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The Effect of Mouthrinses on Salivary Sorption, Solubility and Surface Degradation of a Nanofilled and a Hybrid Resin Composite

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Clinical Relevance

The use of alcohol-containing mouth rinses with a low pH may increase the sorption and solubility of resin composites. The nanofilled resin composite underwent more surface degradation than the hybrid type.

SUMMARY

This *in vitro* study evaluated the effect of mouth rinses on salivary sorption (Sp), solubility (Sl) and surface degradation of a nanofilled (Z350) and hybrid (P60) resin composite. Specimens (6 mm in diameter and 1 mm thick) of a nanofilled and hybrid resin composite were immersed in artificial saliva at 37°C for seven days. Twice a day, the samples (n=5) were immersed in 20 ml of three mouth rinses: Listerine, Plax Mint and Plax. A control group was maintained in artificial saliva. Sp and Sl were evaluated based on ISO 4049:2000(E) and surface degradation by scanning electron microscopy–SEM. The degree of conversion (DC%) of resin composites was obtained by using an FT-IR spectrometer equipped with an attenuated total reflectance crystal (ATR). The data were analyzed using the Student's *t*-test, ANOVA and Tukey test for multiple comparisons. No significant difference in DC% was found between the two resin composites (p<0.05). The highest sorption rate was presented by the nanofilled composite exposed to Listerine (p<0.05). The hybrid composite in the control group (artificial saliva) and Plax presented the lowest sorption (p<0.05). The highest solu-

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bility was presented by the two resin composites exposed to Listerine ($p<0.05$). SEM analysis showed that mouth rinses produced more severe surface degradation in the nanofilled composite.

INTRODUCTION

Today, due to their esthetic features and improved physical-mechanical properties, resin composites are largely used to build-up anterior and posterior restorations. Basically, resin composites are constituted of a polymeric matrix, filler particles and a silane-coupling agent that links the matrix to fillers.[1] As a polymer-based material, resin composite may suffer degradation when applied in the oral environment.[2] As a result, there may be a decrease in properties, such as hardness, flexural strength and elastic modulus.[3-4]

The degradation of resin composites is a complex mechanism that involves water sorption inside the material and other related phenomena, such as thermal and mechanical cycling, crack propagation and attenuation, and it is mainly dependent on the composition of the polymeric matrix and features of its filler particle system.[5-7] Some published studies have shown that nanofilled composites present physical-mechanical properties similar to those of the hybrid type.[8-9] However, the large surface area to volume ratio derived from the nanosilica filler particles present in nanofilled composites may increase its fluid uptake,[7] leading to degradation of the filler-polymeric matrix interface, thus affecting some of its mechanical properties.[3]

Mouth rinses are widely used to prevent and control caries and periodontal disease, even without a dental prescription, and a previous study related that some individuals used mouth rinses with a frequency of up to six times per day.[10] The formulation of commercially available mouth rinses contains various substances, such as water, antimicrobial agents, salts, preservatives and, in some cases, alcohol. The variation in the concentration of these substances will affect the pH of mouth rinses.[11]

Previous studies have shown that mouth rinses with a low pH and a higher alcohol content may affect some physical-mechanical properties of resin composites.[12-13] In this field, Cavalcanti and others[12] found that Listerine, a mouth rinse with an alcohol content of 21.6% and a pH of 4.3, produced a more significant decrease in hardness of TPH resin composite over time than Plax, which is a mouth rinse with an alcohol content of 8.7%, and a pH of 7.0%. Moreover, Weiner and others[13] showed *in vitro* that a heat-treated resin composite gained significantly more weight when soaked in alcohol-containing mouth rinses than in alcohol-free mouth rinses.

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 of 5-20 nm, and nonagglomerated 20 nm silica filler. Polymeric matrix: Bis-GMA, Bis-EMA, UDMA and TEGDMA	A3B
Filtek P 60 (Hybrid)	Filler: 61 vol% silica/zirconia filler with mean particle size of 0.6 μm Polymeric matrix: Bis-GMA, Bis-EMA, UDMA and TEGDMA	A3

Although some published studies have investigated the sorption and solubility phenomena in resin composites,[4-6] little information is available on the influence of mouth rinses with these properties.[14] Therefore, the current study investigated the effect of three commercially available alcohol-containing and alcohol-free mouth rinses on the salivary sorption, solubility and surface degradation of a nanofilled and hybrid resin composite.

METHODS AND MATERIALS

Two resin composites with qualitatively the same polymeric matrix and different types of filler particles were analyzed: nanofilled (Filtek Z350, 3M ESPE, St Paul, MN, USA) and hybrid (Filtek P60, 3M ESPE). The composition of the resin composites is depicted in Table 1. Three mouth rinses, chosen in accordance with the absence or differences in alcohol concentration on their compositions, were used in the current study: alcohol-free—Plax (Colgate-Palmolive, Guilford, UK), alcohol-containing–Listerine (Warner Lambert Health Care, Eastleigh, UK) and Plax Fresh Mint (Colgate-Palmolive). Artificial saliva[5] was used as the control. The composition and characteristics of the substances are described in Table 2. The pH of all the substances was measured in triplicate using a pH meter (HI3220, Hanna Instruments, Woonsocket, RI, USA). To measure the pH, 20 mL of each substance was added to a beaker, and the pH was obtained with a glass pH electrode 1.5 cm in diameter (HI 1131, Hanna Instruments). All the resin composite specimens were light-activated with a quartz-tungsten-halogen unit (Optilux 501, Demetron Inc, Danbury, CT, USA) using an irradiance of 650 mW/cm^2 for 40 seconds. The radiant exposure (26 J/cm^2) was calculated as the product of the irradiance of the curing unit by using a radiometer (model 100, Demetron Inc) and the time of irradiation.

Degree of Conversion (DC%)

Spectra of the unpolymerized and polymerized specimens of each resin composite were recorded with an FT-IR spectrometer (Varian 3100 FT-IR, Varian Inc, Palo Alto, CA, USA) equipped with an attenuated total reflectance crystal (MIRacle ATR, Pike Technologies,

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

Substances	Composition	pH (v/v%)	Alcohol Content
Artificial saliva (S)	KCl, NaCl, MgCl, CaCl, nipacin, carboxymethyl cellulose, sorbitol and deionized water	6.9	0
Listerine (L)	Ethanol, benzoic acid, eucalyptol, menthol, methylsalysilate, thymol	4.3	26.9
Plax Fresh Mint (PM)	Triclosan 0.03%, sodium fluoride 0.025%, Gantrez 0.2%	6.6	6
Plax (P)	Triclosan 0.03%, cetylpyridinium chloride (0.05%)	5.0	0

Madison, WI, USA) operating with 120 scans at a resolution of 4 cm^{-1}. Standard increments of each resin composite were compressed between two polyethylene strips and two glass slides to produce a thin film. Five films of each resin composite were then light-activated with the light tip in contact with the glass slide. FT-IR spectra of the polymerized specimens were recorded 24 hours after storage at 37°C in lightproof containers without the presence of water. The DC% was calculated from the ratio between the height of absorbance peaks of the aliphatic C=C bond (1638 cm^{-1}) to the aromatic C=C bond (1608 cm^{-1}) used as an internal standard obtained from the polymerized and unpolymerized specimens by the following equation:

$$\text{DC }(\%) = 100 \times [1 - (R_{\text{polymerized}}/R_{\text{unpolymerized}})]$$

where R = peak at 1638 cm^{-1}/peak at 1608 cm^{-1}

Sorption (Sp) and Solubility (Sl)

Twenty disc-shaped specimens of each resin composite were built-up by filling an aluminum mold (1 mm thick and 6 mm in diameter). After filling the mold to excess, the resin composite surface was covered with a polyester strip and glass slide compressed with a device (500 g) for 20 seconds to avoid porosities, and it was light-activated from the top. The discs were randomly assigned to four groups (n=5) for each resin composite, placed in a desiccator containing freshly dried silica gel and transferred to an oven at 37°C (Q316B15, Quimis, Petrópolis, RJ, Brazil). After 24 hours, the discs were repeatedly weighed on an analytical balance (AX 220, Shimadzu, Tokyo, Japan) until a constant mass (m_1) was attained, that is, the disc mass variation was less than ± 0.1 mg. After final drying, the thickness and diameter of the discs were measured at four points with a digital caliper (MPI/E-101, Mitutoyo, Tokyo, Japan), and the volume (V) was calculated in mm^3. The discs were then individually placed in plastic vials and immersed in 10 ml of artificial saliva at 37°C. Twice a day, the discs were immersed in 20ml of each mouth rinse for two minutes (12 hour intervals). After immersion in the respective mouth rinses, the discs were washed in artificial saliva. A control group was kept in artificial saliva at 37°C throughout the entire experiment. After seven days, the discs were removed from the vials, washed in distilled water, dried at room temperature for 15 minutes and weighed (m_2). The discs were then placed in a desiccator and weighed daily until the mass variation was less than ± 0.1 mg (m_3). The sorption (Sp) and solubility (Sl) were obtained using the following formula:

$$Sp = \frac{m_2 - m_3}{V}$$

$$Sl = \frac{m_1 - m_3}{V}$$

The units used in the formula were (μg/mm^3).

SEM Analysis

In order to characterize the effect of mouth rinses on the surface degradation of resin composites, three discs from each experimental group and three additional discs that represented the baseline were analyzed by SEM. The discs were dried, sputter-coated and observed by SEM (JSM 5310, JEOL Ltd, Tokyo, Japan) operating at 20kV. The SEM images were taken at 5000x.

Statistical Analysis

The statistical analysis was performed using Statgraphics 5.1 Software (Manugistics, Rockville, MD, USA). After the normal distribution of errors and homo-

Table 3: *Means and Standard Deviations of DC%, Sorption and Solubility (μg/mm^3)*

	Z350		P60	
	Degree of Conversion (%)			
	55.7 (3.8)		59.0 (4.6)	
Media	Solubility	Sorption	Solubility	Sorption
S	3.1 (0.2)^{C}	14.1 (1.0)^{b,c}	2.4 (0.4)^{C}	2.8 (0.3)^{e}
L	6.2 (0.7)^{A}	20.4 (1.7)^{a}	6.4 (0,6)^{A}	8.9 (0.9)^{d}
PM	4.4 (0.8)^{B}	11.9 (2.0)^{c}	3.4 (0.4)^{B,C}	7.3 (1.4)^{d}
P	3.0 (0.3)^{C}	14.8 (1.7)^{b}	2.4 (0.3)^{C}	4.0 (0.7)^{e}

The capital letters represent the statistical significance for solubility values and the lower case letters represent the statistical significance for sorption values. Means followed by the same letters are statistically similar (ANOVA/Tukey test, α=0.05).

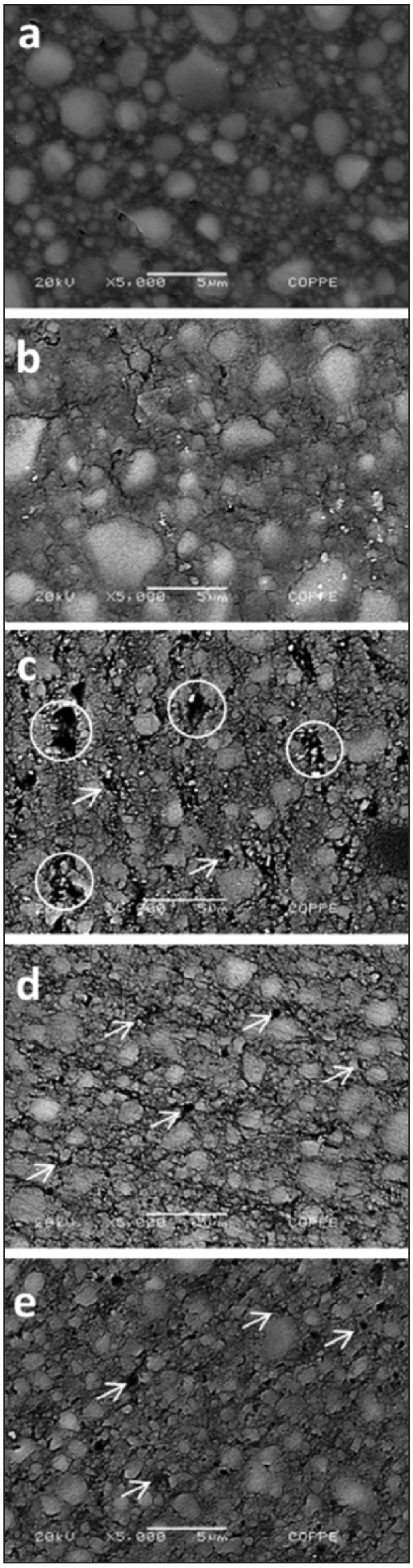

Figure 1: *Representative SEM micrographs of the nanofilled composite surfaces. (a) baseline; (b) artificial saliva; (c) Listerine; (d) Plax Fresh Mint and (e) Plax (original magnification 5000x, dotted line = 5 µm).*

geneity of variances were checked by the Shapiro-Wilk and Levene tests, respectively, the DC% data were analyzed by the Student's t-test and the sorption and solubility data were analyzed by two-way ANOVA and a post hoc test (Tukey's test). The analyses were performed at a significance level of $\alpha=0.05$. In addition, the SEM images were analyzed qualitatively.

RESULTS

Table 3 shows the means and standard deviations of the DC%, sorption and solubility. As regards DC%, the Student's t-test identified no statistically significant difference between the two resin composites ($p>0.05$).

For salivary sorption, two-way ANOVA identified statistical significance for the two main factors: resin composite and mouth rinses ($p<0.05$), as well as for the interaction between them ($p<0.05$). The Tukey test showed that the nanofilled composite exposed to Listerine presented the highest sorption, followed by nanofilled composite in the control group and the composite exposed to Plax ($p<0.05$). The sorption of the nanofilled composite in the control group and the composite exposed to Plax Fresh Mint did not differ from each other ($p>005$). The sorption values of the hybrid composite exposed to Listerine and Plax Fresh Mint were similar ($p>0.05$) and higher than that of the hybrid composite in the control group and the composite exposed to Plax ($p<0.05$).

As regards solubility, the two-way ANOVA also detected a significant influence for the two main factors: resin composite ($p=0.0035$) and mouth rinses ($p<0.05$). On the other hand, the double interaction was shown to not be significant ($p=0.0946$). According to the Tukey test, the two resin composites exposed to Listerine presented the highest solubility ($p<0.05$). The solubility values of the nanofilled and hybrid composites exposed to Plax Fresh Mint were similar and statistically higher than that of the two composites in the control group and the composite exposed to Plax ($p<0.05$), which did not differ from each other ($p>0.05$).

Representative SEM micrographs are shown in Figures 1 and 2. In comparison with the baseline specimens (Figures 1a and 2a), it can be noted that all surfaces presented micromorphological changes. Irrespective of the control group (Figures 1b and 2b) or mouth rinses (Figures 1c-e and 2c-e), several microcracks were seen at the filler-polymeric matrix interfaces. When exposed to the mouth rinses, nanofilled composite presented more severe damage (Figures 1c-e). The voids dispersed throughout the surfaces of the nanofilled composite (white arrows) suggest filler particle loss (Figures 1c-e). The images (marked with circles) viewed in the nanofilled composite/Listerine specimen (Figure 1c) suggest the loss of some aggregated zirconia/silica clusters, ranging from 0.6 to 1.4.

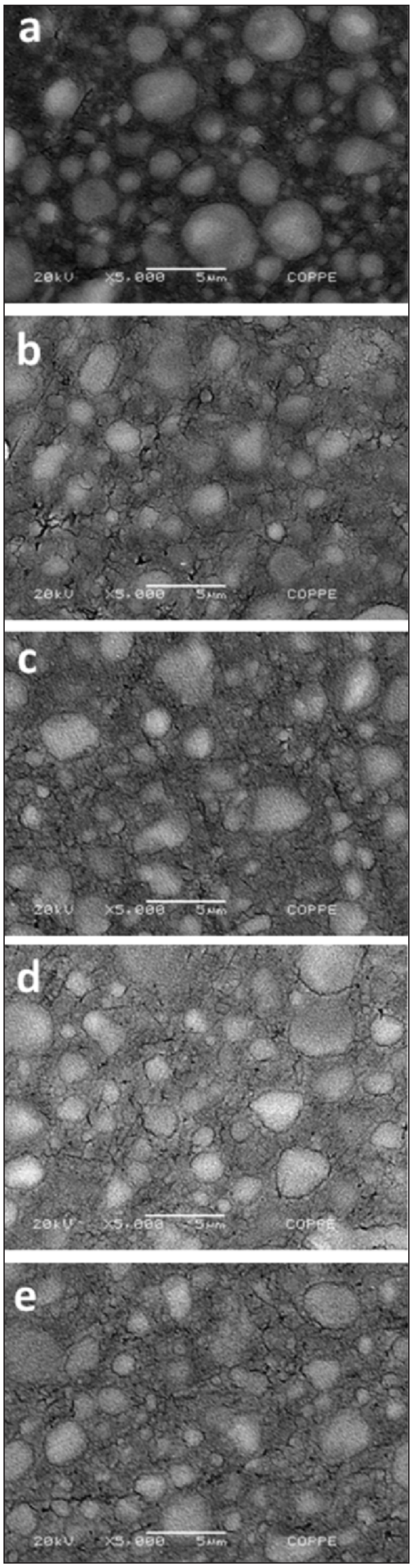

Figure 2: *Representative SEM micrographs of the hybrid composite surfaces. (a) baseline; (b) artificial saliva; (c) Listerine; (d) Plax Fresh Mint and (e) Plax (original magnification 5000x, dotted line = 5 µm).*

DISCUSSION

This *in vitro* study analyzed the effect of mouth rinses on salivary sorption, solubility and surface degradation of a nanofilled and hybrid resin composite. The resistance to degradation in the oral environment plays an important role in the service life of composite restorations.[15] Thus, even when obtained *in vitro*, results that increase the knowledge basis about sorption and solubility phenomena are crucial to predicting the behavior of resin composites when applied in the oral cavity.

In the current study, significant differences in DC% between the two resin composites were not found. Since the two materials had qualitatively the same polymeric matrix, that is, Bis-GMA, Bis-EMA, UDMA and TEGDMA (technical profile of 3M ESPE), this result was expected. Although the DC% represents only the percentage of C=C bonds from dimethacrylate monomers that are broken during resin composite conversion and not the crosslink density developed by the polymeric network,[16-17] it is reasonable to assume that, if the two resin composites analyzed in the current study have the same polymeric matrix and statistically similar DC%, theoretically, they would have developed the same crosslink density in their networks.[18] The polymeric network features are very important, particularly in terms of solubility. In this field, earlier studies have shown a strong correlation between DC% and solubility.[5,7] Based on the aforementioned factors, it was assumed that the differences in sorption, solubility and surface degradation found in the current study were exclusively due to dissimilarities between the filler particle systems present in the resin composites and the composition of the mouth rinses used.

The sorption results obtained in the current study showed interesting aspects; that is, irrespective of the mouth rinse used, including the control group (artificial saliva), the sorption values presented by nanofilled composite were statistically higher than the hybrid composite (Table 3). This suggests that the nanofilled composite is more prone to absorbing fluids than the hybrid type. Even taking into account some differences in the methods used, this result is in agreement with earlier studies that investigated the sorption of nanofilled and hybrid resin composites.[3,7] Curtis and others[3] found that Supreme resin composite (a universal restorative that contains the same patented and proprietary nanotechnology used in Z350, 3M-ESPE) underwent a higher water uptake than Z250, which is a hybrid resin composite similar to P60, after 6 and 12 months of storage. Furthermore, Silva and others[7] showed that Supreme resin composite presented a higher sorption than P60 after seven days of immersion in artificial saliva.

There are two possibilities that could explain this higher salivary gain presented by the nanofilled composite. First, the greater surface area to volume ratio derived from the non-agglomerated 20 nm silica filler could have allowed more artificial saliva to accumulate at the filler-polymeric matrix interfaces,[3,7,19] where there would be the presence of a greater amount of silane with the hydrophilic groups, which is capable of establishing a high level of hydrogen bonds.[20] Second, the saliva accumulated at the interface between the aggregated zirconia/silica cluster filler-polymeric matrix could have diffused into the aggregates through paths created due to poor impregnation of 5-20 nm-sized primary particles by the polymeric matrix.[7,21]

Zhang and Xu[4] showed that two model dental composites (based on Bis-GMA, UDMA and TEGDMA) presented two times higher sorption values in ethanol/water (75:25 v/v%) than in artificial saliva, and these authors claimed that this was due to the easier penetration of ethanol into the resin matrix. In the current study, this finding could be used to explain the highest sorption presented by nanofilled composite exposed to Listerine (45% higher than in artificial saliva only) and greater sorption of the hybrid composite when exposed to alcohol-containing mouth rinses (8.9 and 7.3 µg/mm^3) (Table 2). The alcohol content present in Listerine (26.9%) and Plax Fresh Mint (6%) could probably have softened the polymeric matrix of both composites, thus facilitating the diffusion of saliva into the materials.[22-23] Reinforcing this possibility, Karabela and others[24] showed that five dental resin-nanocomposites (60% silica filler) presented higher sorption in ethanol/water than in water.

Although the sorption values of the hybrid composite were lower than that of the nanofilled composite, it can be noted in Table 3 that, when hybrid composite was exposed to Listerine and Plax Fresh Mint (alcohol content of 26.9 and 6%, respectively), its sorption rates were higher than when it was exposed to Plax and was maintained in the control group. Here, again, the role alcohol played in the sorption phenomenon is clear.

Resin composite solubility can be interpreted as the release of residual monomers and oligomers, as well as the leaching of filler particles and ions from its surfaces.[2] In the current study, the highest solubility was presented by both resin composites exposed to Listerine (alcohol content of 26.9%/pH 4.2). Furthermore, solubility of the nanofilled composite exposed to Plax Fresh Mint (alcohol content 6%/pH 6.6) was found to be higher than when this resin composite was exposed to Plax and was maintained in the control group (artificial saliva only). Moreover, although not statistically significant, sorption of the hybrid composite exposed to Plax Fresh Mint (alcohol content 6/pH 6.6) showed a trend towards being higher than when this composite was exposed to Plax and maintained in the control group (artificial saliva only). Indeed, some previous studies have shown that resin composites are more prone to undergoing monomer release and present high solubility in ethanol/water than in water or artificial saliva.[4,25-26] Even when considering that the experimental conditions used in the current study—alcohol content of the mouth rinses and time of immersion of the specimens—differ from that used in previous studies, it seems clear that the alcohol content influenced the solubility values presented by both resin composites analyzed here. As a good dimethacrylate solvent, alcohol may have swelled the polymeric matrixes of the resin composites, thus increasing the amount of unreacted monomers and oligomers that diffuse out of materials.[25]

Another important aspect that could have taken part in the solubility results observed in the current study is the low pH presented by Listerine (pH 4.2) Basically, the low pH of this mouth rinse may have acted in the polymeric matrixes of the nanofilled and hybrid composite through catalysis of ester groups from dimethacrylate monomers present in their compositions (Bis-GMA, Bis-EMA, UDMA and TEGDMA). The hydrolysis of these ester groups may have formed alcohol and carboxylic acid molecules that may have accelerated degradation of the resin composites due to a lowering of the pH inside their matrixes.[27] On the other hand, the low pH may have also have caused erosion in the surfaces of the filler, accelerating its debonding or, at least, increasing the release of ions from its surfaces.[2] The high level of porosities showed in Figure 1c may be interpreted as a result of this process.

Earlier published studies[28-29] have shown *in vivo* that alcohol-free mouth rinses presented a similar effectiveness on plaque control and gingival inflammation reduction compared to mouth rinses containing alcohol. Moreover, Poggi and others[30] showed *in vitro* that mouth rinses with alcohol may produce cytotoxic effects on human gingival fibroblasts. Based on these published findings and the results presented in the current study, it seems reasonable to advocate for the use of alcohol-free mouth rinses.

CONCLUSIONS

Within the limitations of the current study, it was concluded that the use of alcohol-containing mouth rinses with a low pH may increase the sorption and solubility of resin composites. Furthermore, due to the greater surface area to volume ratio of its filler particle system, the nanofilled resin composite may suffer a higher degradation than the hybrid type.

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