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Surface Roughness of Restorative Materials After Simulated Toothbrushing with Toothpastes Containing Theobromine and Arginine: An In Vitro Study

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ORIGINAL ARTICLE

Surface Roughness of Restorative Materials After Simulated Toothbrushing with Toothpastes Containing Theobromine and Arginine: An In Vitro Study

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ABSTRACT

Objective: This study examined the effect of toothpastes containing theobromine and arginine on the roughness changes of microhybrid composite, nanohybrid composite, and giomer restorative materials. **Methods:** A total of 90 disc-shaped specimens were prepared using microhybrid composite (Arabesk-Ara), nanohybrid composite (Herculite-Her), and giomer (Beautifil II-Gio). The samples were divided into 3 subgroups (n = 10), and initial surface roughness was evaluated with a mechanical profilometer and scanning electron microscopy (SEM). All samples were then subjected to a 1-year brushing simulation via a toothbrushing simulator using toothpastes containing theobromine (Theodent Classic, Theodent) or arginine (Colgate PRO-Relief, Colgate Palmolive); a control group was brushed with distilled water. Afterward, surface roughness measurements and SEM images were re-recorded. The difference in surface roughness was statistically evaluated. **Results:** The toothpaste containing arginine caused the highest increase in surface roughness in all groups. The toothpaste containing theobromine showed the least increase in roughness in the Her and Gio groups. **Conclusion:** Using toothpaste containing theobromine causes the least increase in the surface roughness of restorative materials, while using toothpaste containing arginine causes the greatest increase.

Key words: arginine, composite, surface roughness, theobromine

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INTRODUCTION

Resin composite restorations constitute an essential part of the routine practice of dentists due to the intense demands of patients for aesthetic improvements and new developments that allow for their use on both anterior and posterior teeth.[1,2] The surface quality of restorations used in the mouth is an essential determinant of aesthetic success. In 1984, O'Brien et al. defined the importance of surface gloss and reported a significant relationship between the gloss ratio and the surface roughness of resin-based materials.[3] The brightness is proportional to the ability of the surface to reflect light and is related to the geometric distribution of the light reflected from the surface.[4] The material's high gloss level increases its compatibility with the tooth on which it is placed and with the surrounding teeth.[5] Regarding this compatibility, since a roughness of over 0.2 microns on the surface can be detected with the tip of the patient's tongue, the smoothness of the surface also contributes to patient comfort.[6] In addition, researchers have shown that a roughness above this value is a vital threshold value for bacteria to adhere to the surface, and as a result, plaque can accumulate on the surface.[7] Furthermore, an increase in surface roughness can cause surface staining by coloring agents and lead to aesthetic problems in the resin composite restorations. Such an increase is closely related to the type of restorative material and the polishing systems used.[8,9] Generally, the removal of the organic matrix on the surface of the resin matrix due to brushing causes the wear of resin composites and increases the surface roughness, which results in a surface prone to staining and plaque involvement.[10]

Since toothpaste is one of the basic materials used for brushing teeth, along with toothbrushes, in daily oral care, it has been the focus of attention for many professionals. Toothpaste contains different ingredients, such as detergents, desensitizing agents (e.g., fluoride, theobromine, arginine, etc.), flavorings, and abrasives. Abrasives are important for cleaning teeth, destroying bacteria on the tooth surface, and removing surface stains.[11] Although fluoride toothpastes are widely used in daily brushing, their harms are still discussed. Recent years have seen the emergence of fluoride-resistant S. mutans and, therefore, the possibility of a decrease in the effects of fluoride on this and other acid-producing microorganism species.[12] Such discoveries have led researchers to search for new materials that can function as alternatives to fluoride (e.g., theobromine or arginine).

Theobromine, a water-insoluble, crystaline bitter powder marketed as an alternative to fluoride, is an alkaloid of the cocoa plant and is found in tea, chocolate, and other foods. This active ingredient has an antibacterial effect and reduces plaque formation.[13-15] Research has shown that theobromine content is more effective at increasing the surface hardness of enamel than fluoride.[16] Toothpastes containing theobromine, which have been introduced to the market in recent years, are also effective against caries.[17] Lakshmi et al. reported that compared to fluoridated toothpastes, theobromine showed a significantly greater antimicrobial effect.[18] In another study, Amaechi et al. found that the theobromine additive has a much greater remineralization potential than fluoride and occludes dentinal tubules.[19] Thus, toothpastes containing theobromine may be soothing to patients with dentin sensitivity and reduce the risk of tooth decay. Another proposed alternative is arginine, a natural amino acid that can be found in foods. It is found in microconcentrations in saliva and supports anticariogenic activity by increasing the pH of the oral cavity.[20,21] The potential of arginine to eliminate the deficiencies of fluoride and its synergistic effect with fluoride to support remineralization have led to increases in its use.[22] Although toothpastes with these different active ingredients (e.g., theobromine, arginine, etc.) produced for desensitization are marketed as an alternative to fluoride, the effects of alternative substances on tissues and restorations in the mouth have not been sufficiently investigated.

The aim of this study was to evaluate the effects of toothpaste with theobromine as an active ingredient, whose effect on restorative materials has not been sufficiently investigated, and arginine-containing toothpaste, which has become increasingly popular, on the surface roughness of nanohybrid composite, microhybrid composite, and giomer restorative materials with mechanical profilometry and scanning electron microscopy (SEM). The null hypothesis tested was that toothpastes containing theobromine and arginine would not cause an increase in roughness on the surfaces of restorative materials.

METHODS

Preparation of samples

Microhybrid composite (Arabesk N-Ara, Voco, Germany), nanohybrid composite (Herculite-Her, Kerr, USA), and giomer (Beautifil II-Gio, Shofu, Japan) restorative materials were used in the study. The contents of the materials are given in Table 1. In order to compare the initial and final roughness measurements of the materials with 95% confidence level ($\alpha = 0.05$) and 80% power (β: 0.20), the minimum number of samples to be taken per group was calculated as 10 when the effect size was predicted as 1.04. Restorative materials were placed in a specially prepared 2 mm thick and 10 mm wide disc-shaped standard polyethylene Teflon mold (Figure 1-a) and gently pressed with a glass plate to remove excess. The polymerization was carried out with a II. generation LED light device (Guilin Woodpecker, China) producing light with a power of 800 mW/cm2 and a wavelength of 460–480 nm for 20 seconds. A total of 90 samples (30 for each restorative material) were obtained.

The samples were then polished with the help of coarse, medium, and fine polishing discs (OptiDisc, Kerr, USA). All samples were stored in distilled water at 37 °C for 24 hours. After 24 hours, the initial surface roughness (Ra_0) of the samples was measured and recorded with a mechanical profilometer (Mitutoyo SJ-210, Japan). In addition, a random sample was selected from each restorative material group, and the initial surface image was taken and recorded with the scanning electron microscopy (SEM) device (LEO Evo 40X VP; Carl Zeiss AG, Oberkochen, Germany).

Brushing simulator application

The samples were fixed to polyethylene Teflon molds with a height of 27 mm and an outer diameter of 25 mm using cold acrylic (Imicryl, Turkiye) for the toothbrushing simulator (Moddental, Esetron, Turkiye), then divided into subgroups (n = 10): samples brushed with toothpaste containing theobromine (Theodent Classic, Theodent, USA), samples brushed with toothpaste containing arginine (Colgate PRO-Relief, Colgate Palmolive, USA), and samples brushed with distilled water (control group). The contents of the toothpastes are given in Table 2. An application of 10,000 cycles, corresponding to 1 year of brushing, was carried out on the samples.[11,23] A medium-hard and rounded bristle toothbrush (ExperDent, Turkiye) was fixed to the plastic tips of the brushing simulator. A new toothbrush was used for each sample. The gripper tips, which brushed the samples, performed the brushing at 18 mm/sec frequency, 11 mm working range, and 350 g (3.5 N)[24] load, imitating the force applied by a person. The operations applied to the groups are given in Figure 1.

Table 1. Restorative materials used in the study.

Composite	Type	Compound	Manufacturer
Arabesk N (Ara)	Microhybrid	**Resin:** Bis-GMA, UDMA, TEGDMA **Particle Size:** 0.5-2 µm by weight 76.5% by volume 60%	Voco, Germany
Herculite XRV Ultra (Her)	Nanohybrid	**Resin:** Bis-GMA, TEGDMA **Particle Size:**0.05µm **Filler:** Colloidal silica, barium-aluminum-silicate glass by weight 78%	Kerr Corp., Orange, CA, USA
Beautifil II (Gio)	Giomer	**Resin:** Bis-GMA, TEGDMA, UDMA **Particle Size:** 0.8µm (avarage), 10-20nm (nanofillers) **Filler:** S-PRG by weight: 83.3% by Volume: 68.6%	Shofu, Kyoto, Japan

Table 2. Toothpastes used in the study.

Toothpaste	Manufacturer	Compound
Theodent Classic Toothpaste	Theodent, New Orleans, USA	**Active Ingredients:** Theobromine, Calcium Acetate, Hydrogen Phosphate **Other Ingredients:** Distilled water, hydrated silica, sorbitol, xylitol, glycerin, sodium lauryl sarcosinate, xanthan gum, titanium dioxide, citric acid, peppermint oil, sodium benzoate, stevia extract, sodium bicarbonate, and sugar-free vanilla extract
Colgate PRO-Relief	Colgate Palmolive, New York, USA	**Active Ingredients:** Arginine, Calcium carbonate **Other Ingredients:** Water, sorbitol, sodium lauryl sulfate, flavor, sodium monofluorophosphate, cellulose gum, sodium bicarbonate, tetrasodium pyrophosphate, sodium saccharin, benzyl alcohol, xanthan gum, limonene

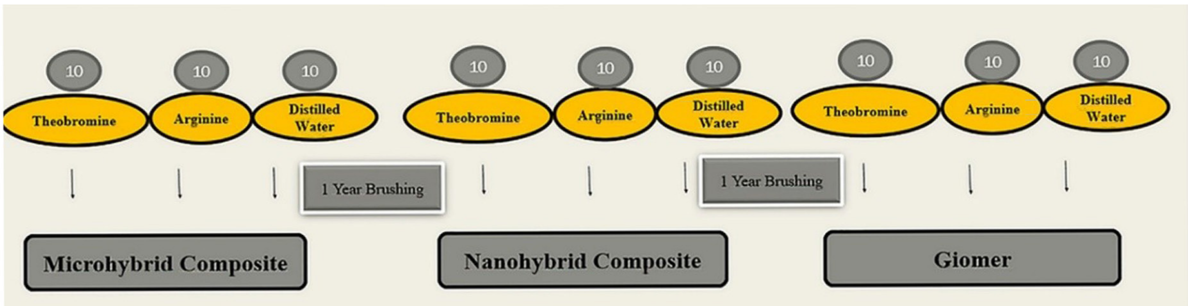

Figure 1. Groups that underwent 1-year brushing simulation.

Measuring surface roughness

After the experimental brushing process was completed in the brushing simulator, surface roughness measurements were taken again to examine the roughness changes on the surface of the samples. Before the measurement, the calibration settings were made with the calibration plate (Mitutoyo 178-601, Japan) in accordance with the company's recommendations. The measurement speed was determined as 0.25 mm/sec, the λc (Lambda C) multiplier was determined as x5, and the measurement length was determined as 1.25 mm. These settings were used to take the most accurate measurement in the 10 mm area, in line with the company recommendations. Measurements were taken from 3 different points of each sample. Then, their average value was taken, and the surface roughness value of the sample was recorded as Ra_1. Care was taken to ensure that the sensitive measuring tip contacted the surface at an angle of 90°.[9,25,26]

SEM images

After all the samples were processed in the brushing simulator, a random sample was selected from each group to view surface changes.[27] The surface coating, consisting of Au-Pd, was applied to the selected samples to make them conductive. The coating process was carried out under a vacuum of 10-1 mBar and a current of 53 mA. Then, the sample surfaces were imaged and recorded with the SEM device (LEO Evo 40X VP; Carl Zeiss AG, Oberkochen, Germany) at x1000 magnification,[28] 24 mm working distance, and 20.00 kV voltage.

Statistical analysis

For the statistical analysis of the data obtained in the study, IBM SPSS Statistics V. 26 for Windows (SPSS Inc., Chicago, IL, USA) program was used. Quantitative data were summarized as the arithmetic mean and standard deviation or median (minimum-maximum) values.

Whether the data obtained from the measurement of the initial surface roughnesses showed normal distribution was checked with the Kolmogorov–Smirnov test. Whether there was a significant difference between the initial (Ra_0) and final (Ra_1) surface roughnesses of the groups was checked with the Wilcoxon signed-rank test. Kruskall–Wallis H and one-way analysis of variance tests were used for multiple comparisons between the groups. For pairwise comparisons, Bonferroni corrected Mann–Whitney U test and Tukey test were used if Kruskall–Wallis H and one-way analysis of variance were significant, respectively. The results were evaluated at a significance level of $p < 0.05$.

RESULTS

Profilometer results

In the data obtained from the comparison of the Her-Ara-Gio groups, it was seen that the group with the lowest initial surface roughness was the Ara group, and the one with the highest value was the Gio group (n = 30). Initial surface roughness values (Ra_0) are shown in Figure 2. In the pairwise comparison of the restorative materials, it was observed that there was a significant difference between the Her, Ara, and Gio groups ($p < 0.05$).

The surface roughness values of the Her, Ara, and Gio groups measured before (Ra_0) and after brushing (Ra_1) with toothpaste and distilled water are shown in Table 3, and ΔRa (Ra_1 - Ra0) values are given in Figure 3. According to these results, theobromine toothpaste applied to each group did not significantly affect the material surface ($p = 0.074$). However, the increase in roughness in the arginine toothpaste and control groups was significant ($p < 0.05$). Overall, there was a significant difference between the increased roughness in the theobromine toothpaste, arginine toothpaste, and control as applied to the Ara and Gio groups ($p < 0.05$).

When the Ra_1 values of the Her, Ara, and Gio groups were compared (Table 4), the Gio group showed the highest Ra_1 value, while the lowest Ra_1 value was seen in the Ara group (theobromine and control subgroups). Considering the pairwise comparisons of the Ra_1 values of the materials, while there was a significant difference between the theobromine and arginine toothpastes in the Her group, there was no significant difference between the applications in the Ara and Gio groups ($p > 0.05$).

SEM results

SEM images of the samples of the Her, Ara, and Gio groups before and after brushing are shown in Figures 4–6. Initial SEM images of the Gio group showed a more porous and irregular surface structure than the other two groups. It was determined that the irregularities of the porous structure on the surface of

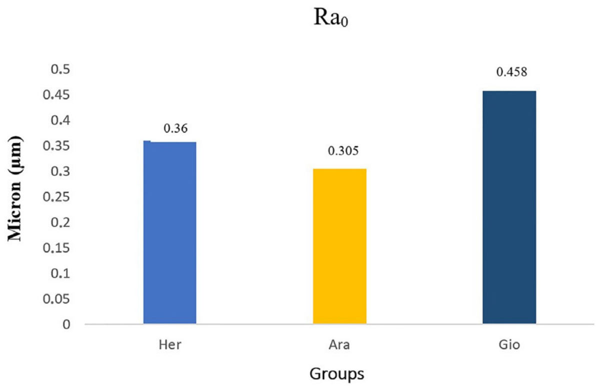

Figure 2. Initial mean surface roughness of restorative materials.

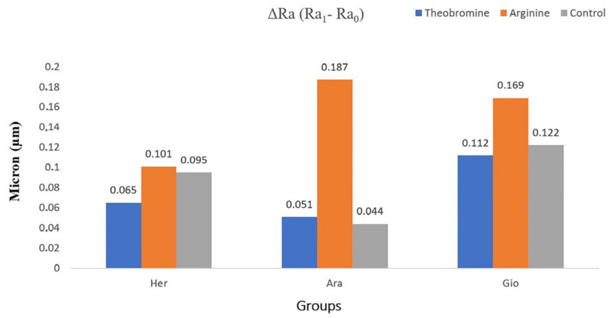

Figure 3. Surface roughness changes after brushing

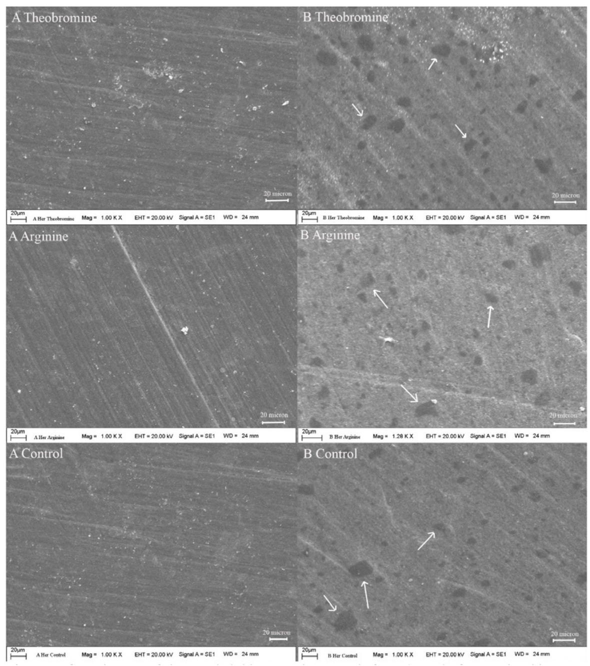

Figure 4. SEM images of the nanohybrid composite (Her) before (A) and after (B) brushing (x1000). Arrows indicate inorganic particles with increased visibility as a result of erosion of the organic matrix.

the Gio group participants increased after brushing. Brushing with theobromine toothpaste, arginine toothpaste, and distilled water caused the visibility of

Table 3. Comparison of the median Ra_0 and Ra_1 values of the groups.

Group	Toothpaste	Ra_0 (µm) Median (min-max)	Ra_1 (µm) Median (min-max)	p*
Her	Theobromine	0.338 (0.235-0.418)	0.403 (0.315-0.567)	0.074
	Arginine	0.402 (0.35-0.507)	0.503(0.413-0.591)	0.028
	Control	0.340 (0.231-0.405)	0.435(0.319-0.572)	0.002
Ara	Theobromine	0.304 (0.245-0.338)	0.355 (0.305-0.456)	0.009
	Arginine	0.310 (0.263-0.516)	0.497 (0.314-0.520)	0.028
	Control	0.300 (0.235-0.403)	0.344 (0.304-0.507)	0.009
Gio	Theobromine	0.450 (0.339-0.528)	0.562(0.459-0.654)	0.007
	Arginine	0.418 (0.32-0.646)	0.587(0.414-0.679)	0.032
	Control	0.506 (0.406-0.638)	0.628(0.422-0.710)	0.009

*: Wilcoxon Test

Table 4. Comparison of the median Ra_1 values of the groups.

Group	Toothpaste	Ra_1 (µm) Median (min-max)	p*
Her	Theobromine	0.403 (0.315-0.567)[a]	0.035
	Arginine	0.503 (0.413-0.591)	
	Control	0.435 (0.319-0.572)	
Ara	Theobromine	0.355 (0.305-0.456)	0.051
	Arginine	0.497 (0.314-0.520)	
	Control	0.344 (0.304-0.507)	
Gio	Theobromine	0.562 (0.459-0.654)	0.535
	Arginine	0.587 (0.414-0.679)	
	Control	0.628 (0.422-0.710)	

a: Significant difference attributed to arginine ($p < 0.05$)
*: Kruskal-Wallis H Test

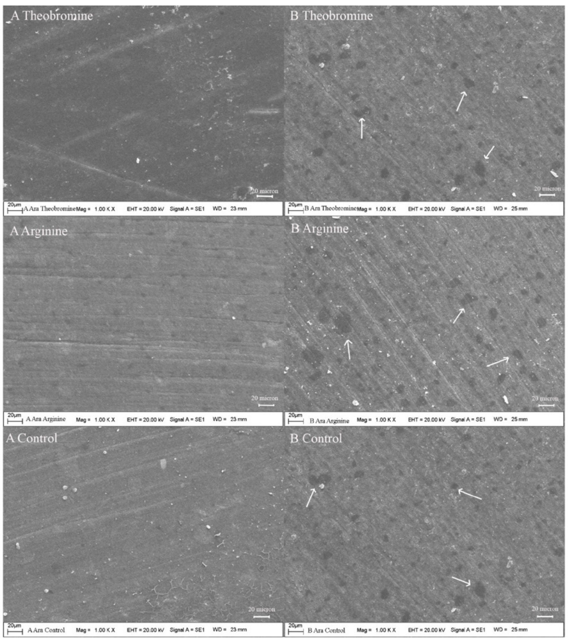

Figure 5. SEM images of microhybrid composite (Ara) before (A) and after (B) brushing (x1000). Arrows indicate inorganic particles with increased visibility as a result of erosion of the organic matrix.

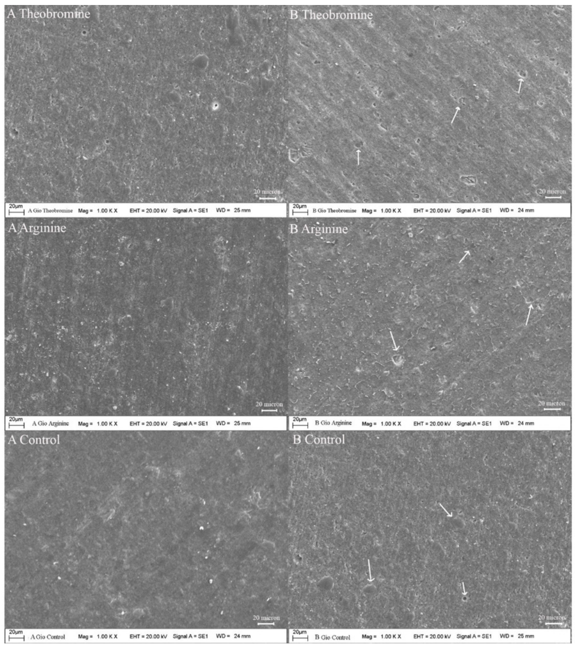

Figure 6. SEM images of giomer (Gio) before (A) and after (B) brushing (x1000). Arrows indicate inorganic particles with increased visibility as a result of erosion of the organic matrix.

inorganic particles to increase, and the lines forming the roughness became more pronounced as a result of the erosion of the organic matrix on the surface of the Her and Ara groups. In addition, the parallel surface lines obtained with the polishing disks became scattered and thickened.

DISCUSSION

Tooth brushing, where toothpastes are used together with toothbrushes, is an important application for oral and dental health.[25] Toothpastes contain various active ingredients that can help address problems that may occur in teeth or periodontal tissues.[29] Today, new active ingredients, such as theobromine and arginine, are used as an alternative to fluoride in some toothpastes produced to relieve sensitivity. The effects of desensitizing toothpastes with active ingredients other than fluoride on teeth and restorations are not well known. Accordingly, this study aimed to investigate the effects of theobromine- and arginine-containing toothpaste on the surface roughness of three different restorative materials in vitro. The null hypothesis of this study was rejected because the surface roughness of the restorative materials increased after the application of toothpaste containing theobromine and arginine.

In this study, microhybrid composite, nanohybrid composite, and glass ionomer-filled composite giomer restorative materials, which are frequently used in teeth restorations today, were preferred as restorative materials. The promising theobromine-containing toothpaste and arginine-containing toothpaste, which are available in the market as alternatives to fluoride, were also preferred. In the quantitative and qualitative evaluations, brushing with distilled water and desensitizing toothpastes increased the surface roughness of the tested restorative materials.

The literature has reported that 10,000 cycles of brushing simulation application correspond to a one-year brushing cycle.[11,23] This study applied a one-year brushing cycle to the samples via a brushing simulator. In the initial surface roughness measurements, Ara was the group with the lowest surface roughness, while the highest roughness was found in the Gio group. The fact that the material with a microhybrid particle size had a less rough surface than the group with nanohybrid particles may be attributed to the type, shape, and degree of hardening of the inorganic fillers.[28] In a similar study comparing the surface roughness values, Say et al. showed that the microhybrid composite group had lower surface roughness values than the nanohybrid composites after polishing systems were applied.[8] In another study, Gönülol et al. compared the effect of different finishing and polishing techniques on nanohybrid and microhybrid composite resins and showed that the microhybrid composite group had smoother surfaces. Their study also demonstrated that composite resins with smaller particle sizes might not exhibit less surface roughness.[30]

When the roughness increases that occurred on the surface of the restorative material after the brushing simulation process were evaluated, toothpaste containing arginine caused the highest surface roughness increase in all three restorative material groups. While toothpaste containing theobromine caused the least increase in roughness in the Her and Gio groups, it was observed that brushing with distilled water caused the least increase in the Ara group. The fact that brushing with arginine-containing toothpaste on all restorative material surfaces increased the surface roughness more than other approaches may be because the abrasives and other active substances in toothpaste interact differently with the structural components of the restorative materials used.[31]

To our knowledge, no study in the literature has examined the effect of theobromine-containing toothpaste on restorative material surfaces. However, a few studies have examined the effect of arginine-containing toothpaste on restorative material surfaces. Garcia-Godoy et al. investigated the surface roughness effect of fluoride-free 8% arginine-based toothpaste on restorative materials and human tooth enamel, finding that, contrary to our study, toothpaste containing arginine did not cause a significant increase in the surface roughness of both tooth and restorative materials.[32] In another study examining the effect of brushing time and abrasives in toothpaste, Monteiro and Spohr reported that three toothpastes containing arginine, fluoride, and stannous fluoride could be used to relieve sensitivity on enamel composites, and that they significantly increased the composite surface roughness after brushing.[11] According to the study's results, arginine-containing toothpaste created more surface roughness than fluoride-based toothpaste, while it created less surface roughness than the stannous fluoride-based toothpaste. Our study observed that the roughness of all restorative material surfaces increased significantly after brushing with arginine-containing toothpaste.

Researchers have suggested that the differences in roughness caused by the effect of brushing on the composite surfaces are due to the variability of the abrasive effect of the toothpastes used in the research, rather than the abrasion resistance between different composite resins.[33] Specifically, Yin et al. stated in their study that abrasives play a prominent role in creating surface roughness differences.[34] As an abrasive, theobromine toothpaste contains hydrated silica,[35] and arginine-based toothpaste contains calcium carbonate.[32] The use of different abrasives may explain the different surface roughness of the composite blocks on which the toothpastes are applied.

When the surface roughness (Ra_1) of the restorative materials was evaluated after toothpaste application, the highest value belonged to the Gio group, while the lowest value belonged to the Ara group. The fact that the highest and lowest Ra_1 values belonged to the control group of the Gio and Ara materials, respectively, may also be due to the highest and lowest initial average surface roughness in these groups, respectively. Other components, such as detergent, that can be included in the paste also affect the surface roughness of teeth and restorations. Related to this, Moore and Addy reported that brushing with detergents alone, apart from the abrasives in the paste, also potentially causes dentin loss. These researchers have argued that changes in the size of silica types used as abrasive in toothpastes can also determine the abrasiveness properties.[36] In one particular study, Ruivo et al. found that the roughness differences on the surfaces of the restorative materials might be due to the differences in the particle size of the restorative materials and structural elements other than the abrasives in the toothpaste.[28] As a result of our study, it can be concluded that the surface roughness differences between the groups after brushing with the theobromine- and arginine-containing toothpastes may have resulted from the differences in the restorative materials, as well as the differences in the other components in the paste other than the abrasive.

The surfaces of the materials used in the study were also examined with the SEM imaging system, so the data obtained quantitatively with the mechanical profilometer were supported qualitatively.[8,28,37] In a similar vein, Say et al. examined the SEM and AFM images of restorative materials after polishing procedures, and they attributed the difference in surface roughness on samples treated with the same filler polishing systems to the type and size of inorganic fillers, the type of resin matrix, and the degree of final hardening.[8] In our study, the roughness differences on the surfaces of the restorative materials detected in SEM images after brushing with desensitizing toothpastes and distilled water may have been due to the different filler types and ratios of the restorative materials. Likewise, when examining the roughness effects of whitening-based toothpastes on the surface of composite blocks with the SEM imaging technique, Ruivo et al. found that the roughness that occurred after brushing was caused by the removal of the surface's organic content and the release of inorganic particles.[28] Similar to this study, when the SEM images of nanohybrid (Her) and micro-hybrid-based composite (Ara) groups were examined, it was observed that the organic matrix covering the surface before brushing eroded after brushing, and the inorganic fillers became more prominent. In our study, compared to the other groups, the SEM images showed the roughest structure in the Gio group, and it was determined that the irregular and porous structure that was present before brushing became more irregular after brushing; thus, the porosity increased.

This in vitro study has a few limitations. First, the study was carried out on two different composite and giomer restorative materials. The effects of the toothpastes used in the study on other restorative materials (e.g., amalgam and ceramics) remain unknown. Second, other effects of toothpastes (e.g., discoloration) on restorative materials have not been evaluated. Third, oral conditions involving such an important factor as saliva could not be imitated.

It has been determined that the desensitizing toothpastes used in our study may increase the roughness of restorations placed in the mouth. According to the results of this study, toothpaste with the active ingredient theobromine caused the least increase in surface roughness in general. When the initial and final surface roughness values were examined, the material with the highest surface roughness was the giomer. The high surface roughness of the giomer may cause aesthetic and functional problems over an extended period. The effects of brushing for longer than one year with toothpastes containing these active ingredients on the restorative materials and tissues in the mouth should be examined with further in vitro and in vivo studies.

CONCLUSION

It has been observed that the surface roughness of all three restorative materials applied with a brushing simulation process corresponding to a one-year cycle increased. In general, it was concluded that the theobromine-containing toothpaste led to the lowest increase in the surface roughness of the materials used in our study. In long-term use, toothpaste containing theobromine may cause fewer side effects resulting from the roughness of restoration surfaces.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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