WATER SORPTION, HARDNESS AND SCANNING ELECTRON MICROSCOPY EVALUATION OF DENTAL COMPOSITE RESINS SUBMITTED TO HIGH-RISK DECAY MODEL AND INTENSIVE TREATMENT WITH FLUORIDE

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ABSTRACT

The aim of this study is to assess through microhardness test, sorption test and morphological analysis, the behavior of composite resin submitted to the influence of pH cycling model and/or topic application of fluoride gels. Samples of microhybrid Z100 (3M), Estelite Σ (J Morita) and nanoparticulated Filtek™ Supreme (3M) composite resins were made. The Digital Scale and Vickers Digital Microhardmeter were employed to verify the water sorption and superficial microhardness, respectively. Samples were submitted to acid challenge and topical fluoride gel application. Other samples were made for the morphological analysis. The resin surface morphology in each phase of this experimental study was observed using scanning electron microscopy (SEM). The ANOVA two-way, ANOVA split plot and Tukey statistical tests were used for the statistical analysis. It was concluded that the type of composite resin can influence the water sorption, microhardness and the degree of morphologic alteration, when submitted to different experimental conditions.

Key words: composite resins, tooth demineralization fluorine compounds, hardness tests, scanning electron microscopy.

ANALISE "IN VITRO" DA SORÇÃO, MICRODUREZA E ANÁLISE EM MICROSCOPIA ELETRÔNICA DE VARREDURA RESINAS COMPOSTAS SUBMETIDAS ALTO RISCO DE CÁRIE E SOB INTENSIVO TRATAMENTO COM FLUORETOS

RESUMO

O objetivo desta pesquisa foi avaliar, através dos testes de microdureza, sorção e análise morfológica o comportamento in vitro de resinas compostas (RCs) submetidas ao desafio ácido e/ou aplicação de soluções fluoretadas. Foram empregadas as RCs: microhíbridas – Z100 (3M), Estelite Σ (J Morita) e nanoparticulada - Filtek™ Supreme (3M), na cor A3 para confecção das amostras. Uma Balança Digital e um Microdurômetro Digital Vickers foram empregados respectivamente, a fim de mensurar a sorção de água e a microdureza superficial. As amostras foram submetidas ao desafio ácido e aplicação tópica de flúor. Para a avaliação morfológica da resina composta novas amostras foram confeccionadas, e para cada fase experimental do estudo a superfície da resina composta foi analisada utilizando um microscópio eletrônico de varredura (MEV). Os testes estatísticos ANOVA two-way, ANOVA split plot e Tukey foram empregados para a avaliação estatística. Concluiu-se que o tipo de resina composta pode influenciar na sorção de água, microdureza e grau de alteração morfológica, quando submetido a diferentes condições experimentais.

Palavras chaves: resinas compostas, desmineralização compostos de flúor, testes de dureza, microscopia eletrônica de varredura.

INTRODUCTION

The development of adhesive materials has meant an important change in restorative philosophy, contributing gradually to the use of composite resin as an alternative to dental amalgam[1], considering the aesthetics, reduced need for preparation and strengthening effect on the remaining tooth achieved by the use of the adhesive technique.

By following a careful adhesive technique and according to the operator's skill, it is possible to make composite resin restorations with satisfactory longevity[2]. Thus, the search for restorations with composite resin has led to an increase in the research and development of new materials, which represent changes in the filler particle size, distribution, orientation and amount incorporated[3,4].

The recently introduced nanoparticle composite resin is gradually becoming a considerable alternative to the widely used microhybrid composite resins[3]. Longitudinal clinical studies reveal acceptable clinical performance of nanofiller resin composites, in stress-bearing posterior cavities[5].

The longevity of restorations using direct adhesive fillings is achieved not only by performing the complex steps correctly, but also by making the patient aware that some foods may stain[6] and degrade composite resin surfaces.

In the oral environment, the microorganisms in dental plaque produce acids such as acetate, propionate and lactate[7], which alter buccal pH. According to the literature[8,9], the surface hardness of composite resins is reduced in acidic solutions.

To reduce the effect of dental demineralization that occurs as a result of bacterial metabolism, fluoride solutions have been used successfully to prevent caries in their initial stages, and are especially recommended against caries in high-risk patients[10]. Studies are found in the literature[11,12] that verify the degradation of restorative resin materials (mainly resin modified glass-ionomer and compomer) when exposed to fluoride solutions.

The surface degradation of aesthetic restorative materials treated with acidulated phosphate fluoride (APF) can influence the color stability[13], and also increase susceptibility to bacterial adhesion. These conditions are observed even in materials that potentially incorporate and release fluoride[14], due to surface disintegration and presence of irregularities[14] that enhance wear and plaque accumulation[15].

Composite resins have different compositions, with varying organic and inorganic components, which in turn involve variations in water sorption depending on the restorative material employed[16].

Material type, storage solution, surface treatment, material surface, and immersion time were significant factors that influenced surface hardness[17]. The need to verify the behavior of association of new composite resins, dynamic demineralization/remineralization cycling and fluoride gels and the absence of experimental design reported in the literature led to the development of this study. Thus, from the data found in the literature, the aim of this study is to evaluate through water sorption analysis, microhardness and morphological pattern using scanning electron microscopy (SEM), the behavior of microhybridic and nanofiller composite resins, under high acid challenge and intensive fluoride treatment. The tested hypothesis is that there are statistical differences between the composite resins tested when evaluated by the sorption analysis, microhardness and morphological pattern, under the abovementioned conditions.

MATERIAL AND METHODS

The composite resins employed for preparing the 90 samples tested in this study are described in Table 1. A single portion of composite resin was placed in a customized stainless steel mould with 5 mm diameter and 2 mm thickness.

Table 1. Composite resins used.

Composite resin	Commercial name manufacturer	Composition
Micro hybrid	Estelite Σ *(J Morita)*	*Organic Matrix:* Bis-GMA and TEGDMA *Filler:* (spherical fillers) Loading 71% by volume. Médium Size: 0.2 μm (0.1-0.3 μm)
	Z 100 *(3M ESPE)*	*Organic Matrix:* Bis-GMA and TEGDMA *Filler:* (irregular fillers) Loading 71% by volume. Maximun Size: 4.5 μm
Nanofiller	Filtek™ Supreme XT *(3M ESPE)*	*Organic Matrix:* Bis-GMA, UDMA, TEGDMA and Bis-EMA *Filler: (*irregular nanofiller) Loading 57,7% by volume *Size:* 20nm clusters of 0.6-1.4/μm

* All the composite resins are composed of zirconia/silica particles.

A glass slide was placed on top of the mould and a second glass slide was placed at the bottom of the mould. A polyester matrix strip was also used between the mould and the glass slide to help its removal.
The materials were cured with halogen light XL 3000 (3M), with intensity of 550 mW/cm^2 according to the curing time specified by the manufactures. After the test specimens were removed from the moulds, they were stored in distilled water at 37 ± 1ºC for 24 h. As the cross-linking reactions continue after the removal of the light source[16-19], the specimens were kept in tightly closed black boxes until the experimental tests. Simulating a high caries challenge, a dynamic demineralization/remineralization cycling model proposed in the literature was employed[20-22]. The samples were stored in these solutions for 10 days, as shown in Table 2.

1. Water Sorption test

The dried samples were weighed (M_i) using digital scales (Mettler Toledo/ AB 2004, Switzerland) and immersed in distilled water for 24h. Then they were dried and weighed again (M_{24}). The pH cycling was applied for 10 days, simulating the demineralization/remineralization cycle. The samples were dried again and the measurements performed ($M_{D/R}$). During this same period, control samples were stored only in distilled water. The water uptake was calculated[23] as described bellow, where FM is the percentage of final mass.

a) Water uptake: water sorption after 24h
$\% FM = [(M_{24} - M_i) \times 100] / Mi$

b) Water uptake: water sorption after pH cycling
$\% FM = [(M_{D/R} - Mi) \times 100] / Mi$

2. Microhardness test

The sample surfaces were divided into 4 quadrants and three indentations were made in each quadrant, using the Digital Vickers (FM – Future Tech) microhardness tester, employing a 50 g load for 30 seconds. The mean values of all 12 measurements of each sample were calculated and finally the mean of each composite resin group was obtained.
The microhardness measurements were taken after the experimental conditions of distilled water storage (24h) and after pH cycling (10d). Five samples were made as a control for all parts of this experimental study.
In order to simulate the intensive treatment carried out on a patient with high-risk decay, topical fluoride (Table 3) was applied for 48 min., simulating a year's preventive treatment. This time (48 min during the year) was calculated following the findings in the literature[24,25]. The values were based on applications of 4 min per session, for 3 weeks, at 3-month intervals over the year.

3. SEM analysis

For the SEM evaluation, another 6 samples were made from each material in order to view the morphological surface of the composite resins in each phase of this experimental study (storage in distilled water, pH cycling and fluorides).
After each experimental step, the samples were cleaned in an ultrasound apparatus (Ultrasonic Cleaner – 1440 D, Ribeirão Preto, SP, Brazil) for 10 min, dried and stored at 37 ±1ºC in the bacterial incubator for 24h. Finally the samples were gold coated using the Denton Vaccum – Desk II (serial number 19539, NJ, USA) and examined by means of scanning electron microscopy (SEM), using 20 KV accelerating voltage.

Table 2. pH- cycling model.

Cycles of demineralization/remineralization		
Solution	**Composition**	**Storage**
Demineralization	[2.2 mM $CaCl_2$, 2.2 mM NaH_2P0_4, 0.05 M acetate buffer, pH regulated to 4.5 with 1M KOH]	6h
Remineralization	[1.5 mM de $CaCl_2$, 0.9 Mm of NaH_2P0_4, 0.15 M of acetate buffer, pH 7.0]	18h

Table 3. Fluoride solutions employed.

Fluoride solution	Commercial name (Manufacturer)	PH (*)	Manufacturer
NaF (neutral) Sodium fluoride	Top Gel	7.0	(Lote) Vigodent S.A (006/05)
APF 1.23% Acidutated phosphate Fluoride	Top Gel Magic Kids	3.6-3.9	Vigodent S.A (002/07)

The SEM photographs (5000x) were rated by blind analysis by two evaluators for extent and type of degradation. Degradation of particles and matrix was rated according to the following criteria: (0) surface intact, particles totally embedded in undisturbed matrix, (1) moderate degradation and (2) severe degradation, little or no matrix around particles, and an excessive number of voids[11].

RESULTS

1. Water sorption analysis

After 24h, the composite resins Estelite Σ (0.33 ± 0.12%); Z100 (0.23 ± 0.10%) and Filtek Supreme (0.18 ± 0.09%) presented mean values that represented water uptake.

The evaluation of mean values for percentage of water uptake (%) (Fig. 1) showed that these values are higher after 10-day pH cycling for all composite resins. The greatest difference in water sorption was observed for nanoparticle (Filtek Supreme: 0.58 ± 0.17%), followed by microhybrid composites with irregular filler (Z100: 0.40 ± 0.12%) and spherical fillers (Estelite Σ: 0.40 ± 0.16%).

The behavior of the control samples (stored only in distilled water) was the same, presenting a similar sorption pattern to the samples submitted to the pH cycling; Estelite Σ (t = - 0.16; gl = 8; p =0.873); Z100 (t = -1.41; gl = 4; p = 0.231); Filtek Supreme (t = 1.58; gl = 7; p = 0.157).

The data were converted to arcsine square root of proportion in order to evaluate the influence of different composite resins and storage environments on the degree of water sorption. These data were estimated using the two-way statistical test Analysis of Variance (the storage environment was the repeated measure) (Table 4).

From the Tukey (5%) statistical test, significant difference was observed between the groups stored in distilled water (24h) and 10-day pH cycling/ distilled water storage. When the composite interaction with the storage condition was evaluated, Filtek Supreme was different from Estelite Σ and Z100 ($p < 0.05$). However, Estelite Σ and Z100 did not differ, regardless of the environment or time of storage.

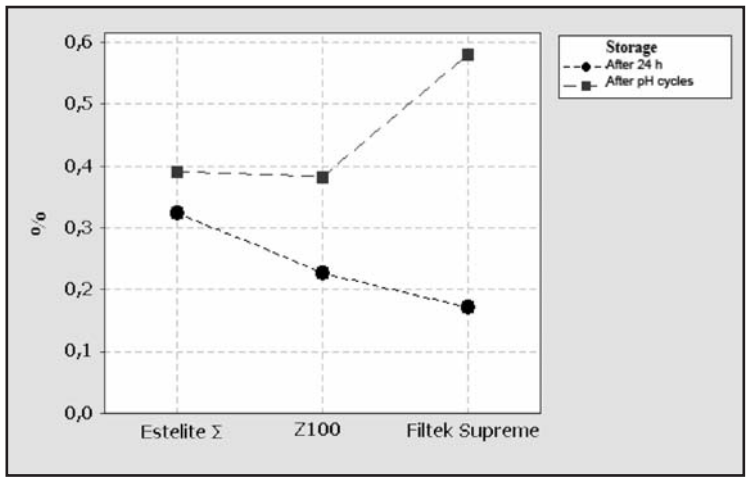

Fig. 1: Mean values of water sorption (% GP) for the composite resins and under two different storage conditions.

2. Microhardness analysis

Statistical differences were found among the mean microhardness values of the composites (Estelite Σ: 38.35± 2.58HV; Filtek Supreme: 60.72±1.57HV and Z100: 96.40±1.71HV). (Table 5).

Considering only the influence of pH cycling, no statistical difference was found between distilled water and de-remineralization storage.

Table 4. ANOVA test for the weight increases due to water sorption (%).

Effect	gl	SQ	QM	F	p
Composite Resin (CR)	2	0.0000111	0.0000055	2.88	0.0643
Storage (S)	1	0.0001334	0.0001334	92.21	0.0001*
CRxS	2	0.0000634	0.0000317	21.90	0.0001*
Residual	57	0.0000825	0.0000014		
Total	***119***	***0.0004001***			

* p<0.05

Table 5. ANOVA 2 factors test for the microhardness data.

Effect	gl	SQ	QM	F	p
Composite Resin (CR)	2	68575.3	34287.7	4761.56	0.001*
Storage	1	5.2	5.2	1.78	0.186
CR x storage	2	14.3	7.2	2.44	0.093
Residual	87	254.7	2.9		
Total	***119***	***69044.0***			

* p<0.05

Table 6. ANOVA split-plot test.

Effect	gl	SQ	QM	F	p
Composite Resin (CR)	2	29482.0	14741.0	5570.43	0.0001*
Fluoride	1	299.3	299.3	91.72	0.0001*
CR x storage	2	138.6	69.3	21.24	0.0001*
Residual	27	88.1	3.3		
Total	***59***	***30133.3***			

* p<0.05

2.1. Influence of Fluoride Solutions

The fluoride solutions caused a significant reduction in the composite resin microhardness values (Table 6).

The ANOVA split-plot test revealed significant statistical difference between the interaction effect (composite/ storage) and fluoride solutions.

Using the Tukey (5%) statistical test, there were significant differences between microhardness values of composite resins after topical application of **Neutral Fluoride** (Estelite Σ: 37.40±2.63HV; Filtek Supreme: 58.60±1.65HV and Z100: 94.70±1.70HV) and **APF** (Estelite Σ: 35.70±1.89 HV; Filtek Supreme: 55.60± 1.90 HV and Z100: 86.00±2.00 HV), except for the Estelite Σ results (Fig. 2).

3. SEM analysis

The morphological analysis of composites illustrates the values found in the microhardness test. The images show slight alteration after pH cycling (Fig. 3-5). The APF gel caused a higher surface degradation pattern for Z100 (Fig. 4) and Filtek Supreme (Fig. 5) than for Estelite Σ (Fig. 3).

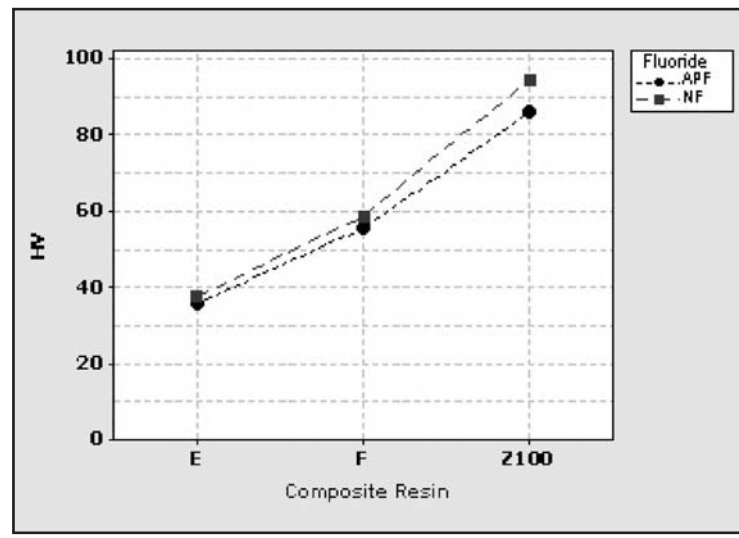

Fig. 2: Mean value alterations of Vickers Hardness (HV) of composite resins with the variable fluoride solution (NF and APF).

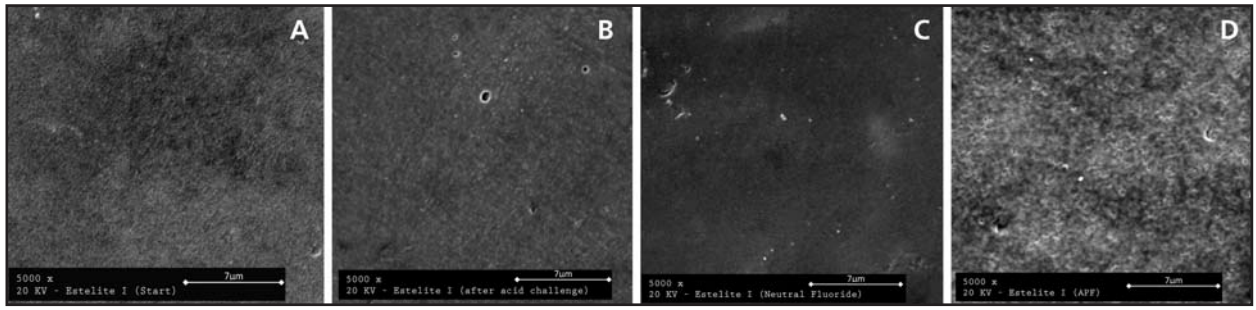

Fig. 3: SEM visualization of microhybrid composite surface - Estelite Σ (spherical filler) after the experimental conditions.

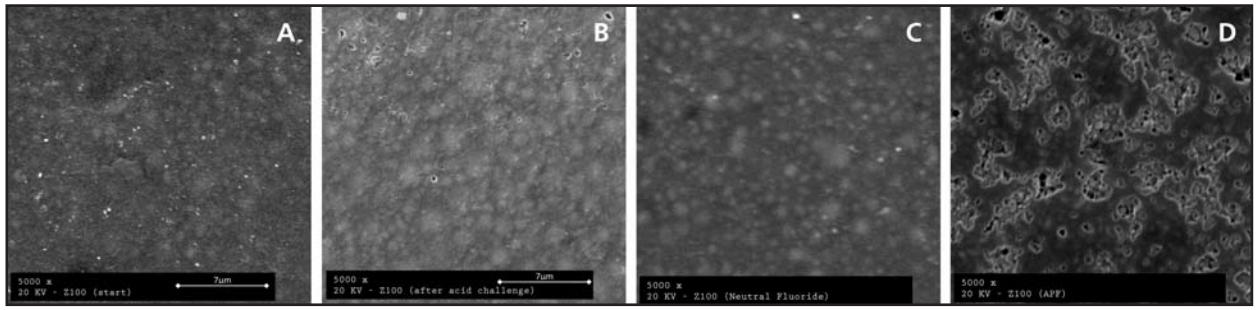

Fig. 4: SEM visualization of microhybrid composite surface – Z100 (irregular filler) after the experimental conditions.

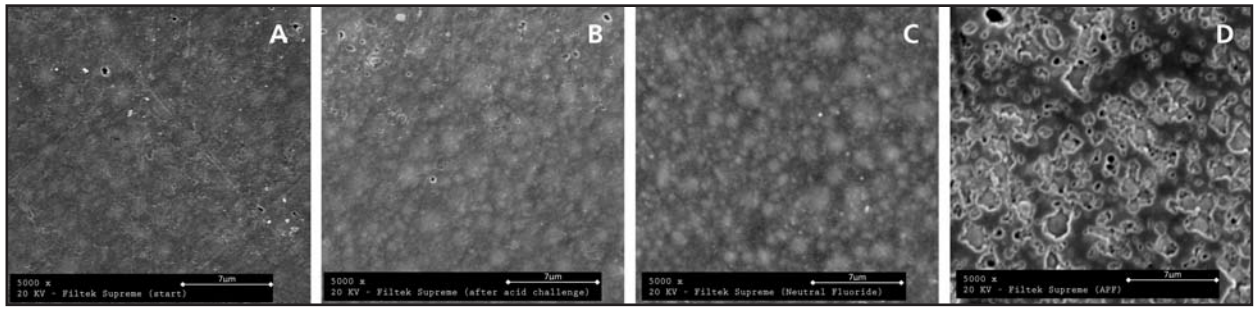

Fig. 5: SEM visualization of nanoparticle composite surface - Filtek Supreme after the experimental conditions.

DISCUSSION

Considering that composite resin is a widely used restorative material, laboratory tests simulating the conditions of the oral cavity are needed to test its behavior in this environment. With this aim, this study evaluated different kinds of composite resins regarding water sorption, acid challenge and influence of fluoride solution, using weight, microhardness and scanning electron microscopy.

From the commercially available composite resins, the microhybrid composite with irregular fillers (4.5 μm) (Z100), microhybrid composite with spherical fillers (0.1-0.3 μm) (Estelite Σ) and the nanohybrid with 20 nm-nanofillers forming clusters with particle size ranging from 0.6 to 1.4 microns (Filtek Supreme) were analyzed. All of these composite resins contain silica/zircon inorganic fillers, being different because of their quantity, shape and size of inorganic particles.

The samples were stored in distilled water for 24h, because according to Swartz et al.[18] high polymerization is reached after this time. Atmadja and Bryant[19] reported that composites continued to polymerize after removal of the light source, and differences between one day and one week were not statistically significant. Water sorption was the first factor to be analysed for an initial understanding of the behavior of composite resins. In this regard, Örtengren et al.[26] reported that chemical reactions between filler particles and water can

result in an increase in the mass of the dental composite material. Mayworn et al.[27] observed that artificial saliva storage increases the composite resin wear resistance, suggesting that composites bulk post-cure takes place and saliva absorption occurs only on the surface of the composites. This effect was confirmed by the authors by comparing the Vickers hardness and Fourier transforming infrared spectroscopy analyses before and after artificial saliva treatment.

The nanoparticle composite resin (Filtek Supreme) was the material that had the lowest water sorption after 24h and the highest water sorption after 10 days in distilled water or in the acid challenge solution. It had the greatest difference between the sorption values (Fig. 1). The microhybrid composite resin (Estelite Σ), formed of spherical fillers, showed the smallest difference between the sorption values after 24h and 10d storage in distilled water or in the acid challenge solution.

Earlier research has shown that the inorganic[16,28] and organic components[16,26] can promote alterations in the level of water sorption and solubility of composite resins. Leakage of filler elements from cured composite materials after storage in water is also observed[28].

The interaction of composite resins according to the environment/ time of storage showed significant statistical differences for sorption (Table 4). Nanoparticle composite resin (Filtek Supreme) has nanofiller clusters, representing approximately 57.7% by volume of inorganic filler (Table 1), consequently it presented the highest values of final sorption, in relation to microhybrid composites tested (Estelite Σ and Z100), which have 71% by volume of inorganic filler in their composition.

Knowing that each composite resin has its own sorption pattern[16,26], their surface microhardness and morphological pattern were also evaluated, as their particles are of different sizes and shapes.

The mean microhardness values were different according to the particular characteristics of each material, thus, the highest Vickers hardness was observed for Z100 (95.70HV ± 1.70) followed by Filtek Supreme (60.70HV ± 0.95) and Estelite Σ (38.70HV ± 3.71) composite resins. Although Filtek Supreme is formed by nanoparticles and has 57.7% inorganic filler by volume, its hardness values are higher than those of Estelite Σ microhybrid composite (71% inorganic filler by volume). Thus, one hypothesis could be that as nanoparticle composite resins have used nanotechnology in their development, allowing the formation of clusters of nanofillers, the final result is filler clusters of 0.6 – 1.4 μm, which is greater than the size of the spherical particles (0.1-0.3 μm) in the Estelite Σ composite resin. According to Leinfelder and Lemons[29], to increase the filler quantity, the manufacturer can employ special processes that agglomerate microparticles. Altering the filler concentration can change many physical and mechanical properties[4,29], such us hardness, abrasion resistance, traction, coefficient of thermal linear expansion, water sorption and shrinkage polymerization. Scougall-Vilchis et al.[4] also found that the ultrastructure, size of filler particles, volume/weight fraction of filler and chemical composition of the composite resins had an effect on Vickers hardness.

A demineralization/remineralization cycling model was used in this study as this process often occurs in the oral cavity. Koulourides and Housch[30], state that the health-disease concept for the caries process is that enamel in a state of losing mineral is on the way to decay, while enamel gaining mineral is in a state of recovery. The microhardness change is an indicator of the balance between these two states. However, the mineral changes that occur in the composite resin under the demineralization/ remineralization process are different from those that occur in dental structures.

If solutions such as tea, cola, and coffee can significantly affect the surface hardness of composite resins[17], the de-remineralization process that occurs frequently in high-risk decay patients can alter the different kinds of composites in different ways. Featherstone et al[21] applied an *in situ* demineralization/remineralization cycling model simulating a high caries challenge, from *in vivo* and *in vitro* studies of demineralization/remineralization patterns. The samples from our study were submitted to this system of pH cycling, which simulates the *in vivo* situation better[20], by using demineralizing solution (pH 4.3-4.7) and remineralizing solution (pH 7.0), according to Ten Cate and Duijsters[20], Featherstone et al.[21] and Serra and Cury[22].

The demineralizing (pH 4.3-4.7) and remineralizing (pH 7.0) solution used in this study, did not promote significant alterations of hardness values, in agreement with Lund et al.[31], although they employed different composite resins. In addition,

the SEM analysis also confirmed the absence of significant difference in the morphology of composite resin after simulation of high-risk challenge.

Because of the proven efficacy of fluoride solutions to control or prevent dental caries[10], the samples were also submitted to intensive topical fluoride application for 48 minutes, simulating a preventive treatment during a year for a high-risk decay patient[24,25].

The storage time of samples in the fluoride solutions varies widely in the literature. While Yap and Mok[32] used 36 hours of storage in fluoride solution, Tanoue et al.[13], kept the composite resins for 32 min in APF, in order to evaluate their stability. Papagiannoulis et al.[33] simulated a 4-year period by applying the APF by storing the composite resin in fluoride solution for 8, 24 and 1440 min.

Papagiannoulis et al.[33] verified that many fluorides affect the morphological characteristics and composition of composites. The pattern of alteration depends on the kind of fluoride solution[33,34] and the restorative material employed[33,35,36]. Yeh et al.[36] analyzed nanocomposites and microhybrid composites treated with fluoride gels and observed that two kinds of acidulated phosphate fluoride gels (Topex and Zap) did not cause surface changes of composite resins, the possible reason being ascribed to the presence of magnesium aluminum silicate clays. In contrast, in the same study *60 Second Taste Fluoride* Gel caused significant roughness increase, microhardness decrease, more prominent filler dissolution, and infrared spectral changes.

This study used NaF (neutral fluoride) gel pH 7.0, which seems to be the least aggressive[11,12,34] to the restorative materials, and the APF gel, pH 3.6-3.9, which according to Delbem and Cury[37] was more efficient than NaF gel in enamel fluoride uptake.

When the APF and neutral fluoride were compared, APF has more influence on the decrease of surface hardness of restorative materials[32]. A reduction in hardness values in these materials was observed, even when neutral fluoride was used, but this effect is less evident compared to the APF[32] and also when the surface of these materials is analysed by SEM[38].

The topical application of APF gel showed considerable degradation of surface and a significant reduction of hardness values for all composite resins studied. A factor that could explain the influence of APF gel on composite resins, would be that APF pH ranges between 3.6-3.9, a pH value which is lower than that found in the demineralizing solution (pH 4.5-4.7) used in this study. Another hypothesis that could be considered is the ability of fluoride to attack the inorganic components (silica-zirconia) of resins. Soeno et al.[39] and Soeno et al.[40] verified that macro-inorganic fillers demonstrated more noticeable etched patterns generated by the APF solution than the microfilled composites.

Studying the influence of APF on the surface of composites, Kula et al.[35] stated that the degree of change in surface is apparently related to the size and type of filler particle in the composite. As observed by Yeh et al.[36], Estelite Sigma (microhybrid composite) was less affected by acidulated and neutral fluoride gels for the nanocomposites studied.

CONCLUSION

Based on the findings of this study it was possible to conclude that there is statistically significant evidence that water sorption varies according to the size, shape and concentration of inorganic filler in the composite resins. The hypothesis that the superficial microhardness and morphological pattern of composites alter after pH-cycling model was rejected, and the hypothesis for the topical application of fluoride solutions on composites was accepted. The fluoride gels caused superficial microhardness reduction on Z100 (microhybrid) and Filtek Supreme (nanoparticle), presenting severe surface degradation, with the most discrepant values for the materials treated with APF, therefore accepting the hypothesis presented.

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