Original Article

The Effect of Herbal Toothpastes on Aged Root Dentin Surface Roughness After Brushing

Pasika Meenamphant[1], Rangsima Sakoolnamarka[1], Issara Wongpraparatana[1], Kopkrit Hataiareerug[1]

[1]Department of Operative Dentistry, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand

Abstract

This study aimed to evaluate the impact of toothbrushing with herbal toothpaste on the surface roughness and loss of aged dentin following toothbrushing with herbal toothpastes. Buccal root dentin specimens were prepared from molars of patients ≥ 60 years old. Specimens were allocated into four groups (n=10) according to the toothpaste used: three herbal toothpastes, HB1, HB2, HB3, and one non-herbal toothpaste, NHB. After undergoing 10,000 cycles of toothbrushing, the surface roughness and surface loss of the specimens were investigated using a contact profilometer. Toothbrushing resulted in a statistically significant increase in surface roughness relative to baseline measurements for all groups (p<0.001). Among these groups, the HB2 group exhibited the highest final surface roughness (Ra_f) value (9.81 ± 0.86 µm), while the NHB group demonstrated the lowest Ra_f value (0.78 ± 0.10 µm). Statistically significant differences in Ra_f values were observed among all groups (p<0.001). The highest surface loss of dentin was found in the HB2 group (0.047 ± 0.012 mm^3), then HB3 (0.039 ± 0.012 mm3), NHB (0.029 ± 0.009 mm^3), and the HB1 group (0.0197 ± 0.005 mm^3). SEM revealed a scratched dentin surface of all specimens indicative of toothbrush bristle abrasion. Among the groups, dentin in the HB2 group exhibited a more pronounced concave area compared to the dentin in the other groups. Analyses of the toothpastes demonstrated variations in shape of their abrasive particles. In conclusion, the use of herbal toothpastes resulted in significantly greater dentin surface roughness compared to non-herbal toothpaste. These findings suggested that abrasive particles in herbal toothpastes may contribute to increased tooth surface wear.

Keyword: Dentin, Geriatric dentistry, Herbal toothpaste, Toothbrushing, Tooth surface roughness

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Doi:

Correspondence to:

Rangsima Sakoolnamarka, Department of Operative Dentistry, Faculty of Dentistry, Chulalongkorn University, Bangkok 10330 Thailand.

Tel: 0814559487 E-mail: aorrangsima@yahoo.com

Introduction

The prevalence of oral health conditions such as tooth wear and gingival recession increases with advancing age in the elderly population.[1] The combined effects of mechanical factors, such as excessive toothbrushing force, and chemical factors such as dietary acid, are likely contributors. One such condition is the non-carious cervical lesion (NCCLs), consisting of pathological tooth surface loss occurring at the cemento-enamel junction (CEJ). The prevalence, severity and progression of NCCLs increases with age.[2,3] The etiology of NCCLs is multifactorial, toothbrushing being among them. Factors related to toothbrushing are, for example, brushing horizontally, using a large brushing force, brushing many times a day, and using highly abrasive toothpaste as well as the stiffness of the toothbrush bristles used.[4-7]

In Thailand, herbal products, including herbal toothpaste, are widely used by elderly people.[8,9] Herbal toothpastes are formulations that incorporate various natural plant-based ingredients such as herbs, spices and plant-derived substances. Toothpastes may incorporate herbal ingredients with potential therapeutic benefits. Examples include streblus asper bark (toothbrush tree) and guava leaf extract, which are believed to exhibit antimicrobial and anti-inflammatory properties. Borneol camphor and licorice extract are also utilized for their antimicrobial effects. Furthermore, essential oils such as pepper mint, eucalyptus, and clove contribute to antimicrobial and antibacterial properties. Additionally, these toothpastes typically contain abrasive substances, which may lead to tooth surface roughness. A few studies[10-12] have investigated enamel surface roughness after using herbal toothpastes, but not in geriatric patients who have root dentin surfaces exposed due to gingival recession. Moreover, most of these studies evaluated surface roughness after brushing with herbal toothpaste over a short period of time such as one month.[12]

Therefore, the aims of this study were: 1. to compare the root dentin surface roughness before and after toothbrushing within the same toothpaste group; 2. to compare the root dentin surface roughness and surface loss after toothbrushing among different toothpastes. The null hypotheses were: 1. There is no difference in surface roughness of dentin before and after toothbrushing within the same toothpaste group; 2. There is no difference in surface roughness and surface loss of dentin among different toothpastes after toothbrushing.

Materials and Methods

Sample size calculation

The sample size was calculated using G*Power (Version 3.1.9.6) with a power of 80% and a 95% confidence level according to data from Korsuwannawong *et al.*[10] The estimated sample size was three per group. To compensate for error (10%) and due to a previous study,10-12 the total sample size per group was 10.

Specimens preparation

Forty extracted molars were obtained from patients aged 60 years or above, who underwent tooth extraction due to periodontal reasons. Prior to the study, ethical approval was granted by the Human Research Ethics Committee of the Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand (HREC-DCU 2023-018). The teeth were stored in a 0.1% thymol at 4°C and used within three months after extraction. Only teeth without caries and cracks were included. Teeth were cleaned with an ultrasonic scaler. The crown of each tooth was removed at the cemento-enamel junction. The buccal root dentin of dimensions 5 mm mesiodistally x 3 mm cervico-apically x 3 mm deep was obtained using a low-speed cutting machine under water irrigation (Isomet 1000; Buehler, Lake Bluff, IL).

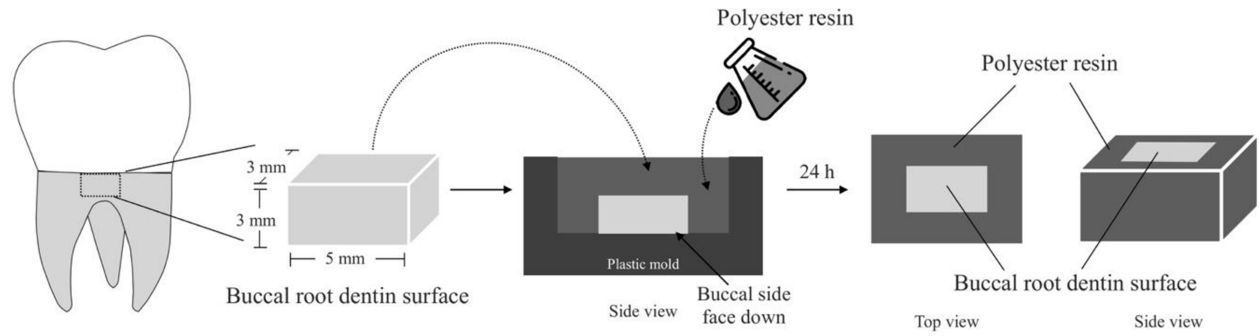

Figure 1 *Specimen preparation*

The dentin sample was placed at the bottom of the plastic mold (12 mm wide x 19 mm long x 8 mm deep) with the buccal side face down. The mold was filled with polyester resin (RA-700; Rungart, Bangkok, Thailand). After 24 hours, all specimens were removed from the mold and the dentin surfaces were plane polished using sandpaper of 600, 800 and 1,000 grit sequentially for 60 s each using a polishing machine (Minitech 233; PRESI, Eybens, France) to remove the excess polyester resin. Specimens were stored in 37°C distilled water until used (Fig. 1).

Specimens were randomly allocated into four groups (n=10) according to the toothpaste used. The toothpastes utilized in this study were carefully selected based on their popularity within the Thai market and their unique formations containing a variety of herbal ingredients. The active ingredients of each toothpaste are shown in Table 1.

Table 1 Toothpastes (data from manufacturers)

Group	Product name	Manufacturer	Ingredients	Abrasive
HB1	Tepthai concentrated herbal toothpaste original flavor (HB1)	Tepthai product Co., Ltd. (Songkhla, Thailand)	Water, calcium carbonate, sodium lauryl sulfate, cellulose gum, sorbitol, Camphor, Borneol, menthol, sodium benzoate, peppermint Oil, clove Oil, Licorice extract, sodium fluoride, Eclipta prostrata leaf extract, Psidium guajava leaf extract, Streblus asper bark	Calcium carbonate
HB2	Dokbuaku original herbal toothpaste (HB2)	Twinlotus company limited (Bangkok, Thailand)	Sorbitol (natural origin from cassava), calcium carbonate (mineral Abrasive), Cuttlefish bone, Toothbrush tree, Clinacanthus nutans, sodium lauryl sulfate (natural origin from coconut oil), hydrated silica (mineral thickener), Orange jessamine, peppermint oil, menthol, eucalyptus oil, sodium benzoate	Calcium carbonate, hydrated silica
HB3	Viset-niyom herbal toothpaste (HB3)	Viset & I Co., Ltd. (Bangkok, Thailand)	Dicalcium phosphate, water, sorbitol, glycerin, saccharin, salt, menthol crystals, peppermint oil, clove oil, cinnamon oil, Glyceriza extract, benzoic acid, sodium benzoate, sodium lauryl sulfate (natural origin from coconut oil), carboxymethyl cellulose, Borneol flakes, Camphor	Dicalcium phosphate
NHB	Colgate maximum cavity protection (NHB)	Colgate-Palmolive (Thailand) Limited (Thailand)	Dicalcium phosphate dihydrate, water, sorbitol, sodium lauryl sulfate, hydrated silica, arginine, sodium monofluorophosphate, flavor, cellulose gum, phosphoric acid, tetrasodium pyrophosphate, sodium saccharin, CI 77891 (titanium dioxide)	Dicalcium phosphate dihydrate, hydrated silica

Initial surface roughness and surface evaluation

The initial surface roughness (Rai) of each specimen was measured using a contact profilometer (Talyscan 150; Taylor Hobson, Leicester, UK). A 2-μm diameter diamond stylus was used at a constant speed of 0.05 mm/s with a 0.7-mN force and a 1.25-mm scan length (0.25 x 5 mm cut-off length).[10,13] To reproduce the measurement location, all specimens were marked on the sides with a permanent marker.

The data were analyzed by TalyMap software (Taylor Hobson, Leicester, UK) which demonstrated surface profile and surface roughness. All specimens were required to have a Rai value below 0.1 µm, as per the guidelines of ISO 11609:2017[14], to ensure a uniform starting point for the toothbrushing. To evaluate the initial surface morphology, two specimens, excluded from the experimental group, were dehydrated, coated with a thin layer of gold using a sputter coater (JFC-1200 Fine coater, Jeol Ltd, Tokyo, Japan) and examined undera scanning electron microscope (Quanta 250; FEI, Hillsboro, OR) at an accelerating voltage of 20 kV and a magnification of 1,000x.

Toothbrushing

The specimen surface was covered with transparent tape (Scotch® Magic™ Tape 810; 3M, Bangkok, Thailand) except for a 2 x 2-mm window to expose the surface to toothbrushing.[15] Toothpaste slurries were prepared according to ISO 11609:2017 by mixing 25 g of each toothpaste with 40 mL of distilled water.[14]

The specimens were put in holder and brushed with the toothpastes using a soft nylon bristle toothbrush (311 GUM® classic toothbrush; Sunstar Singapore Pte. Ltd, Singapore) with a toothbrushing simulator (V-8 cross brushing machine; Sabri Dental Enterprises, Downers Grove, IL) (Fig. 2). The brushing machine was set up with 150 g brushing force, the motion was back and forth at the speed of 100 cycles per minute for a total of 10,000 brushing cycles. A digital push-pull force gauge (SH-100; Wenzhou Sundoo Instruments Co. Ltd, Wenzhou, China) was used to validate the brushing force.

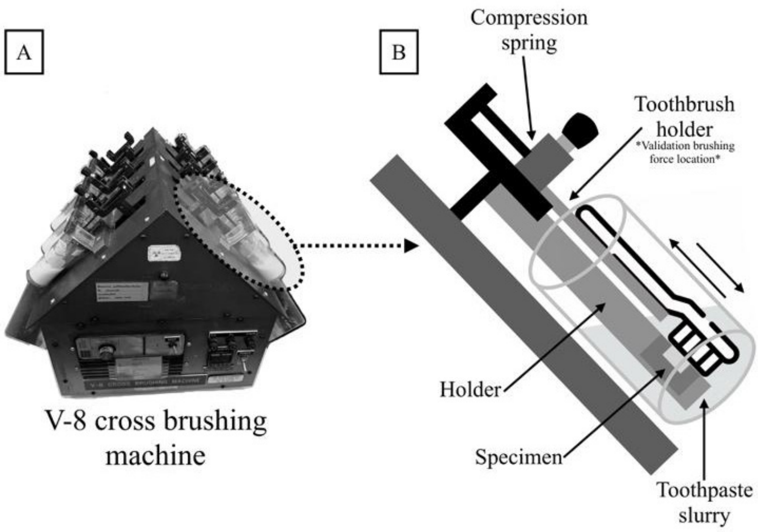

Figure 2 *Toothbrushing: (A) The V-8 cross brushing machine and brushing stations and (B) The experimental setup before undergoing toothbrushing*

Specimens were cleaned in an ultrasonic cleaner (VGT-1990QTD; Guangdong GT Ultrasonic Co Ltd, Shenzhen City, China), and kept in distilled water at 37°C in an incubator (MEM-1-IF160; Memmert, Schwabach, Germany).

Final surface roughness, surface loss and surface evaluation

The transparent tape was removed, specimens were placed on the contact profilometer and Raf values were measured at the same reference point. The surface loss was determined by analyzing the 3D surface profiles of the brushed and unaffected areas using the contact profilometer, which calculated the volume difference between the unbrushed and brushed surfaces, providing a quantitative measure of surface loss. One specimen from each experimental group was randomly selected to evaluate the final surface appearance using the scanning electron microscope at 1,000x as described above.

Toothpaste abrasive evaluation

Slurries of each toothpaste were formulated by adding 25 mg of toothpaste to 40 mL of distilled water and applied as a thin layer to filter paper using a spatula. The filter paper was dried in a desiccator cabinet (Dry-70;

Twosome Trading Corp, Taipei City, Taiwan) for 24 hours and cut into 10-mm squares. The dried toothpaste smear was gold-coated using the sputter coater and examined under the scanning electron microscope using 20 kV at 3,000x.

Statistical analysis

The data were analyzed using SPSS (IBM® SPSS® Statistics for Mac, Version 29.0.1.0, Chicago, IL). The significance level was set at $p \leq 0.05$ and the normality test was examined using Shapiro-Wilk test. Paired t-test was used to analyze the difference in mean surface roughness before and after toothbrushing. One-way ANOVA followed by the Games-Howell post-hoc test was used to analyze the difference in mean surface roughness and surface loss between groups after toothbrushing.

Results

Surface roughness and surface loss measurement

The mean surface roughness before and after the toothbrushing and tooth surface loss are presented in Table 2. Following toothbrushing, all specimens exhibited a significantly higher surface roughness compared to the initial measurements ($p<0.001$). The difference in means of final surface roughness (Raf) between groups was statistically significant for all groups ($p<0.001$). The HB2 group demonstrated the highest Raf values (9.81 ± 0.86 μm) and the NHB group showed the lowest Raf values (0.78 ± 0.10 μm). Regarding the surface loss measurement after toothbrushing, the HB2 group exhibited the highest surface loss (0.047±0.012 mm^3), while the lowest surface loss was observed in the HB1 group (0.019±0.005 mm^3). A statistically significant difference was detected between the HB1 group and both HB2 and HB3 groups ($p<0.01$ and $p=0.003$, respectively), except for the NHB group ($p=0.05$). Concurrently, no significant differences in surface loss were detected between the HB2 and HB3 groups ($p=0.434$) or between the HB3 and NHB groups ($p= 0.246$) (Table 2).

Table 2 Dentin surface roughness before and after toothbrushing, and surface loss after toothbrushing

Group	Surface roughness (μm)		Surface loss (mm^3)
	Initial (Rai) Mean ± SD	Final (Raf) Mean ± SD	Mean ± SD
HB1	0.048 ± 0.004^{a}	5.76 ± 0.67^{bA}	0.019 ± 0.005^{e}
HB2	0.056 ± 0.007^{a}	9.81 ± 0.86^{bB}	0.047 ± 0.012^{f}
HB3	0.059 ± 0.010^{a}	2.70 ± 0.34^{bC}	0.039 ± 0.012^{fg}
NHB	0.051 ± 0.041^{a}	0.78 ± 0.10^{bD}	0.029 ± 0.009^{eg}

Different lowercase letters within rows of surface roughness measurement represent significant differences between before and after toothbrushing with the same toothpaste (p <0.01). Different uppercase letters in columns of final surface roughness measurement indicate significant differences in final Ra among toothpastes after toothbrushing (p <0.01). Different lowercase letters in the column of surface loss measurement signify significant differences in surface loss value among groups (p <0.05). Rai = Initial surface roughness value, Raf = Final surface roughness value.

Surface appearance

Initial surface characterization, as illustrated in Figure 3A, revealed smooth polished dentin surfaces. Following toothbrushing, SEM images obtained at a magnification of 1000x (Fig. 3B-E) displayed dentin surfaces with scratch marks indicative of toothbrush bristle abrasion. Additionally, SEM analysis identified the presence of toothpaste particles adhering to the surface of the specimens, as indicated by white arrows (Fig. 3). The HB2 group showed a deeper concave groove from toothbrush bristles compared to other groups (Fig. 3C).

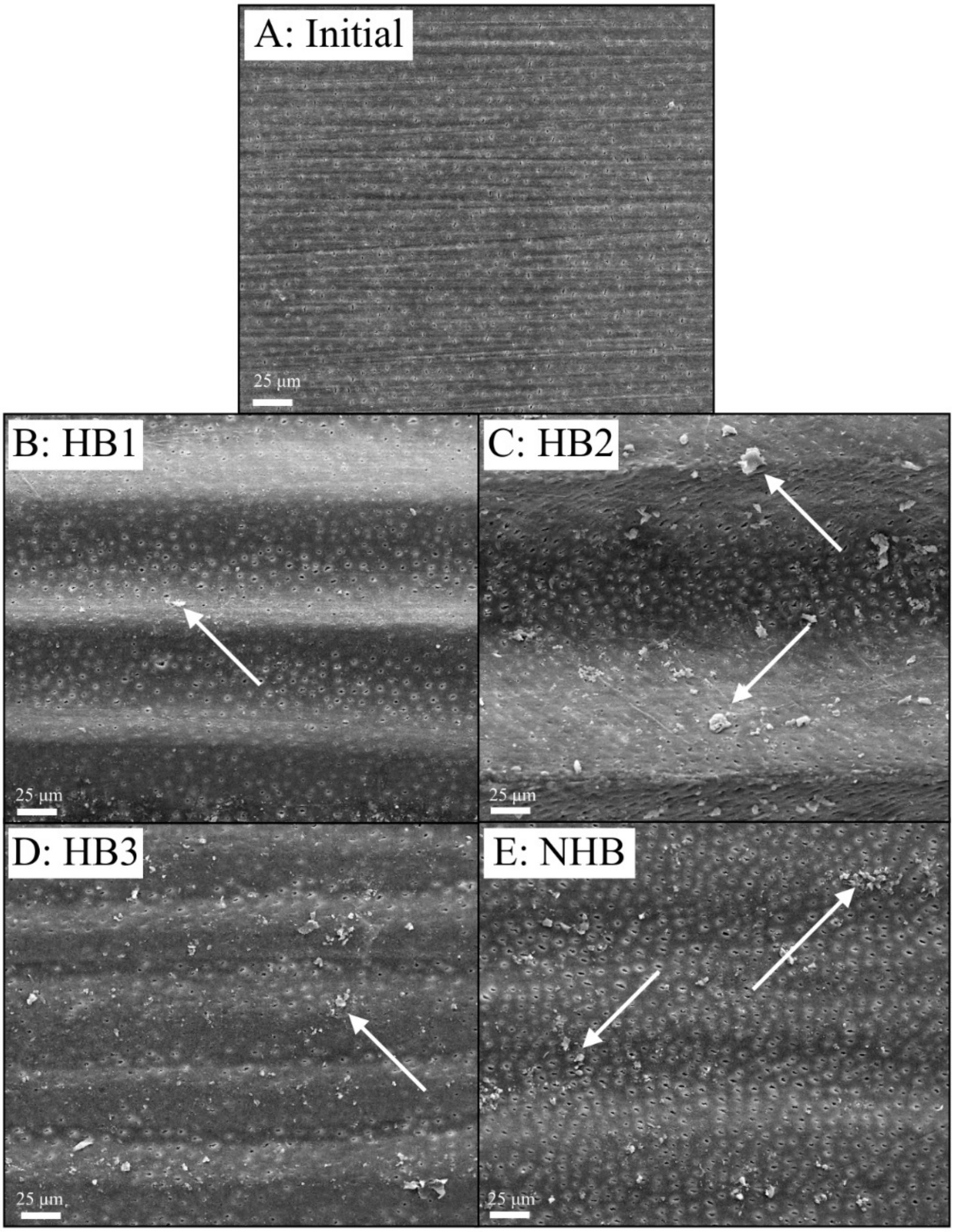

Figure 3 *Dentine surfaces: (A) Initial surface, (B-E) Final surface after toothbrushing (B; HB1 group, C; HB2 group, D; HB3 group, E; NHB group). White arrows showed toothpaste particles adherent to the surface of the dentin. Bar = 25 µm*

Toothpaste analysis

The toothpaste analysis revealed distinct abrasive particles in all groups (Fig. 4A-D). The HB1 group exhibited a uniform distribution of fine particles with particle sizes below 10 microns (Fig. 4A). Conversely, the remaining groups illustrated angular shaped particles. These particles appeared irregular and exhibited variations in dimension, including particles both smaller and larger than 10 µm (Fig. 4B-D).

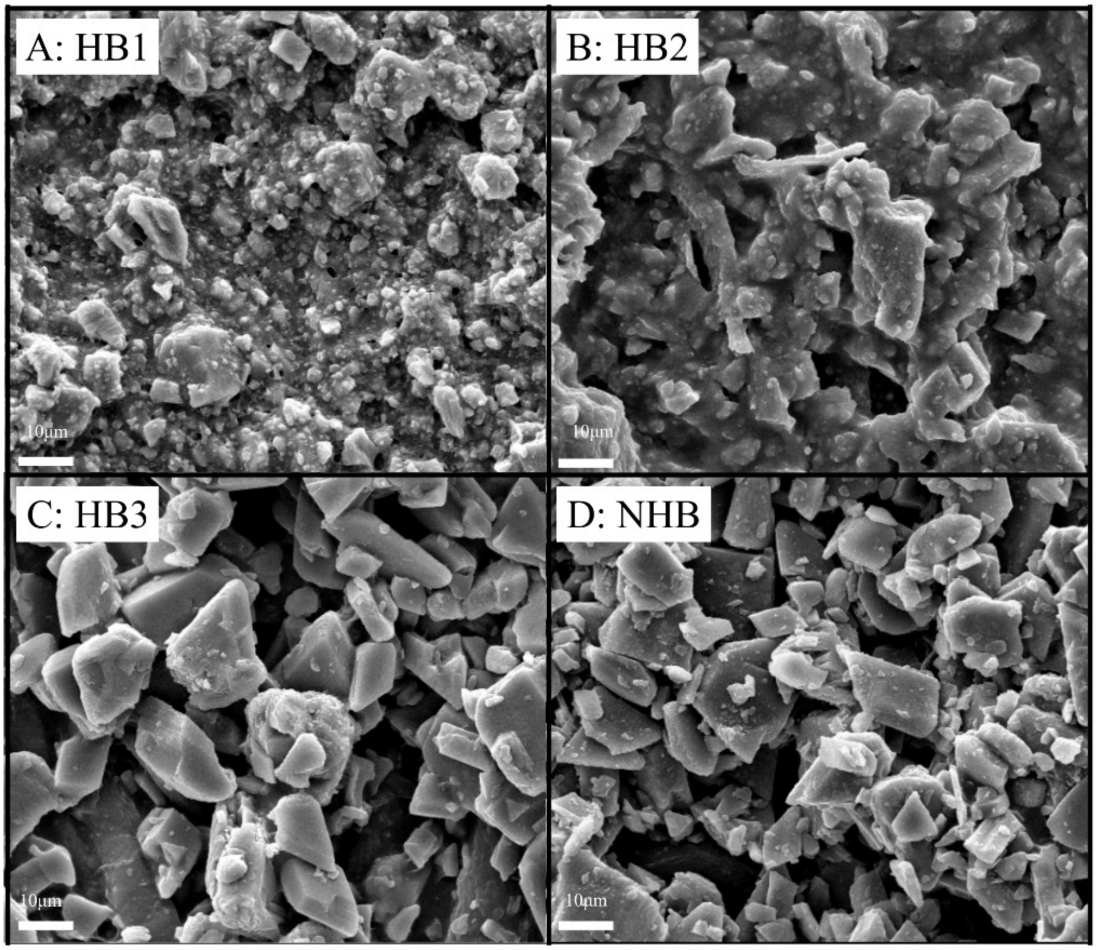

Figure 4 *Toothpaste abrasive particles: (A) HB1 group, (B) HB2 group, (C) HB3 group, (D) NHB group. Bar = 10 µm*

Discussion

This study aimed to evaluate the impact of herbal toothpastes on the buccal dentin surface morphology. Prior to brushing, a polished dentin surface presented a smooth appearance. After brushing with different toothpastes, alterations in surface roughness and morphology were observed. The final surface roughness values (Raf), measured after the toothbrushing, were significantly higher than the initial surface roughness (Rai) in all groups ($p<0.001$), therefore the first null hypothesis was rejected. The Raf values in the herbal groups (HB1, HB2, and HB3) were significantly higher than in the non-herbal group (NHB) ($p<0.001$) (Table 2). These findings are consistent with previous studies[10-12], which examined mean surface roughness after toothbrushing with various herbal toothpastes of enamel specimens. Furthermore, as the differences in Raf values between the groups after toothbrushing were statistically significant ($p<0.001$), the second null hypothesis was rejected.

In the present study, the Raf values in all groups were higher than those reported by Korsuwannawong *et al.*[10] This discrepancy was attributed to differences in the experimental setup. The prior study evaluated changes in enamel surface roughness after brushing with herbal toothpastes, whereas the current study focused on dentin specimens, which have lower surface hardness than enamel.[16] Additionally, the brushing force and the total number of brushing cycles in the present study were higher.

The HB2 group exhibited the highest final surface roughness value and the greatest surface loss among all groups ($p<0.001$). This finding can be attributed to the components of this toothpaste, which include calcium carbonate and hydrated silica as abrasives, in addition to cuttlefish bone. These components have higher Mohs hardness values compared to dentin (dentin = 2-2.5, calcium carbonate = 3, hydrated silica = 2.5-5, cuttlefish bone = 7)[16], which explains why the HB2 group sustained greater surface roughness and loss than in the other groups.

In the HB1 group, which exhibited the second-highest final surface roughness, the toothpaste contained calcium carbonate as an abrasive particle. Calcium carbonate has a higher Mohs hardness than dentin[16], potentially contributing to the increased surface roughness observed in the HB1 group compared to the HB3 and NHB groups. Conversely, the HB3 group used dicalcium phosphate as the abrasive while the NHB group utilized dicalcium phosphate dihydrate. The Mohs hardness of these particles is 2.5. The relatively lower Mohs hardness of dicalcium phosphate and dicalcium phosphate dihydrate (compared to calcium carbonate) may contribute to the observed lesser effect on the dentin surface in the HB3 and NHB groups compared to the HB1 and HB2 groups. Interestingly, the HB1 group displayed the lowest level of surface loss despite containing calcium carbonate, an abrasive material with a Mohs hardness exceeding that of dentin. This observation suggested that the particle size and distribution within the toothpaste formulation play a significant role. In accordance with the previous study[7], the characteristics of the abrasive particles, including hardness, shape, size, size distribution and concentration, all impact on the surface of dental hard tissues. Furthermore, the study by Baig *et al.*[17] found that the spherical abrasive particles resulted in a lower volume loss of enamel compared to angular abrasive particles. Additionally, larger particles were associated with greater damage to the tooth surface. The SEM image of toothpaste (Fig. 4) revealed round shaped particles of similar size in the HB1 group. In contrast, the other groups exhibited particles with irregular shapes. Our morphological observations of the HB1 are consistent with the findings reported by Suriyasangpetch *et al.*[18] This observation of HB1 particles may lead to the finding that HB1 toothpaste resulted in the lowest surface loss.

Johansen *et al.*[19] investigated the relationship between surface roughness and volume loss of acrylic plates after brushing with various toothpastes. The results indicated a weak correlation between these two parameters. Notably, the study found that toothpastes which induce greater volume loss may result in a smoother dentin surface compared to other toothpastes. The hardness and shape of the particles can influence the resulting surface roughness and depth of surface loss. Spherical particles with higher hardness may lead to increased surface roughness. However, due to their shape, they may cause less severe subsurface damage.

The NHB group, exhibiting the lowest final surface roughness value among all test groups, contained hydrated silica and dicalcium phosphate dihydrate as abrasives in toothpaste. The Mohs hardness of hydrated silica ranges from 2.5 to 5, while dicalcium phosphate dihydrate possesses a Mohs hardness of 2.5. In contrast to the NHB group, the dentin in the HB3 group exhibited a significantly higher final surface roughness value. The HB3 toothpaste used anhydrous dicalcium phosphate as its abrasive, while NHB used the dihydrate form. Vranic *et al.*[20] reported that the dihydrate form exhibits softer properties and a milder abrasive effect compared to anhydride form. Consequently, the HB3 group, containing the anhydride form, could potentially have resulted in higher dentin surface roughness compared to the NHB group. Furthermore, the HB3 toothpaste formulation also contained herbal ingredients, which may have contributed to increased dentin surface damage.

The herbal toothpastes contained various other beneficial ingredients. Streblus asper bark, commonly referred to as the toothbrush tree, was incorporated into the herbal toothpastes due to its recognized antimicrobial properties.[21] Cuttlefish bone powder has been suggested as a potential abrasive agent for calculus removal in toothpastes. However, this claim lacks scientific evidence, as no clinical studies have investigated its efficacy.[22] Salt has been traditionally used in oral health practices, exhibiting both calculus removal and anti-inflammatory properties.[23]

In addition to those previously mentioned, the herbal toothpastes contained various other beneficial ingredients. Menthol was included to provide a refreshing and clean taste. Borneol camphor, orange jessamine, and clove oil were incorporated for their antimicrobial properties. Guava leaf and Clinacanthus nutans were selected for their combined antimicrobial and anti-inflammatory effects.[24] Furthermore, certain essential oils such as peppermint oil and eucalyptus oil were added to enhance antimicrobial activity, provide a refreshing sensation, and impart a cooling effect. However, the abrasiveness of many herbal ingredients remains largely unknown due to a limited number of studies investigating their specific effects on tooth surfaces. The potential influences of these herbal ingredients on dentin surface roughness necessitate further investigation to clarify the precise effects.

However, the dentin in the NHB group did not exhibit the lowest surface loss. This observation could be attributed to the irregularity in the shape of the abrasive particles.

Another factor influencing surface roughness during toothbrushing is the abrasive concentration in the toothpaste. A direct correlation between the abrasive concentration of toothpaste and its impact on surface roughness has been reported.[25] Toothpastes with higher levels of abrasive particles tend to exhibit greater abrasiveness on tooth surfaces. Unfortunately, the specific concentrations of the abrasives in the toothpastes used in this study were not disclosed by the manufacturers. This lack of information may contribute to the observed variations in the final surface roughness following toothbrushing.

Relative dentin abrasivity (RDA), a measurement derived from radioactivity methods, is the standard parameter for evaluating the abrasive potential of toothpaste on dentin. Enax *et al.*[26] demonstrated a positive correlation between higher RDA values and increased surface roughness. While the RDA values of toothpastes were not revealed for most groups, the NHB group was reported to have an RDA of 68 according to manufacturer's data, which falls within the medium abrasive range. The absence of RDA data for the other herbal toothpastes limits our ability to fully explain the observed variations in surface roughness. Further investigations into the abrasive properties of these toothpastes, including the determination of their RDA values, would provide more understanding of their impact to dentin surfaces.

The present study investigated the effect of different herbal toothpastes on aged buccal root dentin surface roughness. Specimens were obtained from extracted human molars of geriatric patients. This approach aimed to mimic the clinical scenario of gingival recession commonly observed in the elderly population. Aged root dentin exhibits a higher concentration of calcium, increased hardness, and a greater elastic modulus compared to young dentin. As aged root dentin has different properties, herbal toothpaste may affect its surface roughness differently compared to young root dentin. Further investigation is needed to determine the impact of herbal toothpastes on young dentin surface roughness.[27]

This study focused solely on the effect of toothpaste on surface roughness and loss. However, dental hard tissue loss during toothbrushing is a multifactorial process influenced by various factors beyond the scope of this investigation, such as brushing force and brushing technique.[4-6, 28-32] The International Organization for Standardization (ISO) recommends a force of 150 g for evaluating toothpastes using toothbrushing (ISO 11609:2014). A brushing force of 150 g was employed in this experiment, which aligns with the recommended range for optimal toothbrushing force. Furthermore, the toothbrushing technique used in this experiment was a horizontal brushing motion. Several studies demonstrated an association between horizontal toothbrushing techniques and increased tooth wear.[4,5] On the other hand, some studies[32,33] reported that a horizontal toothbrushing technique may not be significantly associated with tooth wear. Nevertheless, it is important to acknowledge that the horizontal technique used in this study may not fully represent the real toothbrushing movements in daily life. This study aimed to provide crucial information regarding the abrasiveness of these herbal toothpastes. People who prefer herbal toothpastes can benefit from these findings by practicing proper brushing techniques, such as avoiding excessive force and horizontal brushing motions, to minimize the risk of NCCLs. Moreover, this research can provide valuable insights for individuals considering the use of herbal toothpastes, aiding them in making informed decisions about their oral health care.

To ensure the accuracy and reliability of the surface roughness measurements, the contact profilometer underwent a calibration procedure prior to data collection. This study performed 10,000 cycles of toothbrushing which simulated one year of brushing time.[34] These findings warrant further investigation to elucidate the long-term impact of these toothpastes on dentin in a more clinically relevant setting.

Conclusions

This *in vitro* study investigated the impact of various toothpastes on root dentin surface roughness and surface loss after toothbrushing. While the dentin in all experimental groups exhibited increased roughness following toothbrushing, herbal toothpastes were associated with significantly higher roughness compared to the non-herbal control group. Among the toothpastes, the HB2 group exhibited the highest surface roughness and loss, while the NHB group showed the least surface roughness. Notably, the HB1 group showed the lowest surface loss, but which was not statistically significant compared to the NHB group.

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Conflicts of interest

The authors declare no conflict of interest.

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