

Effect of Toothbrushing-mouthrinse-cycling on Surface Roughness and Topography of Nanofilled, Microfilled, and Microhybrid Resin Composites

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Clinical Relevance

The use of alcohol-containing mouthrinses, associated with toothbrushing, may increase the roughness and topography changes of resin composites over time. The microhybrid resin composite was more susceptible to increased roughness than were the nanofilled and microfilled composites.

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SUMMARY

The purpose of this study was to evaluate the influence of toothbrushing-mouthrinse-cycling (TMC) on the surface roughness and topography of three resin composites with different filler particle systems (Z350, nanofilled [Nf]; Durafill, microfilled [Mf], and Empress Direct, microhybrid [Mh]). Twenty specimens of each resin composite (8.0 mm diameter and 2 mm height) were randomly divided into four groups (n=5) according to the mouthrinses: alcohol-free (Plax – P) and alcohol-containing (Listerine – L and Plax Fresh Mint – PM) and artificial saliva (control – AS). The specimens were submitted to TMC for nine

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DOI: 10.2341/13-199-L

weeks. A surface roughness tester and a three-dimensional profilometer were used to measure the roughness (Ra) and the topography (Sa) before and after TMC. The data were analyzed by multifactor analysis of variance and Tukey *post hoc* test ($\alpha=0.05$). In all media, Mh presented greater roughness than Mf ($p<0.05$). The highest value of roughness was presented by Mh immersed in L ($p<0.05$). The lowest values of roughness were presented by Mf ($p<0.05$). The three resin composites presented the highest roughness after immersion in mouthrinses containing alcohol (PM and L) ($p<0.05$). For the three resin composites, the increase in roughness was noticeable after the fifth week. Topographic analysis showed that the smoothest surfaces were present after immersion in AS.

INTRODUCTION

Resin-based composites are currently the most used material in the field of restorative dentistry.¹ Basically, these materials are composed of three chemically different phases: a polymeric matrix of dimethacrylate monomers; filler particles (dispersed phase); and an organosilane, a coupling agent that bonds the fillers to the polymeric matrix. The most used method of classifying resin-matrix composites is based on their filler particle system: hybrid (0.5-3 μm), microhybrid (0.4-1 μm), and microfilled (0.04-0.4 μm).¹ A new class of resin-matrix composite, with filler particles exclusively in the nanoscale, ie, from 0.1 to 100 nm size range, was recently made available. Nanocomposites claim to combine the mechanical behavior of hybrid and microhybrid composites with the improved surface properties of microfilled composites (lower roughness and best gloss retention).^{2,3} Recent *in vitro*⁴⁻⁶ and clinical studies^{7,8} have shown that nanocomposites present similar or better behavior when compared with hybrid composites.

As a polymer-based material, resin-based composites may undergo degradation when exposed to the oral environment. This degradation process may lead to several drawbacks, such as an increase in wear and surface roughness.^{9,10} Among other aspects, the surface topography of resin composites plays a crucial role on the accumulation of dental biofilm.^{11,12} Additionally, it is known that increasing dental biofilm deposits may lead to periodontitis and secondary caries lesions around tooth-resin composite interfaces.¹³

Toothbrushing is the most used and efficient mechanical method for removing dental biofilm from all accessible tooth surfaces.^{14,15} However, published studies have shown that this method may cause tooth^{16,17} and resin composite abrasion.¹⁸ Specifically with resin composites, this abrasion increases the surface roughness, accelerating the staining produced by pigments from beverages and interfering with color appearance over time.¹⁹⁻²¹

In addition to toothbrushing, mouthrinses are widely used to complement the cleaning of the oral cavity and as an antimicrobial agent to prevent and control periodontal diseases. Irrespective of the therapeutic indication, these substances have been used without professional prescription in an attempt to reduce halitosis and to improve the freshness of the oral cavity.²² In general, mouthrinses contain salts, hydrogen peroxides, antimicrobial agents, pigments, emulsifiers, solvents, acids, and alcohol, diluted in an aqueous solution.²³ Previous studies have shown that alcohol-containing mouthrinses negatively influenced the properties of resin composites, such as sorption and solubility,²⁴ roughness,^{25,26} hardness,^{26,27} and color change.^{28,29} Although previous studies have analyzed the effect of toothbrushing and mouthrinses on the roughness of many resin composites,^{3,5,10,16,18,20,25,26} little information is available with regard to the association of these mechanisms.³⁰ Therefore, the purpose of this study was to evaluate the roughness and the topography of resin-based composites submitted to toothbrushing-mouthrinse-cycling (TMC). The research hypotheses were: 1) microfilled and nanofilled composites will present lower roughness and less topographic change than will a microhybrid resin composite, and 2) the alcohol-containing mouthrinses will produce higher roughness and greater topographic change in the resin composites.

METHODS AND MATERIALS

Three resin composites with different types of filler particles were analyzed: Filtek Z350 (nanofilled – Nf), Durafill (microfilled – Mf), and Empress Direct (microhybrid – Mh). Their compositions are depicted in Table 1. Three mouthrinses, chosen based on their alcohol concentration, were used in the current study (Table 2): one alcohol-free (Plax – P) and two containing alcohol (Listerine – L and Plax Fresh Mint – M). Artificial saliva was used as a control (AS). The pH of all substances was measured in triplicate using a pH meter (HI 3220, Hanna Instruments, Woonsocket, RI, USA). The pH was measured by placing a 1.5-cm diameter glass pH

Table 1: Composition of the Resin Composites Analyzed in This Study

Resin Composite	Composition	Shade
Filtek Z350 (nanofilled)	Filler: 59.5 vol% combination of aggregated zirconia/silica cluster ranging from 0.6 to 1.4 μm with primary particles size, 5-20 nm, and nonagglomerated 20-nm silica filler Polymeric matrix: Bis-GMA, Bis-EMA, UDMA, and TEGDMA	A3
Empress Direct (microhybrid)	Filler: Ba-Al-fluorosilicate glass from 0.4 to 0.7 μm (52 vol%) Polymeric matrix: Dimethacrylate, Bis-GMA, UDMA	A3
Durafill (microfilled)	Filler: 40 vol% combination of silicon dioxide cluster ranging from 0.04 to 0.07 μm with prepolymerized particles, size 10 to 20 μm Polymeric matrix: Bis-GMA, UDMA, and TEGDMA	A3
Abbreviations: Bis-EMA, ethoxylated bisphenol A diglycidyl dimethacrylate; Bis-GMA, bisphenol A diglycidyl ether dimethacrylate; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate.		

electrode (HI 1131, Hanna Instruments) into 20 mL of each substance.

Specimen Preparation

Twenty disc-shaped specimens (8.0 mm diameter and 2 mm height) for each resin composite were built up using a Teflon ring matrix. After the matrix was filled, the material was covered with a polyester strip and a glass slab, with a device providing compression (500 g) for 30 seconds to eliminate porosities. The specimens were then light-activated at a central and at four overlapped points on their surfaces, using a quartz-tungsten-halogen unit (Op-tilux 501, Demetron Inc, Danbury, CT, USA) with an irradiance of 650 mW/cm² for 40 seconds. After 24 hours in water storage at 37°C, all of the specimens were sequentially polished with silicon carbide papers: 1200- and 4000-grit (Arotec, Cotia, SP, Brazil) under constant water irrigation (DPU 10, Struers, Ballerup, Denmark). After polishing, the specimens were randomly divided into four groups (n=5) according to the mouthwash and control group (artificial saliva).

Surface Roughness Analysis

All of the specimens had their surface roughness evaluated using a surface roughness tester (Surftest

SJ 201, Mitutoyo, Tokyo, Japan). Five traces of roughness, in different locations, using a 0.8-mm cutoff and a speed of 0.1 mm/s, were recorded for each specimen. The average surface roughness (R_a , μm) was determined for each specimen. The R_a parameter was obtained using the following equation:

$$R_a = \frac{1}{L} \int_0^L |f(x)| dx$$

where the roughness curve is expressed in $y = f(x)$, and L is the sample length.

Topographic Analysis

The topographic analysis was performed using a three-dimensional (3D) profilometer (Form Talysurf 60i, Taylor Hobson, Leicester, UK). For each specimen, an area of 1 mm² (1 × 1 mm) was scanned with a 20-nm z-resolution, employing 4000 steps in the x-axis and a spacing of 2 μm in the y-axis. The roughness of the 3D reconstructed images was obtained using the 3D S_a parameter (average absolute deviation of the surface), using the following equation:

Table 2: Composition and Characteristics of the Substances Used in This Study

Substance	Composition	pH	Alcohol Content, (v/v%)
Artificial saliva	KCl, NaCl, MgCl, CaCl, nipacin, carboxymethyl, cellulose, sorbitol, and deionized water	6.4	0
Listerine	Ethanol, benzoic acid, eucalyptol, menthol, methyl salicylate, thymol	4.1	26.9
Plax Fresh Mint	Triclosan 0.03%, sodium fluoride 0.025%, gantrez 0.2%	6.5	6
Plax	Triclosan 0.03%, cetylpyridinium chloride 0.05%	5.7	0

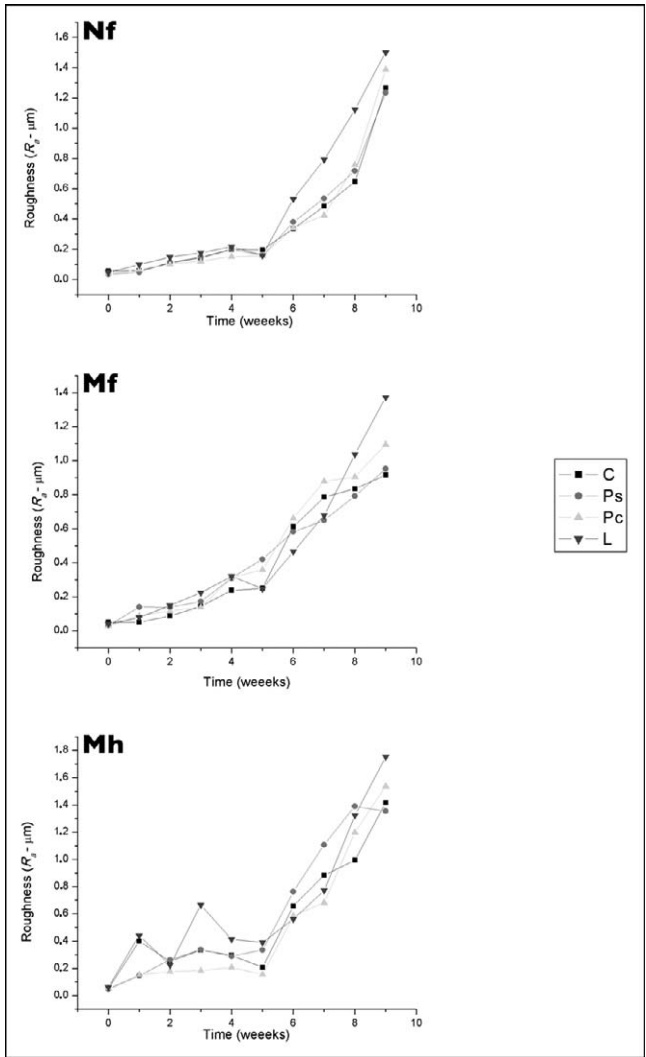


Figure 1. Increase in mean surface roughness (R_a , μm) as a function of time (weeks).

$$Sa = \frac{1}{MN} \sum_{k=0}^{M-1} \sum_{l=0}^{N-1} |z(x_k; y_l)|$$

where z is the height of the measured point in the coordinates x and y .

Toothbrushing-Mouthrinse-Cycling

In the first week, the specimens were submitted daily to TMC as follows: storage for 4 hours in 2 mL of artificial saliva at 37°C, brushing (20 strokes [Oral B 30, Procter & Gamble, São Paulo, SP, Brazil]), storage in artificial saliva at 37°C for 8 hours, brushing (20 strokes [Oral B 30, Procter & Gamble]), and storage in artificial saliva at 37°C overnight (12 hours). Brushing was carried out by using a toothbrushing machine (MEV2, Odeme Biotechnolo-

gy, Joaçaba, SC, Brazil) and a toothpaste slurry in a proportion of 1:2 by weight (18 g of Paradontax [GlaxoSmithKline, Rio de Janeiro, RJ, Brazil] and 36 mL of distilled water). After each brushing, the specimens were abundantly rinsed with distilled water and immersed in 2 mL of mouthrinse or artificial saliva (control) for 2 minutes. The specimens were then washed in distilled water and replaced in artificial saliva. From the second to the ninth week, the specimens were maintained in artificial saliva for seven days and submitted to 280 strokes of brushing and immersed for 28 minutes in mouthrinse or artificial saliva (control). The surface roughness was reevaluated at the end of each week and the topography reevaluated after the ninth week.

Statistical Analysis

The obtained data were analyzed using Statgraphics Centurion XVI software (StatPoint Technologies Inc, Warrenton, VA, USA). Initially, the normal distribution of the errors and the homogeneity of variances were checked by Shapiro-Wilk test and Levene test. Based on these preliminary analyses, the roughness data were analyzed by multifactor analysis of variance (MANOVA) and Tukey *post hoc* test. The baseline and final values of R_a and S_a were submitted to linear regression analysis. All analyses were performed at a significance level of $\alpha=0.05$.

RESULTS

The results of the MANOVA showed that the three independent factors (resin-based composite, mouthrinse, and time of evaluation), as well as all of the interactions, were found to be significant ($p<0.05$). Figure 1 presents the roughness from the baseline to the ninth week for each resin composite in all media. It can be noted that, in general, the increase in roughness was notable after the fifth week. Table 3 shows the mean roughness at the end of the experimental protocol. In all media, Mh presented

Table 3: Final Means (Standard Deviations) of Roughness (R_a , μm) ^a			
Media	Nanofilled	Microfilled	Microhybrid
Artificial saliva	1.27 (0.06) ^{a,A}	0.92 (0.09) ^{a,B}	1.42 (0.22) ^{a,b,C}
Plax	1.24 (0.09) ^{a,A}	0.95 (0.05) ^{a,B}	1.36 (0.19) ^{a,A}
Plax Fresh Mint	1.39 (0.10) ^{a,b,A}	1.09 (0.05) ^{a,B}	1.54 (0.20) ^{b,A}
Listerine	1.50 (0.07) ^{b,A}	1.37 (0.17) ^{b,A}	1.75 (0.24) ^{c,B}
^a In columns, means followed by the same lowercase letters are not statistically different (Tukey test, $p>0.05$). In rows, means followed by the same uppercase letters are not statistically different (Tukey test, $p>0.05$).			

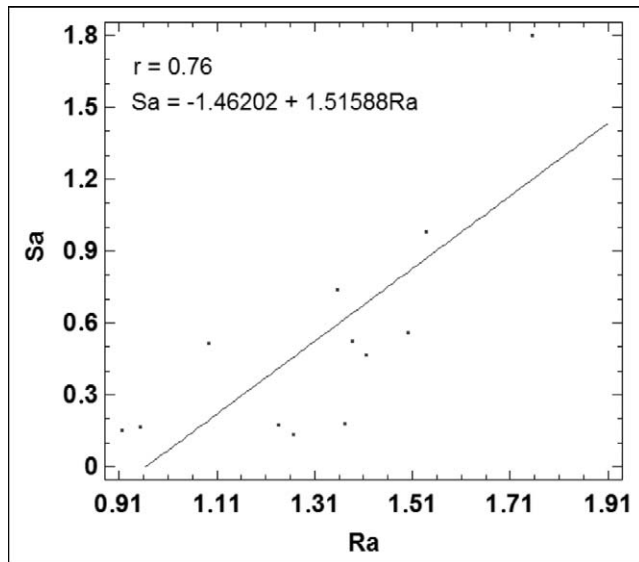


Figure 2. Regression line (linear model) of Ra vs Sa ($r=0.76$, $Sa = -1.46202 + 1.51588Ra$).

higher roughness values than Mf (Tukey test, $p<0.05$). The highest value of roughness was presented by Mh immersed in L (Tukey test, $p<0.05$). The lowest values of roughness were presented by Mf (Tukey test, $p<0.05$). The three resin composites presented the highest roughness after immersion in alcohol-containing mouthrinses (PM and L) (Tukey test, $p<0.05$). Figure 2 shows the plot of Ra against Sa. Linear regression analysis found a significant correlation ($p=0.0038$) between the two parameters ($r=0.76$, $Sa = -1.46202 + 1.51588Ra$).

Representative 3D reconstructed images showing the topography of the resin composites at the end of the experimental protocol are shown in Figure 3. It can be noted that specimens immersed in AS presented the smoothest surfaces.

DISCUSSION

Many published studies have separately analyzed the *in vitro* influence of brushing^{3,5} and mouthrinses^{26,27} on the surface changes of resin composites. However, it is well known that the degradation of resin-based materials in the oral environment is a complex process, which involves mechanical and chemical mechanisms.^{10,24} This was the rationale to employ TMC in the present study. This was performed in an endeavor to simulate actual conditions in the oral environment.

Several published studies have shown that microfilled and nanofilled resin composites present the

lowest roughness immediately after polishing.^{2-4,31} Based on this, clinicians assume that these materials are the most adequate for building up anterior restorations. Moreover, the characteristic of the filler particle system (concentration, size, and shape) is the most crucial factor affecting the wear of resin composite.^{25,32} These aspects conducted the choice of the resin composite used in the present study (nanofilled, microfilled, and microhybrid). This was done in an attempt to investigate whether the type of resin composite is really a matter of concern on the clinical protocol for resin composite restorations. Although it is not a dental clinical procedure, the polishing of all specimens with 1200- and 4000-grit silicon carbide papers at the beginning of the experimental protocol aimed to ensure that all specimens had similar initial roughness values so the final results represented the actual material behavior.⁵ In the present study, the coefficient of variation of the groups ranged from 4.6 to 15.4. These values can be seen as the overall data presenting a low variability, thereby supporting the sample size of $n=5$.

The clinical relevance of surface roughness can be demonstrated in two ways. Firstly, this property is strongly related to the bacterial colonization of surfaces located in the oral environment. A surface roughness above $0.2 \mu\text{m}$ has been reported to increase the colonization and adhesion of bacteria on composite surfaces.¹¹ Moreover, it has been established that a higher surface roughness (difference between peaks and valleys) provides a reduced possibility of dislodging the oral biofilm,^{33,34} a periodontal health concern. Secondly, an increase in roughness can interfere with changes in color and gloss of composite restorations,^{5,18,35} an esthetic concern.

Analysis of Table 3 and Figure 3 indicates that in all media, Mf presented lower roughness and less topographic change than Mh. Moreover, after immersion in AS and L, Nf also presented lower roughness and fewer topographic changes than Mh. These findings lead to the acceptance of the first research hypothesis. The final values of roughness ranged from 0.92 to $1.75 \mu\text{m}$ and are in agreement with previously published results.^{2,5,28} Among other factors, the roughness of resin composites is directly related to the features of their filler particle systems, ie, amount, size, shape, hardness, and interparticle spacing.^{25,36} These aspects can be used to explain the poorest behavior presented by Mh. Even when considering that filler particles and polymeric matrix are bonded with a silane-coupling agent, the first

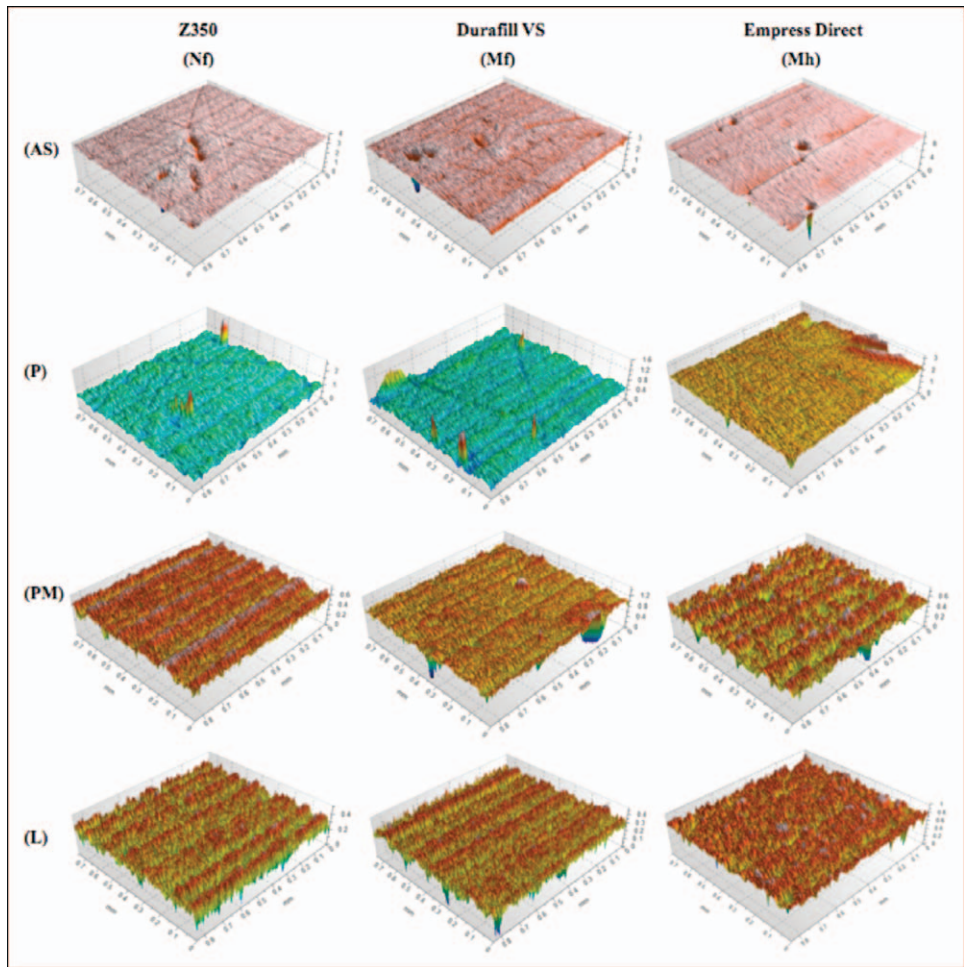


Figure 3. Representative 3D reconstructed images of each resin composite immersed in media (α and β angles = 45° , z axis in μm).

may be plucked out during brushing. Thus, it is possible that, due to the greater size, the filler particle of Mh ($0.4\text{--}0.7\text{ }\mu\text{m}$) protruded more through the composite surface than did the filler particle of the Nf ($5\text{--}20\text{ nm}$) and Mf ($0.05\text{--}0.07\text{ }\mu\text{m}$). This extended protrusion provided longer cantilevers, leading to higher angular moments that facilitated the fillers being pulled out from the material.³⁷ The variation in roughness seen in Figure 1 may reinforce this hypothesis. It is possible that, in some measurements (first and third weeks), the roughness of this resin composite was influenced by the deep valleys produced for some filler particle dislodgment and in a subsequent measurement, the wear of polymeric matrix around these valleys produced a less rough surface.

With the exception of the L group, the final roughness of Nf was always statistically higher than the roughness values of Mf. However, it can be noted that until the fifth week the increasing roughness of

Nf was slightly lower than that of Mf (Figure 1b,c). The higher concentration of filler particles of Nf ($59.5\text{ vol}\%$) may be used to explain this behavior. It is known that when there is a higher content of smaller filler particles (as found with Nf), the distance between neighboring particles is small, which may act as a barrier against polymeric matrix wear.^{37,38} Thus, the current authors hypothesized that the polymeric matrix of Nf was less worn than that of Mf during the first stage of TMC, which had only $40\text{ vol}\%$ of filler particles. On the other hand, it is possible that the continuing TMCs increased the removal of zirconia/silica clusters of 0.6 to $1.4\text{ }\mu\text{m}$, inducing fatigue cracks between fillers and organic matrix and causing gradual matrix destruction and increasing the loss of material.³⁹ Consequently, the final roughness of Nf was increased.

Although Mf had larger filler particles among the analyzed materials (prepolymerized fillers of 10 to $20\text{ }\mu\text{m}$), its roughness was the lowest (Table 3). This can

be explained by the fact that these prepolymerized particles are also formed by monomers (isofillers). Consequently, it is possible that these particles were worn away during TMC, rather than by being plucked out, so that the final roughness of Mf reflected only the size of its primary filler particles of 0.04 to 0.07 μm (40 to 70 nm).⁵ The smoothest surfaces were provided by Mf in all media (Figure 3), which might serve as representative of this phenomenon.

Since the alcohol-containing mouthrinses produced greater roughness and topography changes in the resin composites (Figure 3; Table 3), the second research hypothesis of the present study was accepted. This result was expected and can be explained by the plasticizing effect of ethanol. This polar solvent penetrates into the resin composite, causing material swelling, pulling apart the polymeric matrix chains, and decreasing its crosslinking density, resulting in a decrease in wear resistance^{27,40,41} and potentiating the deleterious effects of brushing. The findings of Almeida and others⁴² may reinforce this discussion. These authors found that the sorption and solubility of a hybrid and a nanofilled resin composite were higher after immersion in alcohol-containing mouthrinses and claimed that this was due to the swelling of their polymeric matrixes produced by the ethanol contained in the mouthrinses and increasing the elution of non-reacted monomers and oligomers from those materials.

Listerine was the medium that produced the greatest increase in roughness in the three resin composites (Table 3). In addition to the greater content of ethanol (26.9 v/v%), it is possible that its low pH (4.1) contributed to this result. It is well established that the ester groups present in dimethacrylate monomers, such as those present in the resin composites in the current study (ie, Bis-GMA, UDMA, Bis-EMA, and TEGDMA), can undergo degradation through hydrolysis in environments with low pH.⁴³ This hydrolysis may produce surface erosion and dissolution, negatively affecting the wear, hardness, and surface integrity by softening the matrix and causing a loss of structural ions.⁴⁴ It is possible that these aspects act synergistically to potentiate the negative effects of toothbrushing, thereby increasing the roughness of the resin composites.

The data provided in Figure 1 are noteworthy. Although the roughness slowly increased from the first to the fifth week for the three resin composites (approximately 15% to 20% of the total roughness),

there was a notable increase from the fifth to the ninth week. Moreover, the final values of roughness (Table 2) were far greater than the values found in other studies that evaluated toothbrushing^{10,39,45} or mouthrinse immersion.^{26,28,46,47} However, Sadaghiani and others⁴⁶ found that when mouthrinses and toothbrushing were combined, the effect on the roughness of modified glass-ionomer restorative materials was at least twice that obtained after immersion in mouthrinses alone. The authors theorized that a superficial layer of material was removed during each episode of toothbrushing, exposing a fresh surface to be attacked by the mouthwash at the next immersion. They further indicated that this interaction between chemical erosion and mechanical abrasion was considered to have contributed to the progressive roughening of the surfaces. Thus, it is reasonable to speculate that the effect described by the former study took place in the present study, since TMC combined toothbrushing and immersion in mouthrinses. The similarities among all curves depicted in Figure 1 suggest a common roughening effect that could be used to reinforce this possibility.

CONCLUSIONS

Within the limitations of the present study, it can be concluded that:

- Models that involve the combined use of toothbrushing and mouthrinse immersion could be more useful to obtain results that are clinically more accurate for roughening resin composites;
- Microfilled resin presented the best behavior (lower roughness and less topographic changes), suggesting that it is more adequate to be used in the surface layer of anterior restorations; and
- Alcohol-containing mouthrinses can increase the roughness of resin composites. Thus, clinicians, especially periodontists, should consider this when prescribing these substances to their patients.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

(Accepted 17 September 2013)

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