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Evaluating the Effect of Different Mouthrinses on Properties of the Enamel and Dental Composite Surfaces

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Abstract: *This study investigated the effects of different solutions (artificial saliva, Listerine Cool Mint-alcohol containing and Colgate Plax-alcohol free) on the nanohardness, elastic modulus and surface roughness of enamel surface and composite materials (Admira Fusion, Clearfil Majesty Esthetic and Mosaic Universal). Specimens of 2 mm depth and 5 mm diameter were stored in solutions for 12 h at 37°C. Baseline and final measurements were obtained using a HYSITRON TI 950 TriboIndenter testing machine. The applied force to each specimen increased from 0 to 1000 µN. For SEM images, one sample in each group was covered with a thin layer of mix of gold and palladium using a sputter coater (Quorum Q150R ES, UK). Scanning electron microscopy (SEM) images were taken at 5000× magnifications to evaluate the surface morphology. Statistical analysis for hardness, elastic modulus and roughness was performed by Two-way ANOVA, Benferroni and Tukey HSD at a significance level 0.05. The results of this study showed that the highest value of surface roughness and lowest hardness and elastic modulus were presented by Admira (p<0.001). Listerine caused significantly increased surface roughness (p<0.001) and decreased hardness and elastic modulus parameters (p<0.001). The mouthrinse containing alcohol caused more significant changes in the nanohardness, elastic modulus, surface roughness values of enamel and composite surfaces.*

Keywords*: Enamel, Composite, Elastic modulus, Nanohardness, Surface Roughness*

1. Introduction

Mouthrinses are commonly used to clean the oral cavity and control plaque formation when used in addition to toothbrushes and toothpastes [1-3]. Mouthrinses contain antimicrobials, water, salts, preservatives, and varying concentrations of alcohol [4]. Mouthrinses are classified according to the active composition:phenolic compounds, bis-biguanides (Listerine Cool Mint ®, (Johnson & Johnson, İstanbul Türkiye)), quaternary ammonium compounds (Colgate Plax Zero, (Colgate Palmolive, İstanbul, Türkiye)), herbal ingredients, germicides, halogens, fluorides and oxygenated agents [5]. In the mouthrinses, the alcohol -especially ethanol- is a common antiseptic agent. Ethanol, besides dissolving dental plaque substances, preserves the formula contents [6]. Nevertheless, it is known that many investigators have reported the side effects of mouthrinses containing alcohol [2, 3, 6]. Previous studies have shown that mouthrinses with low pH negatively affected the properties of the tooth enamel [2, 7]. The changes of hardness and roughness with time in the final values compared to controls observed [2].

Although the improvements in surface properties of dental composites are expected to result in long-term durability of restorations stored in solutions with low *p*H, the differences in the material composition, influence its performance [8]. A current study reported that the surface roughness of nanohybrid composites in a mouthrinse have differences in filler content and filler size, which may have caused them to be affected differently [3]. Also, a previous study demonstrated that the organic matrix structure and filler particles have created differences in the hardness and roughness of resin composites [8]. Additionally, alcohol content in solutions softens the composite resins. This process is associated with the expansion and opening of the polymer matrix of the composites [9]. Previous studies have shown that different mouthrinses containing alcohol lead to decreased hardness values of composite materials [10, 11]. The hardness and elastic modulus of dental materials are highly relevant properties for clinical durability [12]. Generally, several test methods have been recommended to investigate these

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parameters. The most conventional approaches used are the Vickers and Knoop that only displacement at peak load is measured [8, 13, 14]. Besides the microhardness test methods, in Nanoindentation, the indentation depth is measured in situ during loading and unloading times. The load and displacement are monitored throughout the contact cycle on a material surface [15]. With advances in test techniques, the nanoindentation methods allow the evaluation of hardness, elastic modulus of the dental material on a micron to submicron scale [16]. Also, being a quantitively scientific technique, they have the soft-ware supported analysis, relatively non-destructive and repetable measurements [15, 17, 18].

At the end of the dental restoration, the composite surface always requires careful intraoral polishing. Restoration surfaces should be smooth to minimalize plaque retention [3, 19]. Additionally, increased surface roughness accelerates the discoloration of dental materials [19]. Concerning the effect of long-term durability of composites, many investigations subject the surface roughness by different testing methods [3, 19, 20]. One of them, scanning probe microscopy, offers a 3D topography imaging on the material surface. This technique can use the same probe to conduct nanomechanical testing methods [20].

Nevertheless, it has never been observed in the literature thorough examination of the effect of mouthrinses on the enamel and composite samples' hardness, elastic modulus and surface roughness properties with a nanoindentation testing method. Thus, it would be relevant to evaluate the changes caused in teeth and composites when stored in different mouthrinses. Therefore, the aims of this study were to evaluate the nanohardness, elastic modulus and surface roughness parameters of specimens after immersion in different mouthrinses.

2. Materials and methods

2.1. Sample preparation

The materials and mouthrinses used in this study are presented in Tables 1 and 2. Three different composite materials (Admira Fusion, Clearfil Majesty Esthetic and Mosaic Universal) and molar teeth samples were prepared. Hardness, elastic modulus and roughness measurements were obtained using a HYSITRON TI 950 TriboIndenter testing machine. After the immersion in different solutions (Artificial saliva, Listerine Cool Mint, Colgate Plax Non-Alcoholic) for 12 h, these specimens were taken out of the solutions, dried and final measurements were obtained.

Table 1. Description of dental composite materials used in this study

Material	Composition Filler	wt/vol (%)
Admira Fusion (Voco, Germany) (A2), (1933644)	3-dimensionally linked inorganic organic copolymers (Ormocers) Silicon oxide nano filler, glass-ceramic filler (average particle size 0.7µm)	84/69
Clearfil Majesty Esthetic (Kuraray, Japan) (A2), (6M0177)	Bisphenol a glycol dimethacrylate (Bis-GMA), hydrophobicaromatic dimethacrylates, and hydrophobicaliphatic dimethacrylates, dl-Camphorquinone, Silanated barium glass (average particle size 0.7µm) and pre-polymerized organic filler	78/66
Mosaic Universal Composite (Ultradent, USA) (A2), (BHRRV)	Bis-GMA, Zirconia-silica glass ceramic, 20 nanometer silica	-/68

Table 2. Description of solutions used in this study

Solutions	Composition	*p*H (v/v%)	Alcohol Content
Artificial Saliva	NaCl, $MgCl_2(6H_2O)$, KCl, CH_3COOK, $CaCl_2$, $K_3PO_4(3H_2O)$, H_3PO_4, NaF	6.75	-

Listerine Cool Mint	Thymol, eucalyptol, methyl salicylate, menthol, aqua, sorbitol, alcohol, poloxamer 407, benzoic acid, aroma, sodium saccharin, sodium benzoate, CI 42053	4.0	29.2
Colgate Plax	Water, glycerin, propylene glycol, sorbitol, poloxamer 407, flavor, cetylpyridinium chloride, sodium fluoride, potassium sorbate, sodium saccharin, citric acid, CI 42053	5.5	-

Fifteen human extracted teeth without caries, cracks and defects were collected. The middle 1/3 of the buccal surface of the molar teeth were separated using a diamond separator under water cooling. The teeth specimens in artificial saliva and then, they were embedded in a light curing composite using polyethylene molds. The enamel surfaces were polished with 800, 1000, 1200, 2000 and 2400 grit silicon carbide abrasive papers in a water-cooled polishing device (Ecomet 3, Bueller, IL, USA). After polishing procedures, the samples were stored in artificial saliva during the study so that the pieces did not become dehydrated.

Three composite resins (Admira Fusion (Voco, Cuxhaven, Germany), Clearfil Majesty Esthetic (Kuraray Med INC, Okayama, Japan) and Mosaic Universal (Ultradent Products, South Jordan, Utah, USA)) were selected for this study (Table 1). Using polyethylene molds with cylindrical specimens (7x2mm), composite specimens were polymerizated by using a polywave LED light curing unit (Valo Cordless, Ultradent Inc) in the regular mode (1.000 mW/cm^2). The composite groups were finished and polished with Sof-Lex polishing discs (3M ESPE, USA).

For the analysis, the samples of enamel and composites were randomly divided into three groups (n=5) according to artificial saliva (control), alcohol mouthrinse (Listerine Cool Mint, Johnson & Johnson, Istanbul, Turkey) and alcohol-free mouth rinse (Colgate Plax, Colgate Palmolive, Istanbul, Turkey) (Table 2) in 12 h to simulate a 2 min/day for 1 year exposure to mouthrinses [10].

2.2. Testing procedure

Total 60 specimens prepared for these measurements were assessed using a nanoindenter testing device (HYSITRON TI950 TriboIndenter, Bruker Corp., Karlsruhe, Germany) with a Berkovich probe. Air calibration was applied before measuring on samples with the device. Load controlled tests at 1000 μN fixed force was performed with 2s holding time to avoid the creep effect. Five indents in a 30 by 30 array with 5 μm spacing were conducted on each sample [22]. The mechanical properties of the enamel were measured from the middle 1/3 the buccal side of each molar tooth [15].

After each series, the samples were kept in the artificial saliva or the mouthrinses for 12 h and then the measurements were repeated. For the topographic images, the nanoindenter was also operated in scanning probe mode.

One samples in each group were covered with a thin layer of mix of gold and palladium using a sputter coater (Quorum Q150R ES, UK). Scanning electron microscopy (SEM) images were taken at 5000× magnifications to evaluate the surface morphology (Voltage: 10kV; Spot=10.0, WD=11.1).

2.3. Statistical analysis

The statistical analysis was processed with the IBM SPSS V23 software system (SPSS Inc., Chicago, IL, USA). The normality of distributions was assessed with a Shapiro-Wilk test. Two-way ANOVA and Tukey tests were applied to show differences among groups at $p<0.05$ to test the differences in the values of nanohardness, elastic modulus and surface roughness. Two-way ANOVA, Tukey and Bonferroni tests were applied to show differences among groups at $p<0.05$ to test the differences in the values of nano-hardness, elastic modulus and surface roughness changes. Descriptive statistics, including mean and standard deviation values, were calculated.

3. Results and discussions

3.1. Nanohardness tests

Five indentations on each sample were measured and used to calculate nanohardness using. After the nanohardness calculations, the mean and standard deviations of the specimens in terms of GPa are shown in Table 3.

Table 3. Mean and standard deviations of hardness values (GPa)

	Enamel	Admira Fusion	Clearfil Majesty Esthetic	Mosaic Universal	Total
Baseline	4.88 ± 0.18^{A}	2.23 ± 0.09^{EF}	2.58 ± 0.22^{D}	2.64 ± 0.21^{D}	3.08 ± 1.07^{a}
Artificial Saliva	4.65 ± 0.19^{B}	2.02 ± 0.13^{G}	2.55 ± 0.15^{D}	2.55 ± 0.21^{D}	2.94 ± 1.03^{b}
Listerine Cool Mint	3.80 ± 0.13^{C}	1.01 ± 0.21^{I}	2.06 ± 0.10^{FG}	2.15 ± 0.23^{EFG}	2.26 ± 1.02^{c}
Colgate Plax	3.83 ± 0.25^{C}	1.81 ± 0.27^{H}	2.33 ± 0.15^{E}	2.24 ± 0.25^{E}	2.55 ± 0.80^{d}
Total	4.29 ± 0.52^{c}	2.38 ± 0.26^{b}	1.77 ± 0.50^{a}	2.40 ± 0.30^{b}	2.71 ± 1.03

Note: According to Tukey HSD tests, different uppercase letters show significant difference between materials in columns and rows, different lowercase letters show significant difference between materials in columns and rows ($p < .05$).

For hardness measurements, there are various methods in dentistry. The currently more sensitive accepted test method is the nanoindentation [18, 23]. Previous studies have stated that enamel hardness values correlated with the mineral content of the enamel and the differences in test methods [15, 18]. According to one of these studies, it has reported that although the hardness values of buccal, lingual and occlusal enamel surfaces vary, the hardness value of the buccal enamel surface can be between 4-6 GPa [15]. In this study, baseline enamel nanohardness mean was 4.88 GPa.

The enamels in Listerine had lower hardness value than the loss observed in the Colgate group, but the difference was not significant ($p > 0.05$). Although these results are in agreement with that Favaro et al. [2], who observed no significant decrease in enamel hardness between the alcohol-containing and alcohol-free mouthrinses at the same *p*H for 12 weeks of immersion, in present study methodology, *p*H levels of solutions and immersion time were different.

No data could be found in the literature regarding the effects of the different *p*H levels mouthrinses on enamel nanohardness. However, a previous study is reported that acidic beverage exposure caused a significant decrease in the nanohardness of the enamel surface because of the surface erosion [21]. This is in agreement with the results of this study, which revealed that the Listerine and Colgate mouthrinses with low *p*H decreased the nanohardness of enamel.

The hardness of composite materials was related to the filler size and filler weight or volume [1]. Previous studies have reported that the hardness of the composite containing zirconia glass ceramics with 78.5% filler weight is higher than the composite containing 76% prepolymerized organic filler and silane barium glass [14, 25]. However, the hardness results in our study did not show any change depending on the inorganic filler ratio. In our study, the hardness of Mosaic composite was found to be higher than the Clearfil, which has a prepolymerized organic filler, but no statistically significant difference was found between the two composites ($p>0.05$). For this reason, the factors affecting the hardness of composite materials are not only filler ratio and filler type. This idea is compatible with studies showing similar results [1, 11].

Certain results from this study are inconsistent with findings from the available literature regarding the studies stating that the hardness of the material increases as the filler volume increases [14, 25]. This is in agreement with studies where it was shown that filler volume is not the only factor for the hardness of composite materials.

The volumetric expansion in the structure of the composite materials can be the result of the liquid sorption. It is known that as a result of expansion, deformations are observed in the structure and filler particles are released from the organic matrix. The hardness of the composite material is decreased. On the other hand, electropositive elements (Ba, Zr, etc.) in the composite resins tend to react with the water. This situation disrupts the load balance in the silica structure and causes the decreasing in the hardness of the composite materials [26].

Ethanol in mouthrinse causes the expansion of the polymer matrix of the composites, opening the polymer structure and then, softening the composite structure. The concentration of alcohol and application time of the mouthrinse solutions affect the hardness values of composite materials [10, 11]. In present study, mouthrinses affected the nanohardness of the materials. Besides, Listerine and Colgate results were similar for nanohardness of Mosaic composite. This behavior shows that both the solution and the composite structure are important in terms of hardness [27].

3.2. Elastic modulus tests

The mean of the elastic modulus values of the tested materials are shown in Table 4.

Table 4. Mean and standard deviations of elastic modulus values (GPa)

	Enamel	Admira Fusion	Clearfil Majesty Esthetic	Mosaic Universal	Total
Baseline	98.3 ± 2.8^{A}	24.1 ± 1.0^{FG}	27.2 ± 2.0^{E}	26.5 ± 2.3^{E}	44.0 ± 31.6^{a}
Artificial Saliva	94.6 ± 4.1^{B}	21.8 ± 1.5^{HI}	26.2 ± 2.3^{EF}	26.1 ± 2.4^{EF}	42.2 ± 30.6^{b}
Listerine Cool Mint	60.8 ± 3.6^{D}	14.2 ± 0.9^{K}	20.7 ± 1.3^{I}	21.8 ± 2.1^{HI}	29.4 ± 18.6^{c}
Colgate Plax	78.3 ± 2.3^{C}	18.3 ± 1.8^{J}	23.7 ± 1.6^{GH}	23.3 ± 1.6^{GH}	35.9 ± 24.7^{d}
Total	83.0 ± 15.2^{c}	19.6 ± 4.0^{a}	24.5 ± 3.1^{b}	24.4 ± 2.9^{b}	37.9 ± 27.4

Note: According to Tukey HSD tests, different uppercase letters show significant difference between materials in columns and rows, different lowercase letters show significant difference betweem materials in columns and rows ($p < .05$).

It was clearly observed that the alcoholic mouthrinses cause decreasing in the elastic modulus of samples ($p<0.001$). Baseline elastic modulus mean for enamel specimens was 98.3 ± 2.8 GPa. Data indicated lower statistically significant values of mean elastic modulus of enamel in Listerine mouthrinse compared to artificial saliva ($p<0.001$). Samples in Listerine solution showed that the value of Mosaic was higher than Admira. Load-displacement curves obtained during nanoindentation tests for the samples are shown in Figure 1.

The elastic modulus of enamel was related to the surface structure and testing methods [15, 24, 28]. As shown in Table 4, the mean value of elastic modulus for enamel was 98.3 GPa. This result is consistent with the previously reported measurement on the enamel surface [27]. A previous study reported that low *p*H causes the erosion and decrease in elastic modulus of enamel surfaces [24]. Also, another study has reported that elastic modulus of the enamel decreases after being storage in acidic drinks [29]. In this study, the reduction of the elastic modulus values in the enamel surfaces was observed after being stored in mouthrinses.

As a result of this study, alcohol-containing and alcohol-free mouthrinses have decreased the elastic modulus. No data could be found in the literature regarding the effects of the different *p*H levels mouthrinses on the elastic modulus of the composite. However, some studies have shown that the presence of acid may also cause the erosion at the composite surface. It was stated that the filler content was affected by erosion on the composite surface. This might explain why the elastic modulus of the composites in mouthrinses was more sensitive [30, 31].

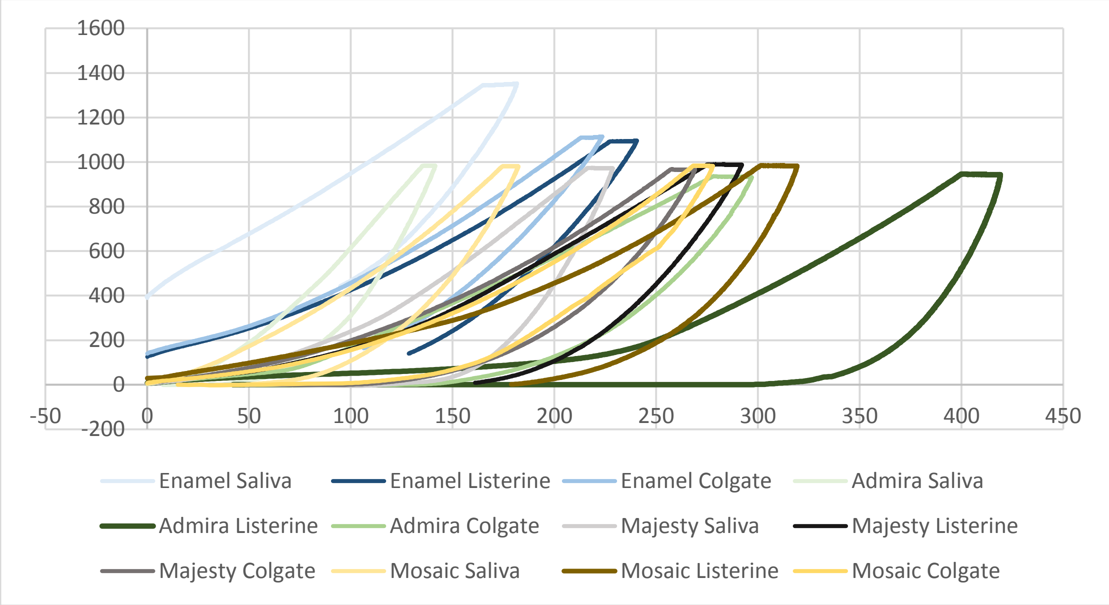


Figure 1. Load-depth curves ofthe tested materials

The figure exhibits twelve different load-unload indentation cycles of samples. The indentation depth increases gradually from the enamel-artificial saliva to Admira-Listerine. Mosaic in the Listerine group had the second highest depth change among all.

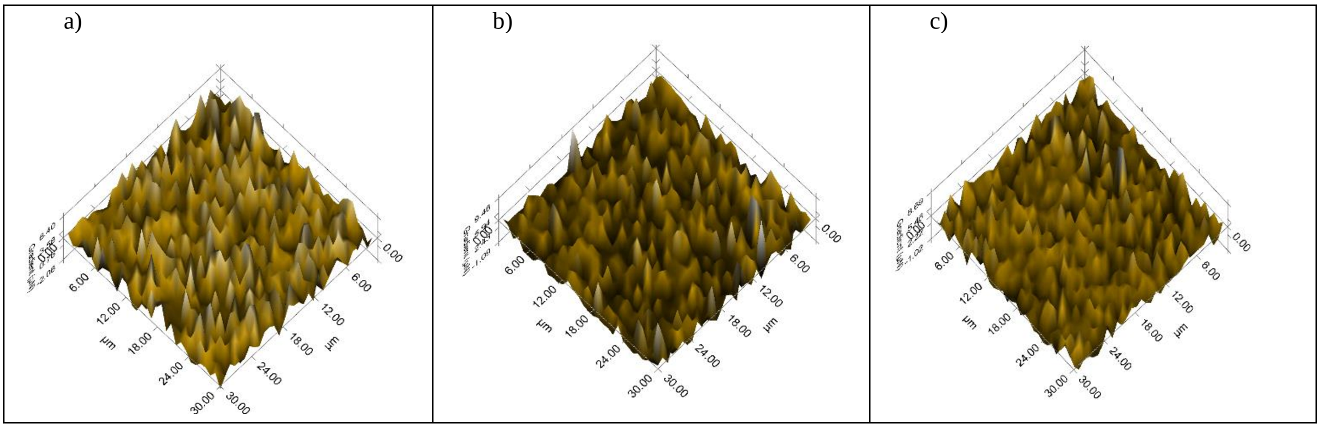
3.3. Surface roughness and topographic images

For surface roughness evaluation, all specimens tested by the Triboindenter testing device. Table 5 shows the mean surface roughness for all materials at baseline and after immersion in solutions. The 3D topographic images of all specimens taken from the Triboindenter are presented in Figure 2.

Table 5. Mean and standard deviations of surface roughness values (nm)

	Enamel	Admira Fusion	Clearfil Majesty Esthetic	Mosaic Universal	Total
Baseline	50.5 ± 7.6^{J}	1423 ± 14.4^{GH}	150.2 ± 11.5^{FGH}	135.5 ± 8.6^{HI}	119.6 ± 42.5^{a}
Artificial Saliva	58.5 ± 7.4^{J}	164.8 ± 18.7^{FG}	171.3 ± 18.6^{EF}	167.4 ± 19.3^{FG}	140.5 ± 51.0^{b}
Listerine Cool Mint	164.2 ± 7.1^{FG}	291.5 ± 8.5^{A}	289.4 ± 8.5^{A}	253.7 ± 7.5^{B}	249.7 ± 53.4^{c}
Colgate Plax	107.4 ± 6.7^{I}	234.0 ± 22.2^{BC}	215.8 ± 8.8^{CD}	198.7 ± 9.9^{DE}	189.0 ± 51.5^{d}
Total	95.1 ± 47.1^{a}	208.2 ± 62.3^{c}	206.7 ± 55.9^{c}	188.8 ± 46.1^{b}	174.7 ± 70.2

Note: According to Tukey HSD tests, different uppercase letters show significant difference between materials in columns and rows, different lowercase letters show significant difference between materials in columns and rows ($p < .05$).



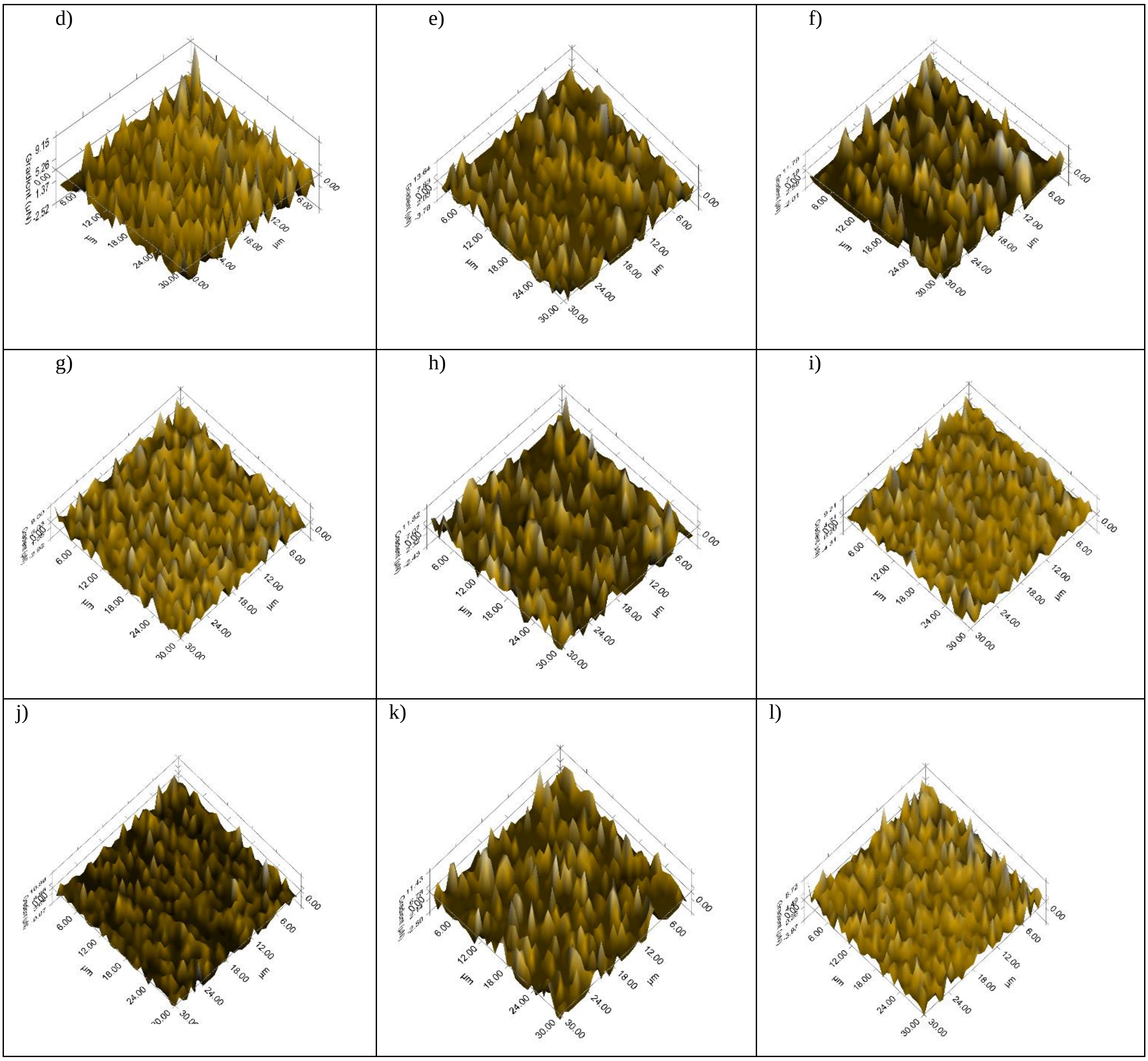


Figure 2. Representive images of surface topography of all specimens. Images represent samples of enamel in (a) artificial saliva, (b) Listerine, and (c) Colgate, Admira in (d) artificial saliva, (e) Listerine, (f) Colgate, Clearfil in (g) artificial saliva, (h) Listerine, (i) Colgate, Mosaic in (j) artificial saliva, (k) Listerine, (l) Colgate

First, our preliminary results revealed that Listerine caused higher roughness values than the other solutions on specimen surfaces ($p < 0.001$). Second, statistically significant differences were observed in the roughness values among the enamel groups in Table 5 ($p < 0.001$). Additionally, the differences within groups of the composite resins at baseline according to the polishing technique are shown in Table 5 ($p > 0.05$). When the types of composite resin were compared, the lowest Ra value for Listerine groups was obtained the Mosaic composite ($p < 0.001$).

The 3D topographic images used to test the surface roughness of all specimens showed that the topographic images were observed to be different from each other. When all composite groups were compared, the greatest irregularities was obtained with the Admira in Listerine.

When 3D images were compared experimentally with the nanomechanical method from the analysis in Table 5 and Figure 2, these topographic images are consistent with the surface roughness mean values of all groups. SEM images of each of the tested materials are shown in Figure 3.

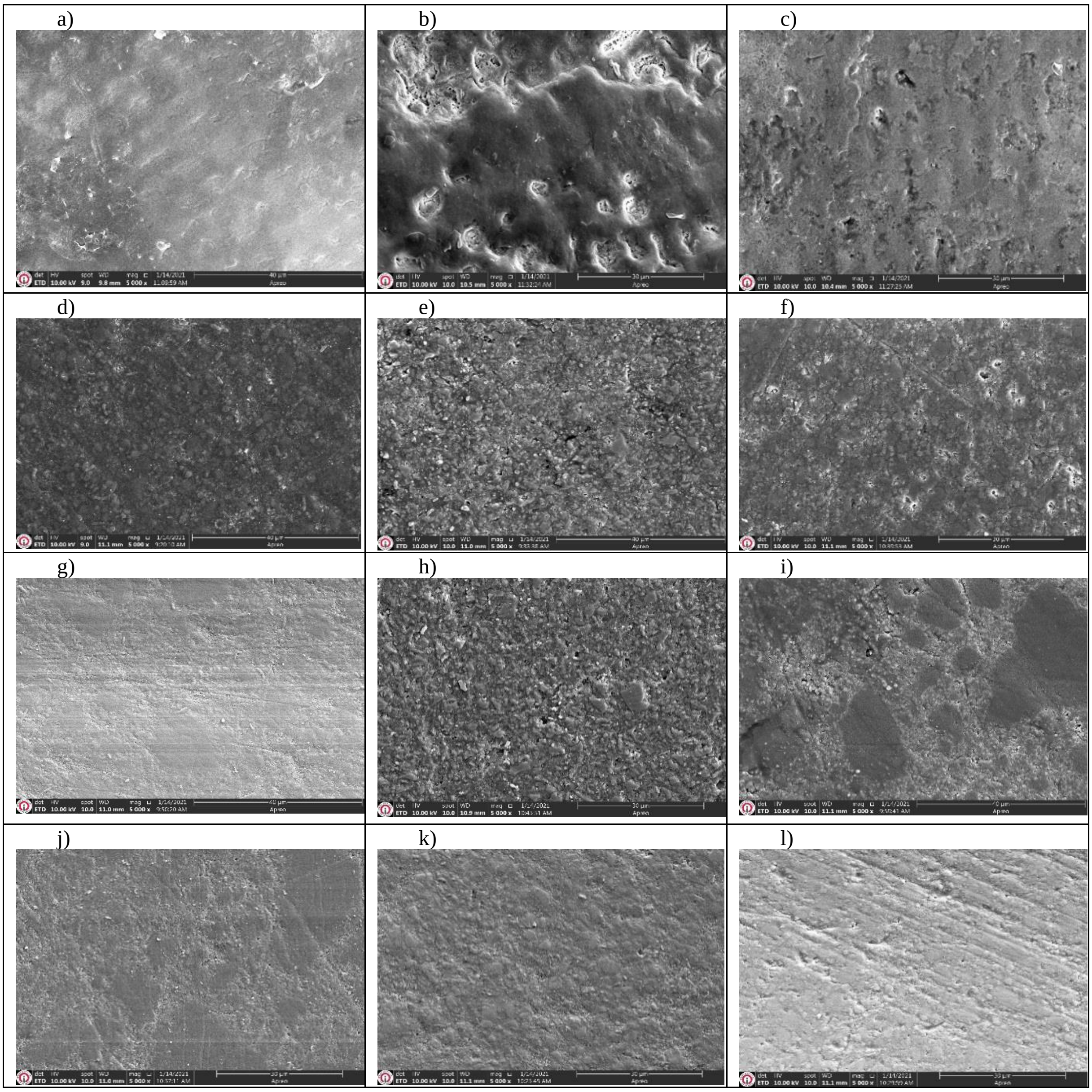


Figure 3. Representative SEM photomicrographs of all specimens at x5000 magnification. Images represent samples of enamel in (a) artificial saliva, (b) Listerine, and (c) Colgate, Admira in (d) artificial saliva, (e) Listerine, (f) Colgate, Clearfil in (g) artificial saliva, (h) Listerine, (i) Colgate, Mosaic in (j) artificial saliva, (k) Listerine, (l) Colgate

The restoration's surface was wanted to be smooth for minimalizing plaque retention by operators [3, 19]. Because of the irregularities, the discolorations occur in the surface of the restoration. Thus, longevity and clinical durability of the restorations can be affected [19]. Many studies have shown the surface roughness evaluations by different testing methods like Profilometer and Atomic Force Microscopy [3, 19, 20]. In the literature, profilometers were frequently used. One of the recent methods for that aim is the nanoindenter by means of AFM, that offers a 3D topography imaging on the material surface. This technique can use the same probe to conduct nanomechanical testing methods [20].

In the oral environment, enamel surface is exposed to several factors. The mechanical properties of enamel affect the *p*H, content and temperature of beverages. The *p*H value critical to tooth enamel is 5.5 [24]. In this study, an increase in the surface roughness of the enamel in mouthrinses was observed. These results were related to low *p*H and alcoholic content in mouthrinses. A previous study investigated the enamel surface properties after immersion in different mouthrinses for 12 weeks of daily application, a digital profilometer was used to calculate the surface roughness [2]. It has also been reported that enamel roughness increases after immersion in all mouthrinses [2].

The surface roughness of composites is related to many factors, such as composite structure, curing method and polishing type [32]. The acceptable value of surface roughness for dental materials is 0.2 µm. Above this value, it causes increased bacterial adhesion and colonization on the material surfaces [3]. All samples in artificial saliva had lower surface roughness than the threshold value. After the same polishing procedure, there were no statistically significant differences among all composite materials at baseline and artificial saliva.

The average of the filler size of Admira and Clearfil are the same and larger than those of the Mosaic composite. The inorganic filler in Admira is silicon dioxide (SiO_2), and according to other studies, SiO_2 on composite content causes high porosity [19, 33]. This might explain why the highest surface roughness of the composites in mouthrinses. However, a previous study has explained that pre-polymerized fillers in nanohybrid composites decreased the surface roughness [34]. In this study, Clearfil has prepolymerized resin fillers.

Concerning the effect of mouthrinses on the surface roughness of resin composites, previous studies have reported that different mouthrinses affected the resin composites differently [3, 26]. One of these studies has reported that alcohol-containing mouthrinse increased the surface roughness of composites. But alcohol-free mouthrinses did not cause any change on the composite's surface roughness [3]. Also, a previous study has reported that there was statistical difference between alcohol-containing and alcohol-free. This change depended on the composite material itself rather than the mouthrinse solution used [26]. In this study, alcohol-containing mouthrinse caused higher surface roughness than alcohol-free.

The different effects of mouthrinses on composite surfaces in different studies can vary depending on the application time, *p*H, acidity and composition of the mouthrinse [3, 10, 26]. Also, the effect of surface roughness can vary depending on the structure of the composite materials [3].

4. Conclusions

According to the results and the limitations of our study, the conclusions below can be drawn:

The performance of the enamel surface was substantially better than all the resin composite groups tested. The mouthrinse containing alcohol caused a greater degradation in nanohardness, elastic modulus and surface roughness in contrast to the alcohol-free mouthrinse and the artificial saliva.

Nanoindentation was found to be a useful method for testing nanohardness and elastic modulus of dental resin composite containing nanoparticles. The evaluation of the 3D topographic images and SEM pictures were in accordance with the surface roughness measurements.

Patients should be aware of the harmful effects of alcohol-containing mouthrinses on their resin composite restorations. Although saliva may provide a protective effect, the exposure of resin composite restorations to mouthrinses may reduce their life span.

If there is a requirement to use a mouthrinse, an alcohol-free brand should be chosen.

Further studies mimicking the oral conditions may be needed to investigate the effects of different mouthrinses on the surface properties of different resin composite materials.

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