

Changes in the permeability and morphology of dentine surfaces after brushing with a Thai herbal toothpaste: A preliminary study

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ABSTRACT

Objectives: The aim of this study was to evaluate dentine permeability after brushing with Twin Lotus[®], Thai herbal toothpaste by comparing with Sensodyne Rapid Relief[®], a commercial desensitizing toothpaste, and also after artificial saliva (AS) immersion or citric acid challenge. **Materials and Methods:** Dentine discs from human mandibular third molars were divided into three groups ($n = 20$) and brushed with either experimental toothpaste or water (control) for 2 min with an automated toothbrush. Then, 10 discs were immersed in AS, and the other 10 discs were immersed in 6% citric acid to simulate the conditions of the oral environment. The dentine permeability of each specimen was measured before brushing and after each treatment using a fluid filtration system. Morphological changes in the dentine were observed using scanning electron microscopy (SEM). **Results:** Both toothpastes significantly reduced dentine permeability, and a crystalline precipitate was observed on the dentine surface under SEM observation. No significant difference was found between the two toothpaste groups with regard to dentine permeability after brushing and AS or acid immersion. **Conclusions:** The dentine permeability reduction caused by the two toothpastes did not differ after brushing or after AS or citric acid immersion.

Key words: Dentine permeability, herbal toothpaste, occlusion

INTRODUCTION

Dentine hypersensitivity is normally caused by exposure of dentine to external stimuli such as temperature changes, air, sour drinks, and sweet drinks.^[1] Enamel and cementum, which normally cover the exposed dentine, can become damaged due to attrition, abrasion, erosion, abfraction, and gingival recession.^[2,3] Dentine hypersensitivity occurs more frequently in the cervical region of the tooth because

the cementum in this area is very thin, ranging from 20 to 50 μm .^[4]

The mechanism of dentine hypersensitivity is explained by the hydrodynamic theory,^[5] which states that the stimulus evokes a fluid shift in the dentinal tubules affecting the pulpal mechanoreceptors, which results

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in the sensation of pain. Desensitizing toothpaste can be used to relieve or reduce the pain of dentine hypersensitivity through one or both of the following mechanisms of action. First, toothpastes may contain potassium salts that have a depolarizing effect on electrical nerve conduction along nerve fibers, which can cause the dentine to be less excitable by stimuli.^[6] Second, toothpastes may contain synthetic active components such as strontium chloride, strontium acetate, or sodium fluoride, which can interact with dentine, causing the formation of a precipitate.^[7]

The aim of this study was to evaluate the ability of Thai herbal toothpaste to reduce dentine permeability compared with commercial toothpaste, Sensodyne Rapid Relief[®], using a fluid filtration system. Changes in dentine morphology were observed before and after brushing using scanning electron microscopy (SEM).

MATERIALS AND METHODS

Experimental design

Tooth extraction

Sixty noncarious human mandibular third molars extracted for surgical reasons (age 18–22 years) were collected under a protocol approved by the Ethics Committee of Rangsit University (No. RSEC 23/53). The teeth were cleaned and stored in 0.1% thymol solution at 4°C within 1 month before the experiment.

Disc preparation

Sixty dentine discs that were each $1000 \pm 100 \mu\text{m}$ in thickness and obtained from areas that were close to the pulp cavity were sectioned perpendicular to the long axis of the teeth using a low-speed, water-cooled diamond saw (Accutom 50, Struers, Copenhagen, Denmark). The thickness of the dentine discs was measured from the dentine surface to the highest pulpal horn using pincer-type calipers.

Dentine permeability measurement

Each disc was placed on Perspex[™] glass (2 cm × 2 cm × 0.5 cm) perforated by an 8-gauge stainless steel tube using cyanoacrylate adhesive. Each specimen was connected to a fluid filtration system using a technique similar to that employed by Sauro *et al.*^[8] under a simulated pulpal pressure of 20 cm H₂O. The occlusal surface of each dentine disc was polished with 600-grit SiC paper for 30 s to create a standard smear layer. The smear layer was subsequently removed by applying 37% phosphoric acid to the dentine surface for 15 s. The etched dentine surface was rinsed and

kept wet to evaluate maximum dentine permeability. The dentine permeability of each specimen was measured for 3 min in triplicate and assigned a value of 100% permeability.

Dentine brushing

The dentine discs were randomly divided into three groups of 20 specimens each. The first group was brushed with the herbal toothpaste (Twin Lotus[®] Co. Ltd., Bangkok, Thailand) (test dentifrice group), which contained the following active ingredients: 0.2% mangosteen peel, 0.5% whole *Hydrocotyle* plant, 1.18% *Clinacanthus nutans*, 2.76% cuttlefish bone powder, and extracts of orange jessamine leaf, and toothbrush tree. The second group was brushed with Sensodyne Rapid Relief[®] (GlaxoSmith Kline, Middlesex, UK) (control dentifrice group). The negative control group was treated the same as the two groups mentioned above, except that deionized water was used instead of toothpaste during brushing. An automated toothbrush (Braun Oral-B Vitality[™] Precision Clean D12.013, Braun GmbH Co, Kronberg, Germany) was used to brush the specimens for 2 min. The brushing device is presented in Figure 1. Individual toothbrushes with medium-hard bristles were used for each group. Again, the permeability of all dentine discs was measured for 3 min in triplicate as mentioned above.

Artificial saliva and acid immersion

The experimental and negative control groups were distributed into two subgroups of 10 specimens each. The dentine discs in each subgroup were individually immersed in test tubes containing 2 ml of artificial saliva (AS) (pH 6.75) at 37°C for 1 h and vibrated continuously at 100 rpm. The AS was composed of methyl-p-hydroxybenzoate (13.145 mM/L), sodium carboxymethyl cellulose (38.140 mM), KCl (8.383 mM), MgCl₂·6H₂O (0.290 mM), CaCl₂·2H₂O (1.129 mM), K₂HPO₄ (4.61 mM), and KH₂PO₄ (2.395 mM). The pH

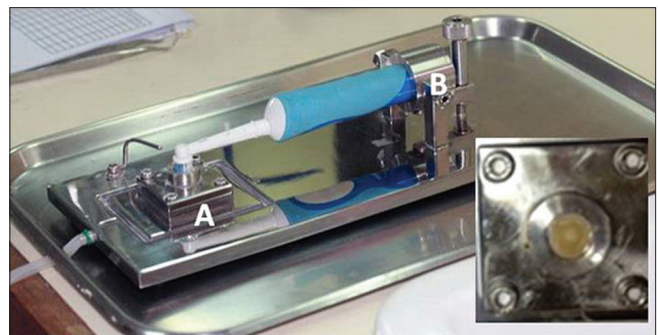


Figure 1: Tooth brushing device (A) a dentine disk holder and (B) a toothbrush holder. The inset shows a top-down view

of the AS was adjusted to 6.75 with KOH. The dentine discs of the other subgroup were immersed in 1 ml of 6% citric acid (pH 1.5) for 1 min at room temperature. The dentine permeability of all specimens after immersion in AS and acid was measured as described above.

Scanning electron microscopy analysis

After brushing and after immersion in AS or acid challenge, three dentine discs in each group were prepared for SEM observation. The specimens were dried in a desiccator for 24 h and then sputter-coated with gold (Sputter SPI, Structure Probe Inc., West Chester, OH, USA). The dentine surfaces were observed under SEM (JSM 6610 LV, JEOL, Akishima-shi, Japan) with an accelerating voltage of 20 KV and a working distance of 12 mm at $\times 2000$.

Data analysis

The linear displacement of the air bubble in each specimen was converted into volume flow ($\mu\text{l}/\text{min}$). The fluid flow across each dentine disc was transformed into hydraulic conductance (L_p , $\mu\text{l}/\text{min}/\text{cm}^2$) according to the formula $L_p = Q/At$ (where Q is the fluid flow in μl , A is the area of the dentine in cm^2 , and t is the time in minutes).^[8] Data are presented as the means and standard deviation values of the $L_p\%$. Repeated measures analysis of variance (ANOVA) was used to compare the mean permeability after the different immersions in the three groups. Tukey's *post hoc* test was used to determine significant differences between the means when the ANOVA test result was significant. The significance level was set at $P \leq 0.05$. Statistical analysis was performed using Statistical Package for the Social Sciences 18.0 for Windows (SPSS Inc., Chicago IL).

RESULTS

Dentine permeability measurements

The dentine permeability results are expressed as percentages of the maximum permeability, which was considered to be equal to 100% after etching with 37% phosphoric acid. Table 1 shows the hydraulic conductance of each group after tooth brushing and after immersion in AS or citric acid.

After brushing

Before immersion into saliva or citric acid, brushing the dentine surface after 37% phosphoric acid etching significantly reduced dentine permeability in both the Sensodyne Rapid Relief® group ($P = 0.000$) and

the Twin Lotus® group ($P = 0.012$). The permeability of the dentine in the control group was slightly reduced, but it was not significantly different from that measured before brushing ($P = 0.436$).

After artificial saliva immersion

The immersion of brushed dentine discs in saliva significantly increased the dentine permeability in the control group, and the differences between the control group and the two treatment groups were significant. However, no significant difference was found between the Sensodyne Rapid Relief® and Twin Lotus® groups ($P = 0.745$).

After acid immersion

Immersion of the brushed dentine in 6% citric acid increased dentine permeability in both the Sensodyne Rapid Relief® and Twin Lotus® groups, and no significant difference was observed between the groups. A significant increase in dentine permeability after acid immersion was found only in the control group.

Scanning electron microscopy evaluation

Most dentinal tubules were obliterated with smear plugs after polishing with SiC paper. After applying 37% phosphoric acid solution to the ground dentine surface for 15 s and rinsing, the smear layer on the surface and smear plug disappeared from the dentine surface [Figure 2a and b].

After brushing

After brushing with two toothpastes, numerous micro-fine granular crystal-like structures were revealed on the dentine surface, and some dentinal tubule openings were occluded. The granular crystals were slightly smaller on the dentine surfaces brushed with Sensodyne Rapid Relief® than on those brushed with Twin Lotus® [Figure 3b and c]. In contrast, on

Table 1: Hydraulic conductance (L_p) after tooth brushing with different treatments and immersion in artificial saliva or citric acid

Treatment	Sensodyne rapid relief®	Twin Lotus®	Water
37% phosphoric acid	100 ^{Ab}	100 ^{Ab}	100 ^{Ab}
Tooth brushing	58.3±13.1 ^{Ba}	47.7±7.3 ^{Ba}	87.4±28.1 ^{Ab}
Immersion in artificial saliva	56.3±22.3 ^{Ba}	52.5±14.4 ^{Ba}	94.2±19.8 ^{Cb}
Tooth brushing	57.3±15.9 ^{Ba}	51.9±12.5 ^{Ba}	97.9±21.2 ^{Ab}
Acid challenge	78.7±36.4 ^{Ba}	70.2±13.6 ^{Ba}	121.1±11.5 ^{Cb}

The values are expressed as a percentage of the maximum permeability obtained with 37% phosphoric acid (100%). The same letter indicates no significant difference, lowercase letters indicate no significant difference within rows, and uppercase letters indicate no significant difference within columns

the dentine surfaces brushed with water [Figure 3a], opened dentinal tubules were found in most areas.

Immersion in artificial saliva

After immersing in AS for 1 h, the group that was brushed with water did not show morphological changes. All dentinal tubules were found to be open, suggesting that no remineralization occurred [Figure 3d]. Both groups brushed with toothpaste exhibited a crystal-like layer on the dentine surface, and approximately half of the dentinal tubules were obliterated with granular deposits [Figure 3e and f].

Immersion in citric acid

After the citric acid challenge, the dentine surfaces brushed with water exhibited smooth surfaces without any debris remnants, and wider tubular openings were observed in most dentinal tubules, indicating more extensive demineralization in this group [Figure 3g]. The groups brushed with toothpaste showed similar

results: The granular precipitate was removed from the dentinal tubule orifices, and increased tubular opening diameters were observed in some tubules [Figure 3h and i].

DISCUSSION

According to the hydrodynamic theory, occlusion of dentinal tubule orifices prevents or reduces dentinal fluid movement due to external factors. Many previous investigations both *in vitro* and *in vivo* have shown reduced dentine permeability following treatment with various dental restorative materials and desensitizing toothpaste.^[9,10] Most herbal toothpastes are reported to have an antiplaque effect on teeth and anti-inflammatory effects on gingival tissue.^[11] Although one study reported that oxalate-containing phytocomplexes such as spinach and rhubarb reduce dentinal permeability,^[12] few reports have assessed the reduction in hypersensitivity mediated by components of herbal toothpastes.

This is the first *in vitro* study of Thai herbal toothpaste; Twin Lotus® in assessing the changes in the permeability and morphology of dentine surface after brushing. When compared to previous studies, the results of this study are similar and dissimilar in many respects. In the control group, where dentine discs were brushed with deionized water and no toothpaste was included, the hydraulic conductance was reduced

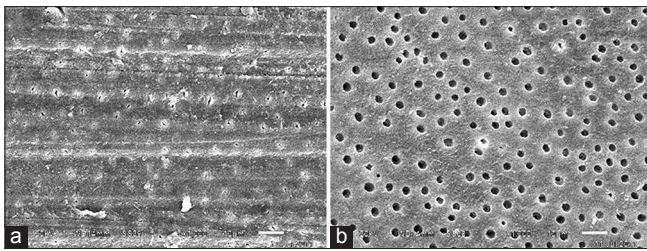


Figure 2: Dentine disk after smear layer creation (a) and after 37% phosphoric acid treatment (b) (×2000)

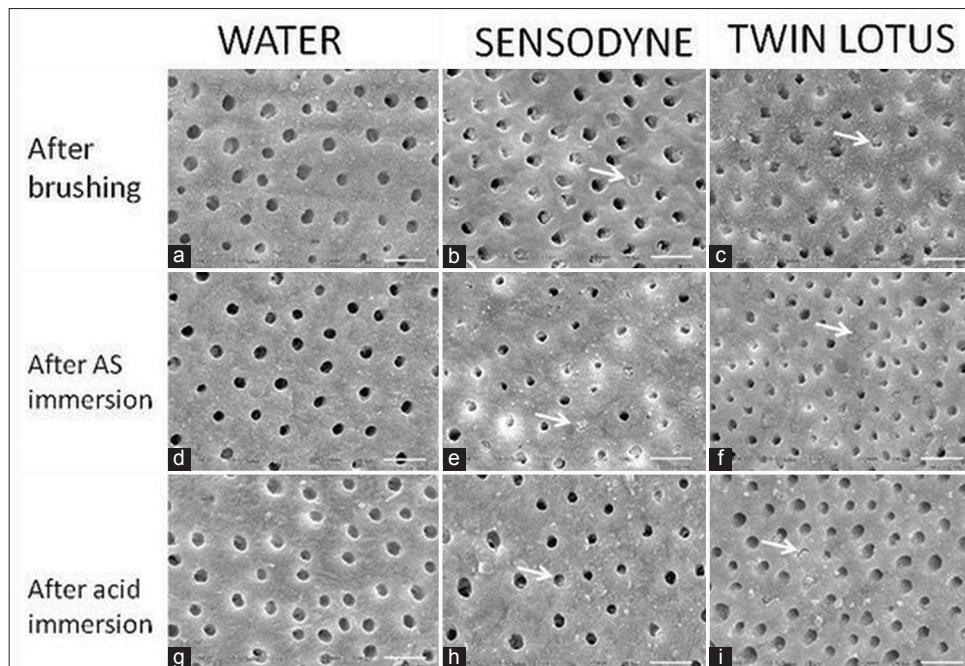


Figure 3: Scanning electron microscopy of dentine surfaces after brushing (a-c). Changes in dentine morphology after immersion in artificial saliva (d-f) or after acid challenge (g-i) (×2000). Arrows indicate dentinal tubules occluded with granular crystals. Scale bar, 10 µm

13% (Lp% value 87%) from the maximum Lp% value. The smear debris from mechanical brushing likely occluded the dentinal tubules and resulted in reduced dentine permeability. The reduced hydraulic conductance (Lp%) observed due to brushing with water also agrees with the results of previous studies indicating that brushing with water reduces dentine permeability by only 5–10%.^[12,13] The morphological changes observed after brushing the dentine surface with water in this study are in agreement with those described in the previous study,^[14] which showed that after brushing a smear-free dentine surface with water, most dentinal tubules remained open, and brushing had no effect on the morphology of the dentine surface.

The Lp% values after brushing with Sensodyne Rapid Relief® and Twin Lotus® were similar (58.3% and 47.7%, respectively) but were significantly lower than the value obtained after brushing with water. This result indicated that occluding granular materials from the chemical constituents in Sensodyne Rapid Relief® and from the herbal components in Twin Lotus® have a significant role in decreasing the permeability of the dentine. The results from the group brushed with Sensodyne Rapid Relief® agree with the results of a study by West *et al.*,^[15] who reported that the desensitizing effects of Sensodyne Rapid Relief® toothpaste are due to occlusion via mineralization from the formation of strontium-substituted Ca hydroxyapatite.

Tubular occlusion due to granular deposition was also found in the group brushed with Twin Lotus®. Most of the components of Twin Lotus® herbal toothpaste are derived from natural plants such as mangosteen, toothbrush tree, *C. nutans*, orange jessamine, and *Hydrocotyle*. These ingredients might occlude and reduce fluid flow through dentinal tubules. In addition, these natural plants are composed of various types of acids^[16] that may chelate with hydroxyapatite, resulting in the formation of a crystalline precipitate that occludes the dentinal tubules. The particle size of the granular precipitate found on the dentine surface brushed with Twin Lotus® was slightly larger than that of the precipitate found on the surface brushed with Sensodyne Rapid Relief®.

After brushing with water, Sensodyne Rapid Relief® or Twin Lotus®, dentine discs were immersed in AS and incubated in a rotating incubator at 37°C, 100 rpm/min, for 1 h. AS has been reported to dissolve occluding depositions on dentinal tubules,

resulting in increased dentinal permeability. We did not observe much alteration in the Lp% value after AS immersion in the groups brushed with either toothpaste, suggesting that the granular deposition on the dentine surface may resist solubilization by AS. Although the immersion period in this study (1 h) was shorter than the immersion times used in other studies (24 h, 2 h),^[14,17] vibrating rotation in AS tends to increase hydrolysis and is closely related to the clinical scenario.

In the acid immersion experiment, we immersed the dentine discs to set criteria, in 6% citric acid (pH 1.5) for 1 min to simulate eating or drinking something sour. Many acids and other components can chemically erode teeth. In particular, citric acid has been documented to be a harmful acid causing enamel and dentine erosion, and it is a common component of fruit and soft drinks.^[18] As expected, dentine permeability in the deionized water group was greater than that observed after 37% phosphoric acid treatment, although no significant difference was observed ($P = 0.891$). This result suggested that the dentine surface is demineralized by citric acid even after only 1 min of etching time, resulting in slight increases in the dimensions of tubular openings, and subsequently, higher Lp% values. The granular products from both toothpastes, which occluded the dentinal tubules, were partially dissolved in this acid, and the Lp% values increased in the Sensodyne Rapid Relief® and Twin Lotus® groups to 78% and 70%, respectively. However, the Lp% values were not different from those obtained before immersion. This finding suggests that the occluding effect of the herbal toothpaste resists acid to the same degree as that of the commercial product containing strontium acetate.

In this study, the dentine permeability reduction found after brushing with Sensodyne Rapid Relief® was lower than that described in a previous study, which reported an approximate 70% reduction in the Lp% value.^[18] This difference may be due to the different methods used for evaluating dentine permeability. Standardizing the methodology used to determine dentine permeability is, therefore, necessary.

CONCLUSIONS

Within the limitations of this study, the results suggest that Thai herbal toothpaste has the potential to relieve tooth hypersensitivity by reducing dentine

permeability due to the effect of dentinal tubule occlusion. This occlusion is caused by deposition of granules that are resistant to solubilization in AS and acidic environments. However, further investigations are required to determine the mechanism by which the granular occluding materials of this herbal toothpaste are formed in the laboratory and also in the clinical trial.

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Conflicts of interest

There are no conflicts of interest.

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