

INVESTIGATION OF THE EFFECTS OF WHITENING TOOTHPASTES ON ENAMEL AND CEMENTUM SURFACES

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ABSTRACT

INTRODUCTION: Toothpastes with whitening properties have emerged as an option to enable and improve the whitening of tooth structure. In recent years, toothpastes have become more specialized and can be classified as therapeutic or cosmetic. One of the most important features regarding the cosmetic function of toothpastes is the capacity to prevent or remove stains on the tooth surface, thereby whitening the teeth.

OBJECTIVES: The purpose of this *in vitro* study was to investigate the effects of different whitening toothpastes on both human enamel and cementum surfaces.

MATERIAL AND METHODS: In total, 140 of human extracted incisor teeth were split from cemento-enamel junction and embedded in gypsum blocks. Ra values were measured with a three-dimensional profilometer. Images were obtained from the samples with a scanning electron microscope. One of the seven groups created was the control group, in which the teeth were brushed with water only, and in six groups (groups 2-7), they were brushed with different whitening toothpastes. Each sample was brushed for 5 seconds for 30 days with an automatic toothbrushing machine.

RESULTS: Regarding the surface roughness in cementum, no differences were detected in the control group. While all toothpastes caused a decrease in roughness, this difference was significant ($p < 0.05$) in group 3 and group 4. For enamel roughness, no differences were detected in the control group. The roughness decreased significantly ($p < 0.05$) in group 3 and group 4. In other whitening toothpaste groups, surface roughness increased but the difference was not significant ($p > 0.05$).

CONCLUSIONS: The results of this *in vitro* study showed that all whitening toothpastes in this study reduced the roughness on the cementum surface. Most whitening toothpastes increased the enamel roughness, while two whitening toothpastes (Splat Special Blackwood and Colgate Optic White) reduced the enamel roughness.

KEY WORDS: scanning electron microscopy, surface properties, tooth abrasion, toothbrushing, toothpastes.

J Stoma 2020; 73, 2: 55-64

DOI: <https://doi.org/10.5114/jos.2020.96116>

INTRODUCTION

Toothpastes with whitening properties have emerged as an option to enable and improve the whitening of tooth structure [1, 2]. In recent years, toothpastes have become more specialized and can be classified as therapeutic or cosmetic [3]. One of the most important fea-

tures regarding the cosmetic function of toothpastes is the capacity to prevent or remove stains on the tooth surface, thereby whitening the teeth [4]. A large number of tooth-cleaning agents have been released, each containing different formulations to increase tooth whitening or cleanliness. Whitening toothpastes may contain chemicals (enzymes, detergents, and oxygenation

JOURNAL OF STOMATOLOGY
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RECEIVED: 05.04.2020 • ACCEPTED: 18.05.2020 • PUBLISHED: 08.06.2020

agents) that can inhibit or directly remove stains on the tooth surface without being stimulated by abrasives [1-5]. These toothpastes often contain hydrogen peroxide, carbamide peroxide, sodium bicarbonate, hydrated silica, or aluminum oxide, individually or in various combinations [6]. Most whitening toothpastes contain many abrasives of different sizes and shapes to cope with stain removal on the teeth. The abrasiveness of toothpaste depends not only on the internal hardness of particles, but also on the particle size and shape of the abrasive components [7]. A positive relationship was found between the abrasiveness of toothpaste and the reduction of superficial spots. However, data comparing different abrasives is not sufficient [8]. As the size of the abrasive particles increases, the abrasiveness of a toothpaste increases also, compared to the same size particle compositions; the silica particles have been observed to show more abrasiveness than those of calcium carbonate [7]. High amounts of abrasive toothpaste can damage the hard and soft tissues and tooth restorations, leading to gingival recession, dental abrasion, and tooth sensitivity [9].

Primary features of periodontitis are clinical attachment loss, radiographically evaluated alveolar bone loss, periodontal pocket formation, and presence of bleeding [10]. In such a situation, there are many patients whose cementum surface is exposed to clinic attachment loss in individuals with periodontitis. This type of a patient is requested to pay more attention to oral hygiene,

but in studies so far, the effect of whitening toothpastes on the cementum surface is minimal. Regular teeth brushing is important for improving oral health; however, data collected so far is not sufficient enough to determine how the cementum surface is affected by bleaching tooth brushing agents. Considering that limited data are available regarding the cementum surface, the purpose of this *in vitro* study was to investigate the effects of different whitening toothpastes on both human enamel and cementum surfaces. As far as the literature is reviewed, this study was the first to evaluate the effects of different whitening toothpastes on enamel and cementum surfaces.

MATERIAL AND METHODS

This study was approved by the Research Ethics Committee of Pamukkale University (number 12, on June 12, 2018). A total of 140 extracted human incisor teeth were collected for the purpose of this research, with informed consents obtained. The enamel and cementum surfaces were then cleaned with prophylactic paste to ensure the elimination of extrinsic stains. The teeth were split from the cemento-enamel junction for a separate investigation of the cementum and enamel. The parts of the teeth were kept in a sterilized artificial saliva for all further experimental procedures. The spec-

TABLE 1. The toothpastes and ingredients used in this study

Groups	Toothpaste	Firm, country	Ingredients
Group 1	Control group (water brushing only)		
Group 2	Sensodyne True White	GlaxoSmithKline, UK	Sorbitol, aqua, glycerin, hydrated silica, potassium nitrate, PEG-6, aroma, titanium dioxide, sodium methyl cocoyl taurate, cocamidopropyl betaine, xanthan gum, sodium hydroxide, sodium saccharin, sodium fluoride, limonene
Group 3	Splat Special Blackwood	Splat, Moscow, Russia	Aqua, hydrated silica, hydrogenated starch hydrolysate, glycerin, maltooligosyl glucoside, sodium lauroyl sarcosinate, cellulose gum, aroma, charcoal powder, capryloyl/ caproyl methyl glucamide, lauroyl/ myristoyl methyl glucamide, sodium benzoate, stevia rebaudiana leaf extract, potassium sorbate, menthol, o-cymen-5-ol, juniperus communis sprout extract, limonene
Group 4	Colgate Optic White	Colgate-Palmolive Company, Australia	Propylene glycol, calcium pyrophosphate, glycerin, PEG/PPG-116/66 copolymer, PEG-12, PVP, silica, flavor, sodium lauryl sulfate, tetrasodium pyrophosphate, hydrogen peroxide, disodium pyrophosphate, sodium saccharin, sucralose, BHT
Group 5	Signal White Now	Unilever, Turkey	Sodium fluoride, hydrogenated starch hydrolysate, aqua, hydrated silica, PEG-32, sodium lauryl sulfate, aroma, cellulose gum, sodium fluoride, sodium saccharin, PVM/MA copolymer, trisodium phosphate, mica, glycerin, sodium laureth sulfate, lecithin, caprylyl glycol, limonene, CI 74160, CI 77891
Group 6	Ipana 3D White	Procter & Gamble, USA	Aqua, sorbitol, hydrated silica, disodium pyrophosphate, sodium lauryl sulfate, aroma, sodium hydroxide, cellulose gum, sodium saccharin, sodium fluoride, carbomer, mica, limonene, CI 77891, polysorbate 80, CI 42090
Group 7	Paradontax Whitening	GlaxoSmithKline, UK	Sodium bicarbonate, aqua, glycerin, hydrated silica, alcohol, cocamidopropyl betadine, mentha arvensis oil, mentha piperita oil, echinacea purpurea, flower/leaf/stem juice, krameria triandra extract, xanthan gum, titanium dioxide, sodium fluoride, chamomilla recutita extract, salvia officinalis oil, commiphora myrrha extract, limonene, sodium saccharin, linalool

imens were embedded in gypsum blocks plaster, with buccal surfaces of the roots and crowns exposed. Since the teeth were divided into two as enamel and cementum, the effect of each paste was investigated for enamel and cementum. Samples were divided into seven groups, and the specimens for cementum ($n = 20$ per group) and enamel ($n = 20$ per group) were randomly distributed to the groups. One group was the control group (group 1; cementum $n = 20$, enamel $n = 20$) and only water brushing (without toothpaste) was applied to this group. Different whitening toothpastes were used in the other six groups (Table 1).

BRUSHING PROCEDURES

Tooth brushing was performed via an automatic toothbrushing machine (Oral-B Genius Pro 10000, Procter & Gamble, USA), with one soft bristle head per sample used (Oral-B Sensi Ultrathin, Procter & Gamble, USA). The brushes were placed on a stabilizer and allowed to brush samples up to 2.4 N of its stopping force. Brushing was performed with a toothpaste slurry, containing toothpaste and water in a ratio of 1 : 3. In each cycle and each toothpaste, 3 ml of the slurry was used to brush each specimen. Assuming that an adult has a minimum of 28 teeth, it gives a total of 56 regions with buccal and lingual/palatal surfaces. When the recommended tooth brushing time is 120 seconds, twice a day, the time applied to a tooth is 4-5 seconds. Accordingly, the samples were brushed for 5 seconds [11] for 30 days.

PROFILOMETER ANALYSIS

A three-dimensional surface profilometer (P-7 Stylus Profiler, KLa Tencor, USA) was used to measure the surface roughness before and after brushing. The profilometer was adjusted to move a diamond pen on the sample surface under a constant load. The scan time for each line was set at 20 seconds, with a constant force of 5 mg on the diamond pen (12.5 microns in radius). Surface morphology was measured with a linear variable differential transformer. The surface roughness was completed by calculating the numerical values of surface profile. The value of Ra defines the overall roughness of a surface and is defined as the average value of all absolute distances of the roughness profiles from the average line in the measuring distance. For each sample, a 15 × 15 mm central area was scanned in 15 lines, 15 mm long, and 1 mm distance between each scanned line. The vertical resolution was 160 angstroms, which represented the accuracy of Ra. The average Ra was calculated from 15 lines as the average roughness of the sample. Surface roughness measurements after the brushing procedure were recorded and the difference between baselines was calculated.

SCANNING ELECTRON MICROSCOPE ANALYSIS

All samples were taken to SEM analysis before and after brushing. The samples were washed with distilled water and slightly air-dried at room temperature. After the fixation of samples, dehydration was achieved by passing through an alcohol series (50%, 70%, 90%, and 100%) for 10 min at each step. The samples were coated with a thin layer of gold in the coating device (Polaron SC 502, Fison Instruments, Uckfield, UK), and images were obtained and recorded by performing surface inspection with a field emission SEM (Gemini 500, Zeiss, Oberkochen, Germany) operating at 1,000 × magnification.

STATISTICAL ANALYSIS

Statistical analyzes were made with SPSS 21 (SPSS, Inc., Chicago, IL, USA) package program. In addition to descriptive statistical methods (mean, standard deviation), one-way analysis of variance post-hoc ANOVA test was used for comparisons between groups. In group evaluations, a paired sample *t* test was applied. The results were evaluated at the significance level of $p < 0.05$.

RESULTS

SURFACE ROUGHNESS

Regarding the surface roughness test, a decrease in roughness of surface was generally detected in the groups examined in cementum. No significant surface differences were detected in the control group, while in group 3 and group 4, the surface roughness decreased significantly. Although roughness decreased in other groups, the results were not significant (Table 2; Figures 1 and 2). Considering cementum Ra differences in the six toothpaste groups, group 7 formed the least value difference, while in group 4, the most Ra difference was observed again.

TABLE 2. Surface roughness values in cementum groups

Cementum groups	Surface roughness value, Ra ± SD (µm)		p value
	Initial	30 days	
Group 1	24.10 ± 13.32	24.45 ± 13.57	0.91
Group 2	32.05 ± 15.93	28.57 ± 11.12	0.87
Group 3	66.80 ± 51.51	37.15 ± 13.15	0.00*
Group 4	59.65 ± 32.49	29.80 ± 21.06	0.02*
Group 5	29.65 ± 18.38	24.60 ± 9.84	0.45
Group 6	41.30 ± 23.67	30.15 ± 14.22	0.13
Group 7	28.50 ± 24.20	25.55 ± 16.57	0.26

* $p < 0.05$

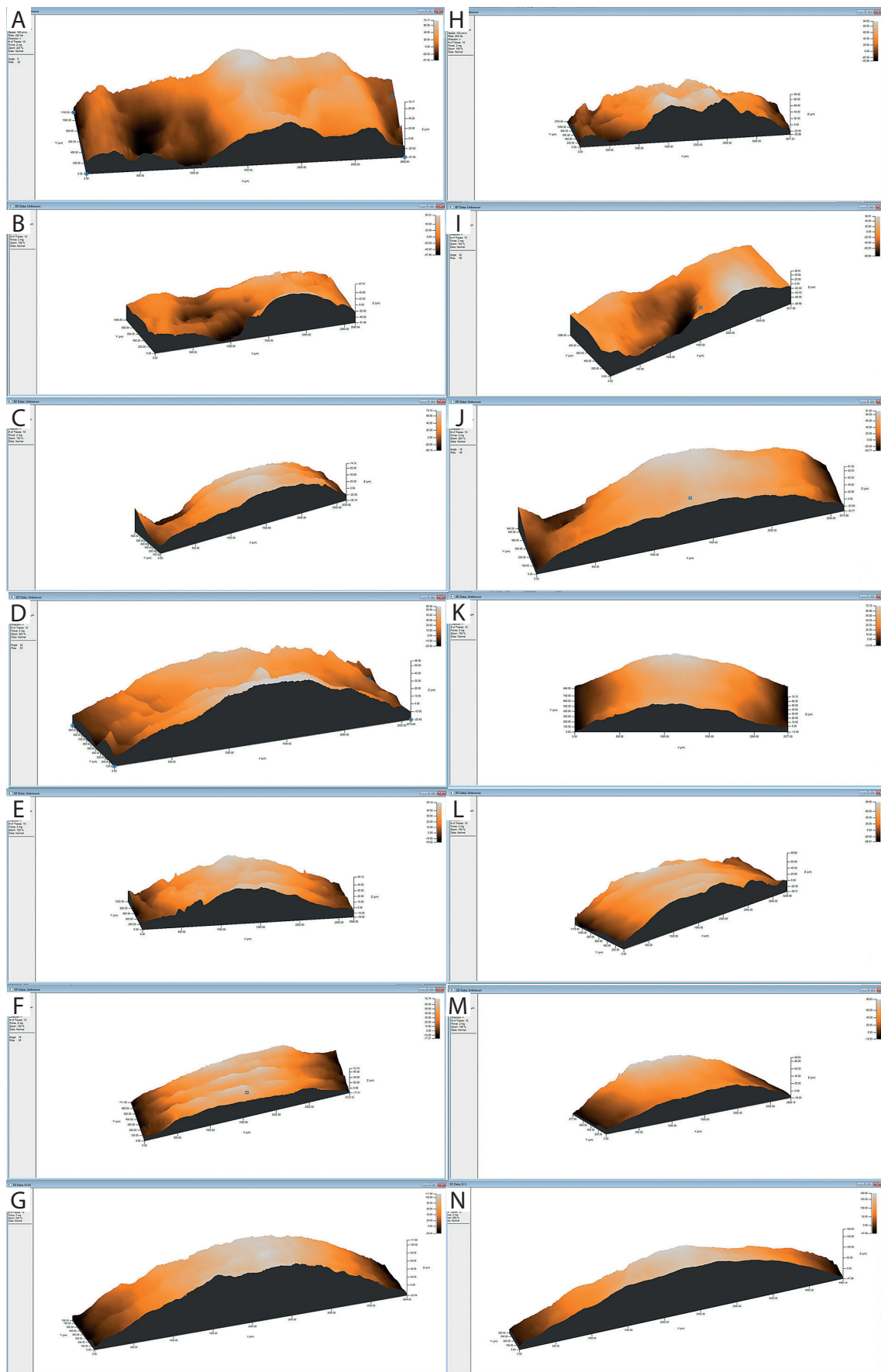


FIGURE 1. Cementum surface analysis samples formed in the profilometer. Left column before brushing, right column after brushing (A, H: group 1; B, I: group 2; C, J: group 3; D, K: group 4; E, L: group 5; F, M: group 6; G, N: group 7)

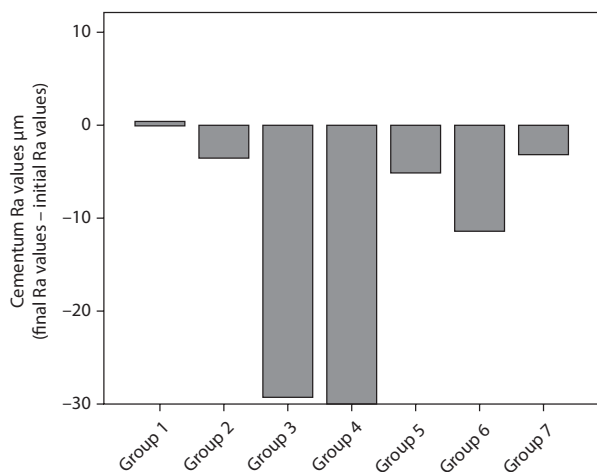


FIGURE 2. The bar chart shows the Ra differences in cementum groups

The roughness values in the enamel varied among the pastes. While there was no significant difference in the control group, the surface roughness decreased significantly in group 3 and group 4. In group 2, group 5, group 6, and group 7, the surface roughness increased, but the difference was not significant (Table 3; Figures 3 and 4). When the initial and final roughness in terms of enamel values were compared between the groups, the group with the highest difference was group 3, whereas the group with the least difference was group 6.

SCANNING ELECTRON MICROSCOPE ANALYSIS

No difference was observed on the surface of both cementum and enamel in group 1. There was a significant change in cementum surfaces. A significant smoothing was observed on the cementum surface in all other groups, except group 1. It was noted that the structure at the beginning was completely different (Figure 5).

In group 3 and group 4, relatively smooth enamel surfaces were observed, compared to initial evaluations. Samples in group 5, 6, and 7 showed similar surface patterns comparing to initial estimations, with some fine scratches (Figure 6).

DISCUSSION

Primary prevention of gingivitis, as primary and secondary prevention of periodontitis, depends on adequate plaque control. This could be achieved by an active participation of patients, following recommended mechanical plaque removal regimen, which is a daily brushing habit. In the dental literature, there are many studies on how a toothpaste affects the surface of dental hard tissues [12-14]. There are various techniques such as weight and volume loss to evaluate the abrasive properties of toothpastes [12]. In this study, we investigated

TABLE 3. Surface roughness values in enamel groups

Enamel groups	Surface roughness value, Ra ± SD (μm)		p value
	Initial	30 days	
Group 1	28.00 ± 17.08	29.15 ± 18.53	0.67
Group 2	34.45 ± 19.75	40.15 ± 25.73	0.19
Group 3	84.55 ± 53.74	53.10 ± 35.97	0.01*
Group 4	60.25 ± 29.68	35.10 ± 17.12	0.00*
Group 5	41.47 ± 29.57	50.35 ± 33.70	0.12
Group 6	33.20 ± 20.71	47.10 ± 32.84	0.06
Group 7	29.40 ± 21.99	36.45 ± 31.17	0.07

*p < 0.05

various types of whitening toothpastes on both enamel and cementum surface. According to the results of our study, all pastes used in the study provided abrasion-related smoothness on the cementum surface and all pastes, except for group 3 and group 4 pastes, increased the roughness of the enamel surface.

Nowadays, it is possible to find a large number of whitening toothpastes with different ingredients. The amount of roughness created by toothpastes in the hard tooth tissues is a matter of curiosity. Many studies have been conducted on the effects of whitening toothpastes on tooth surface roughness and dental restorations. Some of these reveal that whitening toothpastes increase the surface roughness [15, 16], while others show that such pastes do not make a difference in surface roughness [17, 18]. In an animal study of Hilgenberg *et al.*, toothpastes with different contents were compared with the profilometer device, and no statistically significant difference was found. However, statistically significant surface differences were found as a result of using toothpastes after bleaching agent [9]. In a study, the effect of whitening toothpastes on dentin wear was investigated. Colgate Luminous White and Sorriso Xtreme

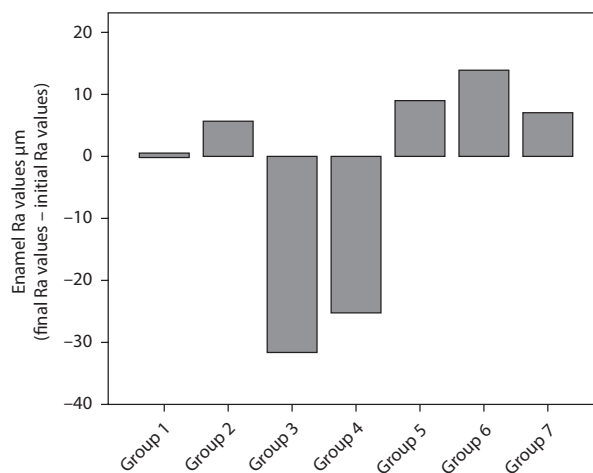


FIGURE 3. The bar chart shows the Ra differences in enamel groups

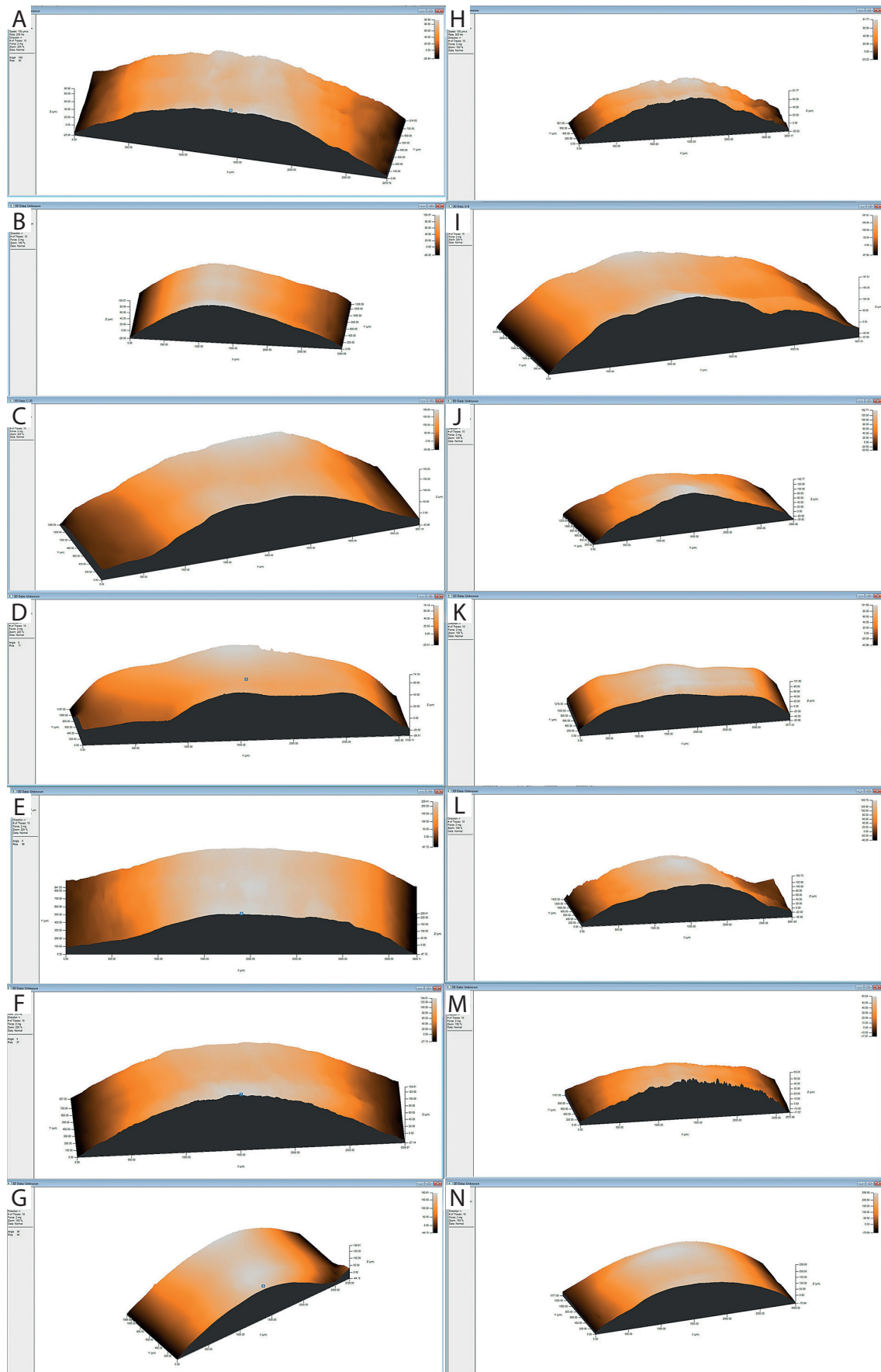


FIGURE 4. Enamel surface analysis samples formed in the profilometer. Left column before brushing, right column after brushing (A, H: group 1; B, I: group 2; C, J: group 3; D, K: group 4; E, L: group 5; F, M: group 6; G, N: group 7)

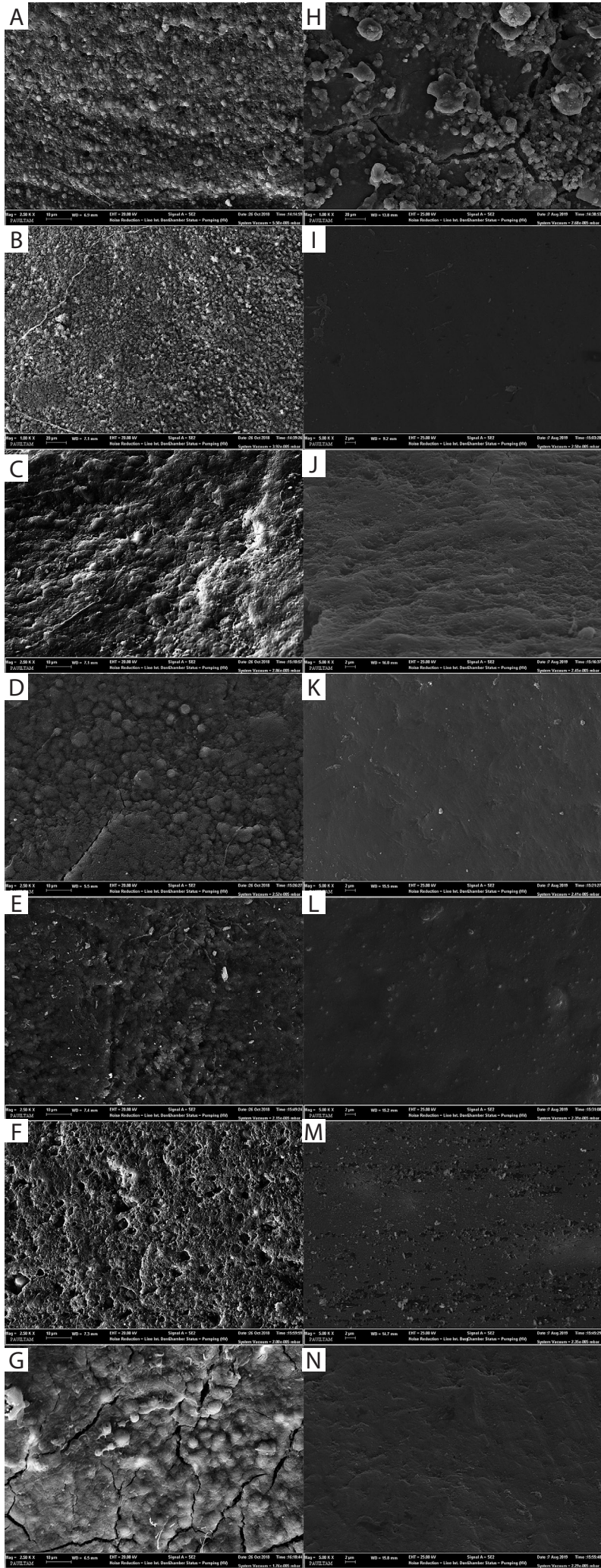


FIGURE 5. Scanning electron microscope photomicrographs of the cementum groups (bar = 20 μ m). Left column before brushing, right column after brushing (**A, H:** group 1; **B, I:** group 2; **C, J:** group 3; **D, K:** group 4; **E, L:** group 5; **F, M:** group 6; **G, N:** group 7)

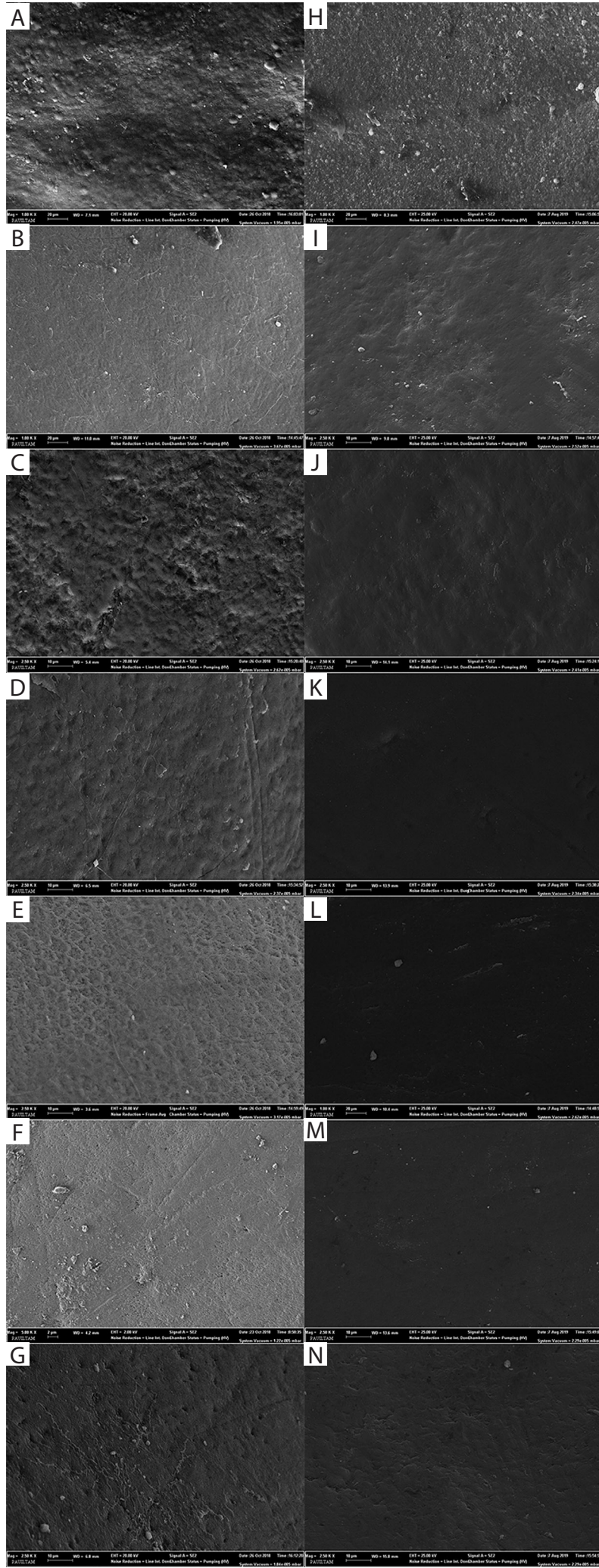


FIGURE 6. Scanning electron microscope photomicrographs of the enamel groups (bar = 20 μ m). Left column before brushing (**A, H**); group 1; **B, I**; group 2; **C, J**; group 3; **D, K**; group 4; **E, L**; group 5; **F, M**; group 6; **G, N**; group 7)

White 4D toothpaste promoted the highest erosive potential [19]. In our study, statistical differences were only observed in group 3 and 4 in terms of surface roughness. In other groups, while the roughness of the enamel increased, the roughness decreased in the cement group and smoother surfaces were observed. But in group 3 and group 4, smoother surfaces were observed both on enamel and cementum surfaces. Cementum is a calcified, avascular mesenchymal tissue that forms the outer covering of anatomic root. Inorganic content of cementum (hydroxyapatite; $\text{Ca}_{10}[\text{PO}_4]_6[\text{OH}]_2$) is 45% to 50%, which is less than that of bone (65%), enamel (97%), or dentin (70%) [20]. Compared to its contents, the cementum has a softer structure than enamel texture. Therefore, different results were obtained in terms of both in the same power and brushing procedure. Group 3 toothpaste contained charcoal powder. Unlike other pastes, hydrogen peroxide was used as a whitening agent in group 4, which showed a reduced roughness. Hydrogen peroxide penetrates the enamel because of its low molecular weight (34 g/mol) and can promote protein denaturing [21]. Many studies have shown changes in enamel morphology associated with the whitening procedure, and revealed areas of depressions, with formation of craters, impaired microhardness, rugosity, and surface wear as well as an exposure of prisms in the areas most affected [21-26]. We assumed that all these demineralization processes provided smoothness on tooth surface with the help of other abrasives of a toothpaste. There was coal in the content of paste used in group 4. Some forms of coal used in oral hygiene procedures have been found to have relatively high abrasiveness [27]. The surface roughness is affected by the applied particle size. Smaller size of particle is possible to achieve more polished surface, but highly polished surface can be achieved by reducing inorganic size of particle [28].

Subgingival instrumentation is considered the gold standard of periodontal therapy and includes three distinct procedures such as debridement, scaling, and root planing. Although it was emphasized that scaling and root planing may decrease bacterial accumulation in subgingival area and can prevent development of gingivitis [29], loss of substance on the cementum surface to ensure smoothness may increase dentin sensitivity in future [30, 31]. However, in order to reach more precise conclusion, erosive wear measurements and mineral content measurements of enamel and cementum are recommended. Besides, *in vitro* models and *in features* such as acquired pellicle and saliva buffering capacity, saliva flow cannot be imitated, so the results of the present study should be confirmed by *in situ* models.

CONCLUSIONS

The results of this *in vitro* study showed that all whitening toothpastes used in this study reduced the rough-

ness on the cementum surface. Most of the whitening toothpastes increased the enamel roughness, while only two whitening toothpastes (Splat Special Blackwood and Colgate Optic White) reduced the enamel roughness. Clinicians should carefully recommend whitening toothpastes to individuals with periodontal disease and clinical attachment loss, in order not to experience tooth sensitivity in future.

ACKNOWLEDGMENTS

This study was supported by the Scientific Research Project Coordination Unit of Pamukkale University (PAUBAP), project number: 2018HZDP043.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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