changes in hardness within treatment groups and differences between groups the data were analyzed using Wilcoxon tests. These analyses revealed a significant decreases in hardness after demineralization in all groups and brushing for the equivalent to 2 weeks with nano
calcium carbonate toothpaste, or miswak toothpaste led to significant increases in hardness (p <0.05), whereas brushing with distilled water did not (p > 0.05). Further significant increases in hardness were observed between the equivalent of 2 and 4 weeks brushing in specimens that were brushed with nano calcium carbonate toothpaste and miswak toothpaste, whereas no significantly increases in hardness were identified in the distilled water group (p>0.05). Finally, enamel hardness values were significantly higher after brushing for the equivalent to 4 weeks in all groups compared with those immediately after demineralization (p <0.05).

In further statistical analyses using Kruskal-Wallis multiple comparisons tests, decreases and increases in enamel hardness values within and between the three treatment groups were analyzed (Table 2). These analyses showed no significant between-group differences in initial hardness (p>0.05) However, after brushing for the equivalent of 2 and 4 weeks, hardness values differed significantly between control and toothpaste groups.

### Table 2. Kruskal-Wallis tests of changes in enamel hardness between the three brushed groups.

|                      | Sig.     |
|----------------------|----------|
| Initial enamel hardness | 0.387**  |
| Enamel hardness after demineralization in 0.3% citric acid | 0.083**  |
| Enamel hardness after brushing for the equivalent of 2 weeks | 0.000*   |
| Enamel hardness after brushing for the equivalent of 4 weeks | 0.000*   |

*P<0.05; **P>0.05

Post Hoc Mann-Whitney tests were performed to compare hardness values between brushed groups (Table 3). These analyses again showed significant differences between distilled water group and the nano calcium carbonate toothpaste group, between the distilled water group and miswak toothpaste brushed groups after brushing for the equivalent to 2 weeks (p < 0.05). Similar differences were identified after the equivalent of 4-weeks brushing, with significant differences between all groups at this time point (p<0.05).

### Table 3. Post Hoc Mann-Whitney tests of changes in enamel hardness between brushed groups.

|                      | Sig.     |
|----------------------|----------|
| Enamel hardness after brushing for the equivalent of 2 weeks | Distilled water vs. nano calcium carbonate toothpaste groups 0.000* |
|                      | Distilled water vs. Miswak toothpaste groups 0.000* |
|                      | Nano calcium carbonate vs. Miswak toothpaste groups 0.000* |
| Enamel hardness after brushing for the equivalent of 4 weeks | Distilled water vs. nano calcium carbonate toothpaste groups 0.000* |
|                      | Distilled water vs. Miswak toothpaste groups 0.000* |
|                      | Nano calcium carbonate vs. Miswak toothpaste group 0.000* |

*P<0.05

### 4. Discussion

Mechanical properties of enamel, such as hardness, are influenced by mineral contents [10]. In particular, concentration of calcium plays a major role in the mechanical properties of enamel, and
changes in calcium concentrations have been correlated with decreases in hardness. Calcium contents of enamel surfaces can be reduced by dissolution of calcium due to exposure to solutions with low pH. Accordingly, this study showed significant decreases in enamel hardness after demineralization with 0.3% citric acid solution (pH 3.25) for 3 min, as shown previously in human teeth by Zhou et al. [10]. Also, demineralization of hydroxyapatite has been shown at pH below 5.5 and this process initiates below-surface lesions. Other factors that influence demineralization included plaque accumulation, and buffer capacity and flow rate of saliva [4]. In this study, 0.3% citric acid solution was used to demineralize enamel surfaces.

Decreases in enamel hardness following demineralization were indicative of lesion formation beneath the surface of the enamel. These lesions are reversible, indicating that calcium on the losses on enamel surfaces can be stopped and lost calcium structure can be replaced through remineralization. However, remineralization requires sufficient calcium, phosphate and fluoride ions, and neutral pH and adequate quantity of good quality saliva [2,4].

In this study, demineralized enamel specimens were brushed with distilled water, nano calcium carbonate or miswak toothpaste. Hardness was increased by kinds of toothpaste although brushing with distilled water failed to increase hardness significantly, reflecting the absence of minerals. However, the paucity of effects of distilled water confirmed that brushing alone is not an effective intervention. An agreement, Rios et al. showed no increase in enamel hardness after uncontrolled brushing without toothpaste in-situ [11].

Calcium, phosphate, and fluoride content of saliva and plaque played important roles in the formation of caries, and high calcium concentrations are likely preventive [12]. Saliva conditions in the oral cavity are influenced by food, which can alter buffer capacity and destabilize the quality and quantity of saliva. Therefore additional mineral intake is required to support the remineralization process. The present toothpaste contain calcium and fluoride ions, which are required for remineralization [4,6,9]. This study also showed that nano calcium carbonate toothpaste produces a superior increase in enamel hardness, with significant improvements after the equivalent of 2 and 4 weeks likely reflecting the presence of calcium on enamel surfaces. Similarly, Balakrishnan et al. compared three calcium containing toothpaste products and showed an increase in enamel hardness [3]. In addition, the considerable superiority of the nano calcium toothpaste may be attributed to similar sizes of nano particles to the hydroxyapatite in enamel allowing greater access to sub enamel surface lesions than nitrate-size calcium carbonates, although the 4 week duration of brushing has been shown to increase hardness regardless of the toothpaste used. Accordingly, Nakashima et al. (2009) showed increased calcium content and hardness of enamel lesions after exposure to nano calcium carbonate paste for the equivalent of 4 weeks [6].

Increased hardness was also evident after only 2 weeks of brushing with herbal toothpaste containing miswak, which contain fluoride required for remineralization, as previously reported by Ezoddini-Arakadan [9]. Fluoride binds calcium ions to form fluorapatite and replaces demineralized hydroxyapatite, and the present data agrees with that reported by previous studies. These investigators showed remineralization and increased the hardness of demineralized enamel surfaces after soaking in 5% and 10% miswak extract for 1 week, again reflecting the presence of fluoride, which binds calcium and phosphate to replace hydroxyapatite [13,14].

The present nano calcium carbonate toothpaste was superior to the miswak toothpaste, likely because the effects of fluoride are limited to the availability of calcium, and because nano-sized calcium carbonate particles have greater access to sites of demineralization. Also, Nakashima et al. showed that the release of calcium ions from nano calcium carbonate was faster than that from nano-sized calcium carbonate, leading to higher calcium levels at the enamel surface [6]. The present study shows that brushing with nano calcium carbonate toothpaste for the equivalent to 2 and 4 weeks restores enamel hardness to within this range with the measured hardness of 288 ± 3.82 KHN and 328 ± 2.46 KHN, respectively. Although brushing with miswak toothpaste also restored hardness to within the reported range for human tooth enamel, this study showed the hardness of only
296 ± 1.86 KHN after the equivalent of 4 weeks. This data indicates that kinds of toothpaste prevent the expansion of sub-enamel lesions by triggering remineralization.

5. Conclusion
From the present analyses, we conclude that brushing with nano calcium carbonate toothpaste or miswak toothpaste for the equivalent of 2 and 4 weeks restore the hardness of demineralized tooth enamel. However, even after the equivalent of 4 weeks brushing, neither toothpaste restores the initial hardness completely. Finally, our data show that toothpaste containing nano calcium carbonate offers superior protection against caries than miswak toothpaste.

6. References
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