

# Effect of pH Cycling Followed by Simulated Toothbrushing on the Surface Roughness and Bacterial Adhesion of Bulk-fill Composite Resins

DC Somacal • FB Manfroi • MSG Monteiro • SD Oliveira  
HR Bittencourt • GA Borges • AM Spohr

## Clinical Relevance

Toothbrushing slightly increases the surface degradation of conventional and bulk-fill resins initially caused by a cariogenic challenge. However, these superficial changes do not suggest significant differences in bacterial adhesion on the composite resin surfaces.

## SUMMARY

The aim was to evaluate, *in vitro*, quantitatively and qualitatively, the effect of pH cycling and simulated toothbrushing on surface roughness (Ra) and bacterial adhesion (Cn) of bulk-fill composite resins. Thirty specimens of each composite resin, 5 mm wide and 4 mm high, were obtained: group 1 (control): Filtek Z250 (Z250); group 2: Filtek Bulk-Fill (FTK);

group 3: Tetric N-Ceram Bulk-Fill (TTC); and group 4: Aura Bulk-Fill (AUR). After 24 hours, the specimens were polished and then alternated with demineralization/remineralization solutions for 15 cycles of 24 hours each at 37°C. Then the specimens were submitted to simulated toothbrushing. The Ra and Cn measurements were quantitatively analyzed in three stages: after polishing (Ra0 and Cn0), after pH

Deise Caren Somacal, DDS, MS, Department of Restorative Dentistry, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Brazil

Fernanda Borguetti Manfroi, DDS, MS, PhD, Department of Restorative Dentistry, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Brazil

Marina Silveira Gregis Monteiro, graduate student, Department of Immunology and Microbiology, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Brazil

Sílvia Dias de Oliveira, MS, PhD, Department of Immunology and Microbiology, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Brazil

Hélio Radke Bittencourt, MS, PhD, Department of Statistics, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Brazil

Gilberto Antonio Borges, DDS, MS, PhD, Department of Restorative Dentistry, Uberaba University, Uberaba, Brazil

\*Ana Maria Spohr, PhD, Department of Dental Materials, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, Brazil

\*Corresponding author: Avenida Ipiranga, 6681, Porto Alegre, Rio Grande do Sul 90619-900, Brazil; e-mail: anaspohr@terra.com.br

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cycling (Ra1 and Cn1), and after simulated toothbrushing (Ra2 and Cn2). The Ra values were submitted to two-way analysis of variance, followed by the Tukey test ( $\alpha=0.05$ ). The Kruskal-Wallis test, followed by multiple comparisons, was applied for Cn analysis. Surface topography and bacterial adhesion were observed by scanning electron microscopy (SEM). Z250, FTK, and TTC showed no significant change in Ra regardless of the treatment performed; AUR obtained increased Ra at Ra2 ( $p<0.05$ ). FTK differed from the others at Cn0 and Cn1 ( $p<0.05$ ). At Cn2, there was no difference among the composite resins. SEM images showed the exposure of fillers and microcavities at Ra1 and Ra2. There was greater bacterial adhesion at Cn1 for Z250 and FTK. It was concluded that the pH cycling caused surface degradation of all composite resins, which was potentiated by simulated toothbrushing. However, only AUR presented an increased Ra. Bacterial adhesion occurred on all composite resins after pH cycling; however, after simulated toothbrushing, adhesion of dispersed bacteria was similar for all the composite resin groups.

## INTRODUCTION

Composite resins are widely used in restorative dentistry to restore tooth structure lost due to carious lesions, trauma, or abrasive processes and for aesthetic reasons.<sup>1</sup> The desire to facilitate clinical practice resulted in bulk-fill composite resins. These materials are suitable for insertion in a 4- or 5-mm bulk placement, depending on the brand, without prolonged polymerization time. Therefore, bulk-fill composite resins eliminate incremental techniques and potentially reduce the amount of work and clinical steps required.<sup>2</sup>

One of the factors related to the success of composite resin restoration is the capacity of the material to be resistant to degradation in the oral environment.<sup>3</sup> An imbalance between the demineralization and remineralization process, associated with the presence of cariogenic bacteria, salivary dysfunction, and ingestion of fermentable carbohydrates, favor the occurrence of dental caries.<sup>4</sup> These chemical characteristics of the oral cavity may influence the properties of restorative materials.<sup>5,6</sup>

The study of surface degradation of the materials is important since it can facilitate the accumulation of bacterial plaque.<sup>7</sup> An association between surface roughness and bacterial adhesion was found to exist

for resinous materials.<sup>8</sup> Greater bacterial adhesion was observed in direct composite resin restorations when compared with indirect composite resin restorations<sup>9</sup> and glass-ionomer cements.<sup>10</sup> This susceptibility increases the patient's risk of secondary caries adjacent to the restoration margins in addition to the formation of biofilm, which contributes to the chemical and mechanical degradation of composite resin restorations.<sup>11</sup>

Consequently, there is a necessity to evaluate restorative materials under the challenging chemical conditions that occur in the oral cavity, such as pH cycling. In addition, it is important to evaluate pH cycling in association with the mechanical process of toothbrushing since it is a common daily oral hygiene habit.<sup>12</sup> *In vitro* studies used the pH-cycling model to mimic the effect of oral conditions on the surface of restorative materials.<sup>13-17</sup> This model is characterized by the dynamics between periods of demineralization and remineralization, simulating a cariogenic challenge<sup>17,18</sup> proposed for laboratory studies by Featherstone and others<sup>19</sup> and modified by Serra and Cury.<sup>20</sup> On this research line, studies examined restorative materials such as glass ionomers and polyacid-modified resins throughout the pH cycling.<sup>13,15,16</sup> Other studies submitted composite resins to pH cycling and analyzed their effect on the materials' surface after simulated toothbrushing.<sup>14,17</sup> Modification of surface roughness occurred when composite resin was submerged in different acidic solutions, with pH values ranging between 6.6 and 2.5.<sup>21</sup> However, no studies were found in the consulted literature regarding the effect of pH cycling followed by simulated toothbrushing on bulk-fill composite resins.

Therefore, this study aimed to evaluate the effect of pH cycling on surface roughness and biofilm formation of three bulk-fill composite resins in comparison with a conventional composite resin, with and without simulated toothbrushing. Complementary analysis using scanning electron microscopy (SEM) to evaluate surface topography and biofilm formation was also performed. The study was conducted under the following null hypotheses: pH cycling followed by simulated toothbrushing does not significantly influence 1) surface roughness or 2) biofilm formation on the composite resins.

## METHODS AND MATERIALS

### Obtaining Composite Resin Specimens

Three bulk-fill composite resins and a conventional composite resin were used (Table 1). The composite

Table 1: Composite Resins Used in the Study

Material/Shade	Filler Content (% Wt/Vol)	Organic Matrix	Manufacturer	Lot Number
Filtek Z250, Microhybrid/A2 (Z250)	Zirconia/silica, 0.01 $\mu\text{m}$ to 3.50 $\mu\text{m}$ (84.5/60)	TEGDMA, Bis-GMA, UDMA, Bis-EMA	3M ESPE (St Paul, MN, USA)	1628100449
Filtek Bulk Fill, Nanofiller/A2 (FTK)	Zirconia, 4 to 11 nm; silica, 20 nm; ytterbium fluoride, 100 nm (76.5/58.4)	AUDMA, AFM, DDDMA, UDMA	3M ESPE	1632700708
Tetric N-Ceram Bulk Fill, Nanohybrid/IVB (TTC)	Barium/silica/aluminum; ytterbium fluoride, 0.04 to 3 $\mu\text{m}$ , (77/55)	Bis-GMA, UDMA, Bis-EMA	Ivoclar Vivadent (Schaan, Liechtenstein)	U27917
Aura Bulk Fill, Nanohybrid/Universal (AUR)	Barium alumino-borosilicate and silica (65/81)	UDMA, Bis-EMA, Bis-GMA, TEGDMA	SDI (Bayswater, Australia)	150931

Abbreviations: TEGDMA, triethyleneglycol dimethacrylate; Bis-GMA, bisphenol-A-glycidyl methacrylate; UDMA, urethane dimethacrylate; Bis-EMA, ethoxylated bisphenol-A-dimethacrylate; AUDMA, high-molecular-weight aromatic dimethacrylate; AFM, addition-fragmentation monomers; DDDMA, 1,12-dodecanediol dimethacrylate.

resin specimens were made using a silicone matrix with orifices of 5 mm in diameter and 4 mm in height. Thirty-three specimens were obtained for each composite resin: group 1 (control) Z250 (Z250); group 2: Filtek Bulk Fill (FTK); group 3: Tetric N-Ceram Bulk Fill (TTC); and group 4: Aura Bulk Fill (AUR). The matrix was positioned on a glass plate and filled with composite resin. The Z250 composite resin was inserted in two increments of approximately 2-mm thickness. The FTK, TTC, and AUR composite resins were each inserted in one increment of 4-mm thickness. A polyester strip was placed on each composite resin, followed by a glass plate, in order to obtain a flat surface. The composite resin increments were light cured using the LED light unit Radium-cal (SDI, Bayswater, Australia) for 20 seconds at a distance of 1 mm from the specimen surface. The light intensity was 1000 mW/cm<sup>2</sup> and monitored by a radiometer (Model 100 Demetron, St Louis, MO, USA).

The composite resin surface in contact with the polyester strip was finished with polishing discs (Sof-Lex Pop On, 3M ESPE, St Paul, MN, USA) of medium, fine, and superfine grain. Each disc was applied for 15 seconds and by only one operator. After polishing, the specimens were ultrasonically cleaned in distilled water for 10 minutes and then stored in distilled water at 37°C for 24 hours.

### Surface Roughness Test

The initial surface roughness (Ra0) of the specimens in each group (n=15) was measured with a roughness tester SL-201 (Mitutoyo SurfTest Analyzer, Tokyo, Japan). Three consecutive measurements of the specimen were taken in different regions (one

central, one right, and one left), with a cutoff of 0.25. The mean values of roughness (Ra, in  $\mu\text{m}$ ) were obtained for each specimen.

### pH Cycling

Each specimen alternated with the cycles of demineralization and remineralization solutions simulating cariogenic alteration. The cariogenic alteration was composed of 15 cycles, and each cycle consisted of immersing the specimens in the demineralization solution for six hours and subsequently immersing them in the remineralization solution for 18 hours. Between one solution and the other, the specimens were washed with deionized water for one minute. Each specimen was immersed in 10 mL of the above-mentioned solutions, based on the methodology applied by Valinoti and others.<sup>22</sup> At the end of the pH cycles, the specimens were subjected to a new surface roughness reading (Ra1).

### Simulated Toothbrushing

A simulated toothbrushing machine, developed by the Idea Institute of the University, was used for this study. Each specimen was fixed in the center (orifice) of an acrylic plate (55×25×4 mm), enabling the specimen to remain 1 mm beyond the edge of the orifice that housed the specimen. Utility wax was applied to fix the specimens. Each plate was placed in an acrylic tank that was attached to the brushing machine. The acrylic tank was filled with a mixture composed of 1 g of toothpaste (Colgate Total 12, Colgate-Palmolive, São Bernardo do Campo, Brazil) per 1 mL of distilled water. Soft bristle Classic Colgate toothbrushes (Colgate-Palmolive) were used, and a load of 200g was applied. The speed of

brushing was 250 cycles per minute, carried out in 10,000 cycles of simulated toothbrushing. The toothbrushes were changed after every four brushed specimens from each group. After the brushing cycle, the specimens were washed in running water and ultrasonically cleaned in distilled water for 10 minutes, followed by drying with compressed air. The roughness of the surface was measured again (Ra2). The surface roughness reading was perpendicular to the brushing direction of the toothbrush bristles. For the correct positioning of the specimen in the brushing machine and to ensure repeatability in the same direction (perpendicular to the brushing), a mark with a diamond bur and high-speed hand piece was made on the border of each specimen.

### Surface Topography Analysis by SEM

Three specimens (n=3) from each composite resin were used. Of the three specimens, one was analyzed by SEM after polishing, the second after pH cycling, and the third after pH cycling and simulated toothbrushing. The specimens were dried in a dehumidifier with silica gel for 72 hours, fixed with double-sided carbon adhesive tape (SPI, West Chester, PA, USA), and the top surface sputter coated with gold (Balzers, Balzers, Liechtenstein) before SEM observation (JSM 6060, JEOL, Tokyo, Japan) under 20,000× magnification.

### Biofilm Analysis

The specimens of composite resin were sterilized with ethylene oxide gas (ETR Sterilizer, Porto Alegre, Brazil). The colony-forming units (CFU/mL) were evaluated in triplicate for each group (n=9) after the following treatments: polishing (Cn0), pH cycling (Cn1), and pH cycling and simulated toothbrushing (Cn2). Biofilm adhesion was qualitatively evaluated by SEM in duplicates for each group (n=6) after the following treatments: polishing, pH cycling, and pH cycling and simulated toothbrushing. Polishing, pH cycling, and toothbrushing of the specimens were performed according to the procedures described above.

*Streptococcus mutans* ATCC 25175, stored at -20°C, was obtained and cultivated in BHI (brain-heart infusion) broth for 24 hours at 37°C in a bacteriological incubator. An aliquot of this primary culture was inoculated into a fresh BHI broth and incubated for an additional 24 hours at 37°C. Subsequently, 100 µL of this culture, containing approximately 10<sup>6</sup> CFU/mL, were added to 1 mL of BHI broth supplemented with 1% sucrose. The composite resin specimen was then placed in the

culture and incubated for 24 hours under microaerophilic conditions at 37°C. The specimens were carefully removed from the culture and washed in 1 mL of 0.85% saline solution, removing the planktonic cells and keeping only the cells adhered to the surface of the specimens; this process was performed twice.

**Bacterial Counts**—After the washes, the specimens were placed in the ultrasonic bath for 10 minutes in order to disintegrate the biofilm. Subsequently, the saline solution containing the disaggregated cells was diluted to 10<sup>-6</sup>. The bacterial counts were determined spotting 10 µL of the first three dilutions and spreading 100 µL of the last three on BHI agar in triplicate. These two techniques were used according to the expected cellular concentrations at each dilution. The BHI agar plates were incubated at 37°C for 24 hours, followed by CFU/mL count. The assays were adapted from Nakamura and others<sup>23</sup> and Yoshihara and others.<sup>24</sup>

**Biofilm Analysis by SEM**—The specimens were immersed in the fixative solution (2.5% glutaraldehyde), where they were kept for seven days. After fixation, the specimens were washed three times for 30 minutes each in 0.2 mol/L phosphate buffer and distilled water in a ratio of 1:1. The specimens were then dehydrated by immersion in 30%, 50%, 70%, 90%, and 100% ethanol and dried in a silica gel dehumidifier for 72 hours. Surfaces were sputter coated with gold (Balzers) and observed by SEM (JSM 6060 LV, JEOL) under 500× to 2000× magnification. Qualitative analysis was started with the smaller magnification (500×), selecting areas with a higher concentration of bacterial biofilm.

For image assessment, a single observer, blinded to the identities of the experimental groups, classified them according to the presence of bacteria. Using PowerPoint (Microsoft Corp, Redmond, WA, USA), each image was placed on a slide and presented on a computer in the form of a presentation. The images were arranged in order according to the level of contamination found so that the first was the least contaminated and the last was the most contaminated. The image occupying the middle position in each group was then selected.<sup>25</sup>

### Statistical Analysis

Surface roughness data were analyzed by two-way repeated measures analysis of variance (ANOVA) (material×treatment), followed by the Tukey test. The CFU/mL count was analyzed by the Kruskal-Wallis nonparametric test, followed by multiple

Table 2: Surface Roughness (Mean  $\pm$  Standard Deviation) of the Composite Resins After the Different Treatments<sup>a</sup>

Material	Surface Roughness, $\mu\text{m}$		
	Ra0	Ra1	Ra2
Z250	0.15 $\pm$ 0.07 aA	0.15 $\pm$ 0.06 Aa	0.17 $\pm$ 0.05 aA
FTK	0.17 $\pm$ 0.08 aA	0.15 $\pm$ 0.09 aA	0.18 $\pm$ 0.05 aA
TTC	0.15 $\pm$ 0.06 aA	0.19 $\pm$ 0.06 aA	0.20 $\pm$ 0.06 aA
AUR	0.19 $\pm$ 0.08 aA	0.19 $\pm$ 0.08 aA	0.27 $\pm$ 0.11 bB

Abbreviations: Ra0, after polishing; Ra1, after pH cycling; Ra2, after pH cycling followed by toothbrushing.  
<sup>a</sup> Means followed by different lowercase and uppercase letters represent significant differences according to the Tukey test ( $\alpha=0.05$ ).

comparisons. The significance level was 5%. The software used was SPSS 10.0 (SPSS Inc, Chicago, IL, USA).

## RESULTS

### Surface Roughness

According to the two-way ANOVA, the material factor ( $p=0.016$ ) and treatment factor ( $p=0.001$ ) were significant. The interaction between the factors was not significant ( $p=0.265$ ) (Table 2).

The four composite resins did not differ significantly from each other at Ra0 and Ra1. At Ra2, AUR obtained significantly higher surface roughness than the other composite resins that was also higher than at Ra0 and Ra1 ( $p<0.05$ ).

### Surface Topography Analysis by SEM

The polished surfaces of the composite resins Z250, FTK, TTC, and AUR are shown in Figure 1 (A1 and B1) and Figure 2 (C1 and D1), respectively. After pH cycles, there was removal of the organic matrix and exposure of the fillers (black arrows), especially on the surface of Z250 (Figure 1A2), TTC (Figure 2C2), and AUR (Figure 2D2), in which the fillers became more evident compared to the polished surfaces. It is also possible to observe cracks (circles) and microcavities (white arrows) on the surface of all bulk-fill composite resins (Figures 1B2, 2C2, 2D2). After pH cycles followed by simulated toothbrushing, greater degradation of the organic matrix occurred in all composite resins, and the filler exposure and microcavities were more evident (Figures 1A3, 1B3, 2C3, 2D3).

### Bacterial Counts

According to the Kruskal-Wallis analysis, followed by multiple comparisons, there were higher bacterial counts for FTK in relation to those for Z250, TTC,

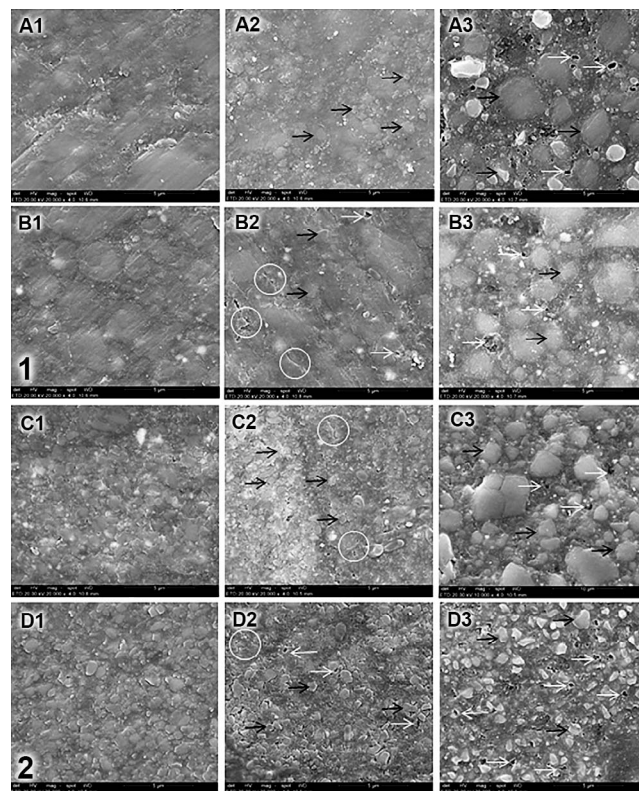


Figure 1. Scanning electron microscopic images (20,000 $\times$ ) of Filtek Z250 (A) and Filtek Bulk Fill (B) after polishing (1), after pH cycling (2), and after pH cycling and simulated toothbrushing (3). Circles, cracks; black arrows, fillers exposed; white arrows, microcavities.

Figure 2. Scanning electron microscopic images (20,000 $\times$ ) of Tetric N-Ceram Bulk Fill (C) and Aura Bulk Fill (D) after polishing (1), after pH cycling (2), and after pH cycling and simulated toothbrushing (3). Circles, cracks; black arrows, fillers exposed; white arrows, microcavities.

and AUR at Cn0 and Cn1 (Table 3). At Cn2, there were no significant differences in bacterial counts among the composite resins.

At Cn1, there was a significantly greater bacterial count for Z250 than at Cn0, followed by a significant decrease at Cn2. For FTK, there was a significantly decreased bacterial count at Cn2 than at Cn1. Bacterial counts for TTC and AUR did not differ significantly at Cn0, Cn1, or Cn2.

### Biofilm Analysis by SEM

After polishing, dispersed bacteria were present (white arrows) on all composite resins and agglomerate formation (black arrows) on Z250 (Figure 3A1) and AUR (Figure 4D1).

After pH cycles, there was greater bacterial adhesion, with the formation of multiple agglomerates on Z250 (Figure 3A2), FTK (Figure 3B2), and

Table 3: Median Values of Bacteria Count of the Composite Resins After the Different Treatments<sup>a</sup>

Material	Bacterial Count, CFU/mL10 <sup>6</sup>		
	Cn0	Cn1	Cn2
Z250	7.00 aA	14.00 aB	1.80 aA
FTK	31.60 bBC	39.00 bC	12.70 aAB
TTC	0.60 aA	3.50 aA	2.30 aA
AUR	2.00aA	2.60aA	2.00aA

Abbreviations: Ra0, after polishing; Ra1, after pH cycling; Ra2, after pH cycling followed by toothbrushing.  
<sup>a</sup> Medians followed by the same lowercase and uppercase letters do not differ significantly by the multiple comparison test.

AUR (Figure 4D2). There were dispersed bacteria on TTC (Figure 4C2).

Bacterial adhesion was less after simulated toothbrushing on Z250 (Figure 3A3), FTK (Figure 3B3), and AUR (Figure 4D3) than in the images obtained after pH cycles. There were dispersed bacteria on Z250, FTK, and TTC, while some bacterial adhesion remained on the surfaces of AUR (Figure 4D3).

## DISCUSSION

The present study evaluated, qualitatively and quantitatively, the effect of pH cycling, followed by simulated toothbrushing, on surface changes and the bacterial adhesion of bulk-fill composite resins.

After pH cycling, which simulates cariogenic challenge, the SEM images showed modifications on the surface topography of the composite resins. Filler exposure, cracks, and microcavities occurred on the composite resins, with different intensities among the materials. These findings are related mainly to degradation of the organic matrix by the action of the cariogenic challenge solutions.<sup>26</sup>

The water in the solutions penetrates the polymer structure and chemically degrades the polymer, forming oligomers and monomers. The progressive degradation of this matrix leads to the formation of pores through which oligomers and monomers are released. In addition, there is degradation of the organic matrix by the acidic pH of the demineralization solution. Catalization of the ester groups of the dimethacrylates occurs, favoring the hydrolysis of these groups and formation of molecules of carboxylic acid and alcohol, which accelerate the degradation of the composite resin.<sup>26</sup> As a consequence, there is exposure of the fillers.

Absorption of the water in the solutions causes an increase in osmotic pressure at the organic matrix/filler interface, favoring formation of cracks on the

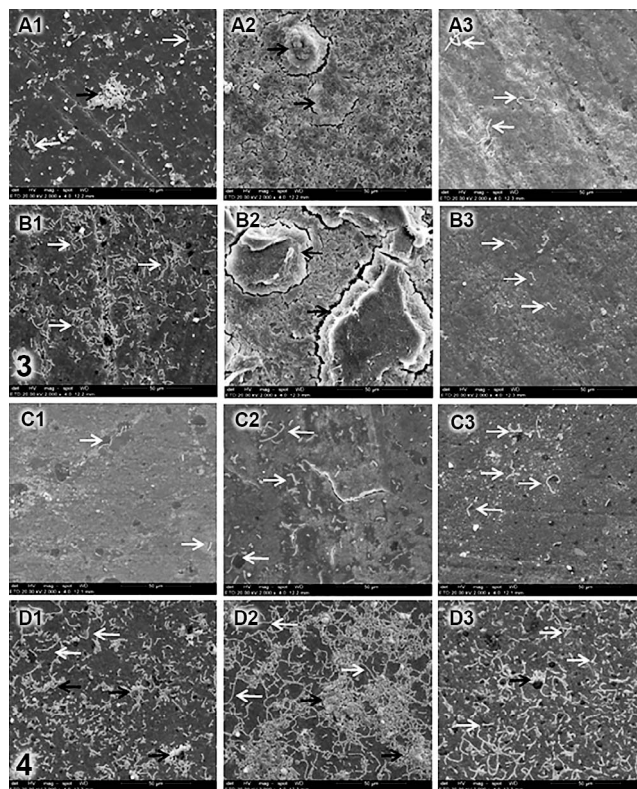


Figure 3. Scanning electron microscopic images (2,000 $\times$ ) of biofilm on Filtek Z250 (A) and Filtek Bulk Fill (B) after polishing (1), after pH cycling (2), and after pH cycling and simulated toothbrushing (3). White arrows, dispersed bacteria; black arrows, agglomerates of bacteria.

Figure 4. Scanning electron microscopic images (2,000 $\times$ ) of biofilm on Tetric N-Ceram Bulk Fill (C) and Aura Bulk Fill (D) after polishing (1), after pH cycling (2), and after pH cycling and simulated toothbrushing (3). White arrows, dispersed bacteria; black arrows, agglomerates of bacteria.

surface of the composite resins.<sup>27,28</sup> This absorption depends on the composition of the organic matrix and the quality of the bond between the organic matrix and fillers.<sup>29</sup> These cracks were also detected in another study.<sup>27</sup> Moreover, water absorption can generate hydrolytic degradation of the silane, favoring detachment of the fillers from the organic matrix and, consequently, the formation of microcavities.<sup>28</sup> Microcavities were also observed in another study that evaluated nanofiller and hybrid composite resins in contact with low-pH solutions.<sup>30</sup> According to Göpferich,<sup>26</sup> polymer degradation initially occurs superficially, with changes in surface morphology, followed by cracking and increased surface roughness. Although pH cycling caused changes in the composite resin surface in the present study, these changes did not result in a significant increase in surface roughness measurements, showing that the

composite resins evaluated were effective in maintaining the surface roughness against the pH variations.

The association between pH cycling and simulated toothbrushing was also evaluated since toothbrushing is part of common daily oral hygiene. Simulated toothbrushing abrasion is a methodology established in the literature; it is an important *in vitro* wear factor that simulates clinical conditions. According to Sexson and Phillips,<sup>31</sup> a patient performs approximately 15 cycles for each session of brushing. Thus, if maintaining an oral hygiene routine consisting of two daily brushing sessions, then 10,000 cycles are performed by the end of one year, which is the number of cycles applied to the specimens. The toothpaste used was Colgate Total 12, which has low abrasiveness on composite resins.<sup>32,33</sup>

SEM images demonstrated that the association of pH cycling with simulated toothbrushing caused a greater change on the surface topography of the composite resins. An increase in the exposure of fillers as well as the formation of microcavities was observed. Simulated toothbrushing with toothpaste causes wear on the composite resin surface through the abrasion process.<sup>33</sup> Several mechanisms are related to this wear: 1) organic matrix wear, 2) loss of fillers because of bond failure with the organic matrix, 3) loss of fillers due to shear of exposed fillers, 4) loss of fillers as a result of cracks in the organic matrix, and 5) exposure of voids intrinsic to the restorative process.<sup>34</sup> Therefore, toothbrushing potentiates the superficial degradation initially caused by pH cycling.

The pH cycling followed by simulated toothbrushing caused a significant increase in surface roughness only for the AUR composite resin. Therefore, the first hypothesis was partially rejected. Arithmetic roughness is a measure quantified by parameters of length (ampleness) and width (spacing) of irregularities or a combination of both.<sup>35</sup> It is suggested that the greater degradation of the organic matrix of the AUR favored the increase of its surface roughness when it was submitted to the toothbrushing abrasion mechanism. Some of the fillers exposed on the surface were removed by the shear process, leaving the surface with microcavities (Figure 2D3), which increased the parameters of height and width of the surface, consequently increasing surface roughness.<sup>34</sup> AUR contains the TEGDMA monomer, which reduces the material's viscosity while increasing its water absorption.<sup>36</sup> Degradation of the composite resins by hydrolysis is related to polymerization of the material and the monomeric composi-

tion. TEGDMA was shown to be more susceptible to hydrolysis than were Bis-GMA and Bis-EMA, leading to increased material wear and surface roughness.<sup>29</sup> While Z250 also contains TEGDMA, it did not show significant surface roughness alteration after pH cycling followed by simulated toothbrushing, which suggests that there may be differences in the percentages of this monomer in the compositions of the composite resins.<sup>29</sup>

One of the important aspects of the surface roughness study is related to bacterial adhesion and retention. After polishing (Ra0), all composite resins obtained surface roughness less than 0.2  $\mu\text{m}$ . After pH cycling (Ra1) and simulated toothbrushing (Ra2), surface roughness of the composite resins was approximately at the stated threshold surface roughness of 0.2  $\mu\text{m}$ ;<sup>7</sup> this can be considered a positive result since surface roughness above 0.2  $\mu\text{m}$  favors an increase in plaque formation on the material surfaces.<sup>37</sup>

The present study also evaluated bacterial adhesion on the composite resin surfaces. The accumulation of biofilm favors the formation of secondary caries at the restorative interfaces, which is the main factor responsible for failures of composite resin restorations.<sup>38</sup>

SEM images showed agglomerates of bacteria after pH cycling on Z250 and FTK composite resin surfaces (Figures 3A2 and 3B2, respectively). The agglomerates of bacteria were more intense for FTK. Conversely, there were only dispersed bacteria for TTC (Figure 4C2) and AUR (Figure 4D2). These findings corroborate the results of bacterial counts after pH cycling. According to the surface roughness means, similarities in relation to the bacterial adhesion would be expected, but this was not confirmed. Yuan and others<sup>39</sup> observed that bacterial adhesion is not always related only to the surface roughness but also to surface energy. Besides that, *S mutans* have greater propensity for adhesion to substrates with high surface energy. One factor that influences the surface energy is the composition of the fillers of the composite resins.<sup>40</sup> The filler in Z250 and FTK is silica and zirconia particles, TTC uses barium-silica-aluminum particles, and AUR uses silica and barium alumino-borosilicate particles. However, other factors contribute to bacterial adhesion, such as size and shape of fillers,<sup>41,42</sup> monomer composition,<sup>42,43</sup> and amount of residual monomer.<sup>41</sup> A possible explanation for higher bacteria adhesion obtained for FTK could be related to the particular monomer mixture and fillers of this material. Further investigation is necessary to establish which

component is more relevant in the process of bacterial adhesion for each composite resin.

There were only dispersed bacteria on the surface of all composite resins after pH cycling followed by simulated toothbrushing. These findings are more evident for Z250 (Figure 3A3) and FTK (Figure 3B3), which showed a significant decrease in bacterial counts after toothbrushing in relation to pH cycling. There was no significant difference in bacterial counts between the treatments for TTC and AUR. The SEM images corroborate these findings since dispersed bacteria were predominant for the three treatments. Therefore, the second hypothesis was partially rejected.

Possible explanations can be drawn for the decrease of bacterial counts and the presence of dispersed bacteria instead of agglomerates of bacteria on the surface of the composite resins after toothbrushing. First, the presence of 0.3% Triclosan in the fluoridated toothpaste, which has a broad spectrum of antimicrobial action, may influence agglomeration. In bacteriostatic concentrations, this substance prevents protein synthesis and, in bactericidal concentrations, disorganizes the cytoplasmic membrane of the bacterium, leading to a loss of structural integrity and a leakage of intracellular content.<sup>44</sup> At lower concentrations (0.2% to 0.5%), Triclosan affects the metabolism of some species, such as *S mutans*, and may have influenced the lower bacterial adhesion on the surface of the composite resins.<sup>45</sup> In a second possible explanation, the presence of sodium fluoride in the toothpaste, in low concentrations, exerts subtle antimicrobial action,<sup>46</sup> presenting direct and indirect effects on *S mutans*. In the direct effect, sodium fluoride prevents an increase in the number of *S mutans* through the inhibition of critical metabolic processes.<sup>47</sup> In the indirect effect, sodium fluoride reduces environmental acidification in the biofilm. Another study found a reduction in *S mutans* when combining an acidic pH with the presence of sodium fluoride.<sup>46</sup>

Despite the easier and faster restorative procedures obtained with the bulk-fill composite resins, these materials are affected by pH cycling. Ideally, patients should maintain adequate oral hygiene and follow a low-sugar diet. Noncompliance with these factors increases the cariogenic challenges imposed on the restorative interfaces.

## CONCLUSIONS

Within the limitations of the present study, it is possible to conclude that pH cycling caused degradation of the surface of composite resins. Simulated

toothbrushing potentiated this degradation. However, only Aura Bulk Fill presented an increase in surface roughness after the simulated brushing regimen. Bacterial adhesion occurred on all composite resins after pH cycling; however, after simulated brushing, adhesion of dispersed bacteria was similar for all the composite resin groups.

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## Conflict of Interest

The authors of this article 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.

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