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# Microhardness and Roughness of Infiltrated White Spot Lesions Submitted to Different Challenges

ÉY Neres • MD Moda • EK Chiba • ALF Briso
JP Pessan • TC Fagundes

### Clinical Relevance

Infiltrated white spot lesions seem to resist mechanical abrasion and artificial accelerated aging, but they are not resistant to a new cariogenic challenge.

## SUMMARY

**A white spot lesion is the first clinical sign of a caries lesion and represents mineral loss from the enamel subsurface. The purpose of this study was to evaluate the microhardness and surface roughness of white spot lesions after application of a resin infiltrant and subjection to different challenges. Caries-like lesions were induced in bovine enamel discs (n=50), and the specimens were randomly divided into five study groups (n=10): demineralized enamel (negative control, G1), infiltrated enamel (G2), infiltrated enamel submitted to brushing (G3), infiltrated enamel submitted to pH cycling (G4), and infiltrated enamel submitted to artificial aging (G5). Half of each enamel surface was used as its own positive control. Roughness data were analyzed using the Kruskal-Wallis test followed by the Dunn test. Results from microhardness were analyzed by two-way analysis of variance, followed by the Tukey test for multiple comparisons. The level of significance was set at 5%. Microhardness and roughness values obtained from the test side of the specimens were significantly lower compared with the sound enamel for all groups. Microhardness values obtained for G2, G3, and G5 were not significantly different. Values found for G1 were significantly lower compared with those for G2, G3, and G5. The lowest microhardness values were observed for G4, which was significantly different from**

Émerson Yoshio Neres, DDS, Department of Restorative Dentistry, São Paulo State University (Unesp), School of Dentistry, Araçatuba, Brazil

Mariana Moda, DDS, PhD student, Department of Restorative Dentistry, Araçatuba Dental School, UNESP – Univ Estadual Paulista, Araçatuba, Brazil

Erika Kiyoko Chiba, undergraduate student, Department of Restorative Dentistry, São Paulo State University (Unesp), School of Dentistry, Araçatuba, Brazil

André Luis Fraga Briso, DDS, MS, PhD, associate professor, Department of Restorative Dentistry, São Paulo State University (Unesp), School of Dentistry, Araçatuba, Brazil

Juliano Pelim Pessan, DDS, MS, PhD, assistant professor, Department of Pediatric Dentistry and Public Health, São Paulo State University (Unesp), School of Dentistry, Araçatuba, Brazil

*Ticiane Cestari Fagundes, DDS, MS, PhD, assistant professor, Department of Restorative Dentistry, São Paulo State University (Unesp), School of Dentistry, Araçatuba, Brazil

*Corresponding author: R. José Bonifácio, 1193, Araçatuba, SP, 16015-050, Brazil; e-mail: ticiane@foa.unesp.br

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**the other groups. Surface roughness was not significantly different between G2 and G3. The resin infiltrant presented superiority over the unprotected white spot lesions, as they were more resistant to mechanical and aging challenges. However, resin infiltration was not able to reestablish the properties of sound enamel and was not resistant to a new cariogenic challenge.**

## INTRODUCTION

White spots are recognized as initial carious lesions, which could develop into cavities. Different management approaches have been applied to treat white spot lesions on smooth surfaces, as early as possible, to avoid invasive treatment with cavity preparation.[1] These include remineralization of the lesion with topically applied fluoride products[2] or use of a microabrasion technique, which is especially indicated for remineralized superficial lesions.[3] Although microabrasion is an effective method for eliminating a white spot lesion, minimal wear occurs in healthy tissues.[3]

More recently, a noninvasive alternative treatment was proposed, based on caries infiltration with a hydrophobic resin, which has a refractive index close to that of sound enamel, therefore masking the white spot by infiltrating the porous enamel.[4] This treatment has also been proposed to inhibit demineralization because the diffusion pathways for cariogenic acids are blocked, therefore sealing the white spot lesions.[4] The infiltrant resin is a product that allows for the treatment of carious lesions in early stages without invasive measures.[5]

However, the oral environment is constantly under mechanical and chemical challenges that may affect the tooth structure or restorative materials. Toothbrushing is a mechanical challenge for the prevention and control of oral diseases, such as dental caries and periodontal disease. Nevertheless, there is increased wear during brushing compared with sound enamel in demineralized enamel.[6,7] In this regard, *in vitro* toothbrushing has been a useful tool for testing the resistance to wear of sealant materials.[6,7] Moreover, pH cycling models are also used to evaluate treatment options for white spot lesions, since these models mimic pH alterations of the oral environment.[8] Additionally, artificial accelerated aging systems have been used to simulate aging of the tooth structure and/or restorative materials because it simulates the chemical and physical environments that could partially replace the oral cavity conditions.[9]

Considering the scarce literature on studies evaluating the effect of resin infiltrants applied on white spot lesions submitted to different challenges, this study evaluated the microhardness and surface roughness of infiltrated white spot lesions after exposure to toothbrushing, a pH cycling regimen, and artificial aging. The null hypothesis was that the type of challenge does not influence the microhardness and surface roughness of white spot lesions and infiltrated enamel.

## METHODS AND MATERIALS

### Sample Preparation

This study was previously approved by the local animal ethical committee (protocol #2014-01115). Sound permanent bovine incisors obtained from steers aged 24 to 30 months old were collected and stored in 0.1% thymol solution. Specimens without white spots, cracks, or any other defect were selected and then cleaned using slurry pumice and brush. The tooth roots were removed under abundant water irrigation. The crowns were then mounted in a cutting machine using a cylindrical diamond tip (Dinser Ferramentas Diamantadas, São Paulo, Brazil). Discs containing enamel and dentin were cut, each with a 5.7-mm diameter, from the middle third of the buccal surface of each tooth.

The specimens were fixed on an acrylic base, and the dentin surfaces were flattened using a polishing machine (Arotec, Cotia, Brazil) with 320-grit aluminum oxide sandpaper (Buehler Ltd, Lake Bluff, IL, USA) under water cooling at low speed (100 rpm) until a 2.2-mm thickness was obtained. Then, the enamel surfaces were flattened and polished using 400-, 600-, 800-, and 1200-grit sandpaper, reaching enamel thickness of 1.3 mm. Final polishing was performed with felt disks and 1 μm diamond paste (Extec Corp, Enfield, CT, USA) for 3 minutes.

### Analysis of Surface Microhardness

Baseline surface microhardness was determined by the mean of five indentations produced 100 μm apart from each other in the center of the specimen with a Knoop (KHN) diamond indenter and a load of 25 g and dwell time of 10 seconds, using a microhardness meter durometer (Buehler 5114, Buehler) and specific software for image analysis (OmniMet, Buehler). Only specimens with baseline values between 300 and 380 KHN were selected for the study.

Table 1: *Composition and Application Steps of Resin Infiltrant (Icon, DMG, Hamburg, Germany) According to the Manufacturer's Instructions*

| Composition | Application Steps | Batch No. |
|---|---|---|
| Icon-etch: (HCl 15%) pyrogenic silicic acid, surface-active substances | Apply Icon-Etch. Let set for 2 min. Rinse off with water for 30 s. Air dry. | 711766/ 708255/ 702131 |
| Icon-dry: 99% ethanol | Apply Icon-Dry. Let set for 30 s. Air dry. | |
| Icon-infiltrant: methacrylate-based resin matrix, initiators, and additives | Apply Icon-Infiltrant. Let set for 3 min. Light-cure for 40 s. Apply Icon-Infiltrant. Let set for 1 min. Light-cure for 40 s. | |

### Induction of White Spot Lesions

Prior to the experiment, two layers of acid-resistant nail varnish (Risqué, Barueri, São Paulo, Brazil) were applied on half of the enamel surface. This protected surface served as sound enamel (positive control). Each specimen was immersed in 32 mL of a demineralizing solution containing 50 mM acetate buffer solution and 1.28 mM $Ca(NO_3)_2\_4H_2O$, 0.74 mM $(NaH_2PO_4)\cdot 2H_2O$, and 0.03 ppm F at pH 5.0,[10] for 24 hours at 37 °C. Subsequently, specimens were removed from the solution and thoroughly washed with deionized water. This treatment produced white spot lesions on the enamel surface.

### Experimental Groups

The 50 specimens were divided into five groups (n=10) as follows: negative control (not treated, G1), infiltrated enamel (G2), infiltrated enamel submitted to brushing (G3), infiltrated enamel submitted to pH cycling (G4), and infiltrated enamel submitted to artificial aging (G5).

Resin infiltrant (Icon, DMG, Hamburg, Germany) was applied on the specimens of G2, G3, G4, and G5 and light cured at 1100 mW/cm$^2$ using a light-emitting diode device (Kavo, Poly Wireless, Joinville, Brazil), following the manufacturer's instructions. Composition and manufacturer's instructions are shown in Table 1. Then, specimens were polished using medium, fine, and superfine aluminum oxide abrasive Sof-Lex discs (3M-ESPE Dental Products, St Paul, MN, USA) in a low-speed hand piece under air cooling for 20 seconds. All specimens were stored for 7 days at 37 °C and 100% relative humidity. After this period, specimens from G1 and G2 were analyzed for microhardness and surface roughness, and specimens from G3, G4, and G5 were subjected to challenges.

### Toothbrushing

Toothbrushing was performed on half of the surface of discs of G3 using a mechanical brushing machine (150 g axial load, 5 strokes/s; Elquip Maq Escovação, São Carlos, Brazil) with slurries of dentifrices and water (1:3 w/w, Colgate Total 12, Colgate-Palmolive, São Paulo, Brazil, 1450 ppm as NaF).[6] The specimens were submitted to 10,000 strokes of toothbrushing, over a total of 45 minutes. Specimens were removed and ultrasonically cleaned with water for 10 minutes.

### pH-Cycling Model

Ten discs were individually submitted to a pH cycling model at 37 °C over 7 days. The pH cycling consisted of immersing the specimens in 35.5 mL of demineralizing solution: (2.0 mmol/ L Ca, 2.0 mmol/ L P, 0.075 mol/ L acetate buffer, 2.22 mL/ mm$^2$ of enamel surface) for 6 hours, alternated with immersion in 17.75 mL of remineralizing solution: (1.5 mmol/ L Ca, 0.9 mmol/ L P, 0.15 mol/ L KCl, 0.02 mol/ L cacodylate buffer, pH 7.0, 0.25 mL/mm$^2$) for 18 hours for 5 days. Then, specimens were kept for 2 more days in a fresh remineralizing solution, completing 7 days of treatment.[11] Specimens were washed in deionized water for 30 seconds between demineralizing and remineralizing cycles.

### Accelerated Artificial Aging

The accelerated artificial aging process was performed in an ultraviolet (UV) accelerated aging chamber (EQUV, Equilam Ind Com Ltda, Diadema, Brazil), according to ASTM G154. The accelerated aging process consisted of alternating periods of UV light (8 hours) and condensation (4 hours), under heat (65 °C±3 °C or 45 °C±3 °C) and 100% humidity. Specimens were subjected to a total of 252 hours of aging and 168 hours of UV-B irradiation with a 313-nm emission peak.[9]

### Measurement of Microhardness and Surface Roughness of Enamel Surface

After challenges, a final microhardness measurement on the test side of each specimen was performed as previously described.

Table 2: *Means ± Standard Deviations Surface Microhardness (KHN – kg/mm$^2$) of Enamel Discs According to Treatment Group (1 to 5) and Area of the Specimen (Control or Test)*[a]

| Groups | Control | Test |
|---|---|---|
| 1: Not treated | 345.0 ± 32.7$^{Aa}$ | 117.8 ± 13.4$^{Bb}$ |
| 2: Infiltrated | 351.2 ± 22.6$^{Aa}$ | 228.3 ± 15.4$^{Ba}$ |
| 3: Infiltrated and brushing | 341.3 ± 20.6$^{Aa}$ | 227.2 ± 10.9$^{Ba}$ |
| 4: Infiltrated and pH cycling | 352.7 ± 23.0$^{Aa}$ | 30.9 ± 3.8$^{Bc}$ |
| 5: infiltrated and artificial aging | 344.1 ± 14.8$^{Aa}$ | 237.3 ± 19.0$^{Ba}$ |

[a] *Capital superscript letters indicate significant differences between control and test areas; lowercase superscript letters represent significant differences among treatment groups in each column (Tukey test,* $p<0.05$*).*

Table 3: *Means ± Standard Deviations Surface Roughness (Ra μm) of Enamel Discs According to Treatment Group (1 to 5) and Area of the Specimen (Control or Test)* [a]

| Groups | Control | Test |
|---|---|---|
| 1: Not treated | 0.03 ± 0.005$^{Aa}$ | 0.05 ± 0.03$^{Ba}$ |
| 2: Infiltrated | 0.03 ± 0.007$^{Aa}$ | 0.12 ± 0.05$^{Ba,b}$ |
| 3: Infiltrated and brushing | 0.04 ± 0.009$^{Aa}$ | 0.22 ± 0.08$^{Bb}$ |
| 4: Infiltrated and pH cycling | 0.03 ± 0.015$^{Aa}$ | 0.65 ± 0.30$^{Bc}$ |
| 5: Infiltrated and artificial aging | 0.04 ± 0.026$^{Aa}$ | 0.09 ± 0.02$^{Ba}$ |

[a] *Capital superscript letters indicate significant differences between control and test areas; lowercase superscript letters represent significant differences among treatment groups in each column (Dunn test,* $p<0.05$*).*

The nail varnish on the enamel surfaces was carefully removed using acetone-soaked cotton wool. Surface roughness was characterized by the average roughness (Ra), which represents the arithmetic mean value of all absolute distances of the roughness profile from the center line within the measuring length. Three measurements were recorded on half of each surface using a roughness meter Surftest SJ 400 – Mitutoyo (Mitutoyo American Corporation, Kanagawa, Japan). The cutoff value (distance traversed by the stylus over which the data were collected) for surface roughness was 0.25 mm.

### Scanning Electronic Microscopy (SEM) Analysis

Two specimens of each material were mounted on aluminum stubs, sputter-coated with gold (Balzers SCD-050 sputter coater, OC Oerlikon Corporation AG, Pfäffikon, Switzerland) and submitted to SEM analysis (Evo LS15, Carl Zeiss, Oberkochen, Germany) at 70× magnification of the most representative center area of the specimen.

### Statistical Analysis

The software StatView version 5.0 (SAS Institute, Cary, NC, USA) was used for the statistical analysis. The assumptions of equality of variances and normal distribution of data were checked using the Bartlett and Shapiro-Wilk tests, respectively. The microhardness results were analyzed using two-way analysis of variance, followed by the Tukey test for multiple comparisons. Since homogeneity was not achieved, the roughness data were analyzed using the Kruskal-Wallis test, followed by the Dunn test. The level of significance was set at 5%.

## RESULTS

Microhardness and surface roughness values of the test groups (G1–G5) were significantly lower compared with those obtained for sound enamel ($p<0.05$). Microhardness of G1 (no treated white spot lesion) was significantly lower compared with G2, G3, and G5 ($p<0.05$). The lowest microhardness values were observed for G4 (submitted to pH cycling), which was significantly different from all other groups ($p<0.05$). The mean (standard deviation [SD]) microhardness for all groups is presented in Table 2.

Regarding surface roughness, G4 (pH cycling) presented the highest values, which was significantly different compared with the other groups ($p<0.05$). The mean (SD) surface roughness is displayed in Table 3.

SEM images (Figures 1 through 5) showed major differences between sound enamel and test groups, mainly for the group that was infiltrated and submitted to pH cycling (Figure 4). In contrast, a similar appearance was found for groups only infiltrated and infiltrated with brushing (Figures 2 and 3).

## DISCUSSION

Changes in microhardness and surface roughness in direct restorative materials, especially resin-based materials, have a direct influence on the longevity of a restoration.[9] When such changes occur in white spot lesions infiltrated by resin infiltrant, the loss of strength and smoothness of the material can leave such surfaces unprotected and favor the development of new caries lesions.[6,12] The null hypothesis was partially rejected, since the resin infiltrant increased the microhardness of white spot lesions, except after pH cycling. Additionally, the highest surface roughness was also found after pH cycling.

Previous studies have used test methodologies similar to those used in this work to evaluate the surface of infiltrated enamel.[13-15] The present data

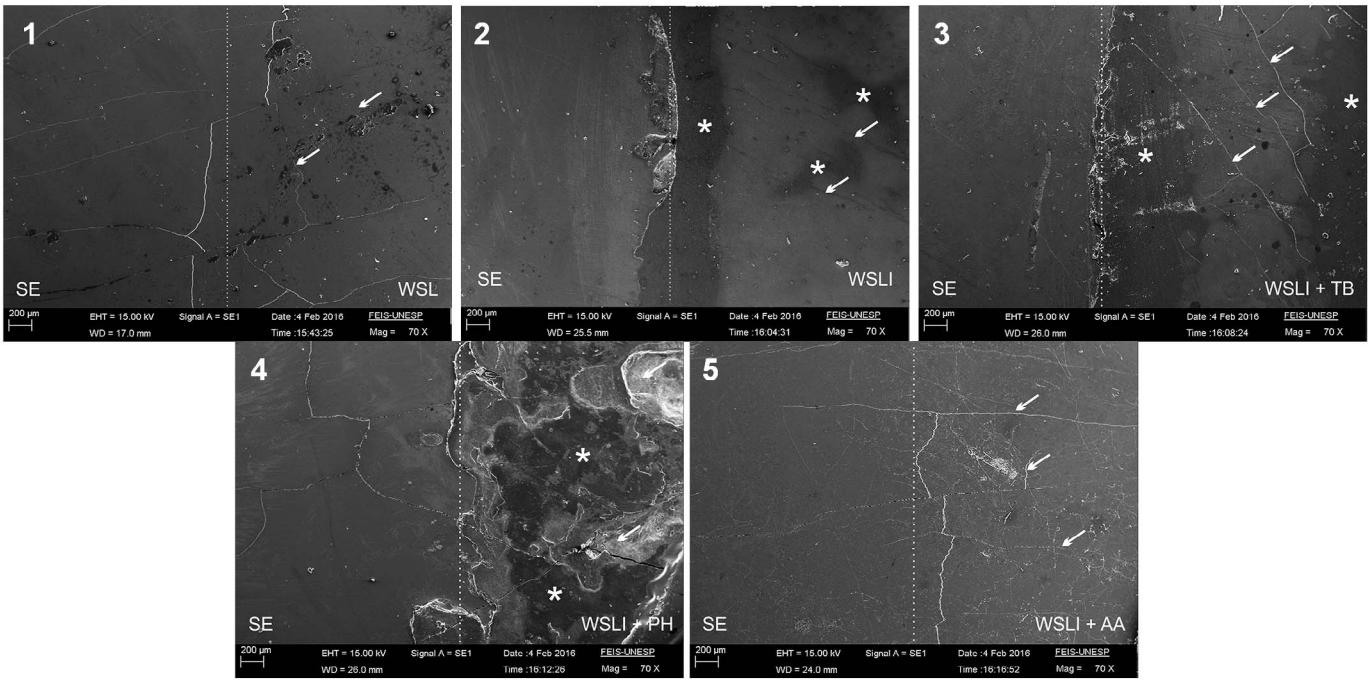


Figure 1. *SEM image from G1. Right side represents the white spot lesion. Left side is sound enamel. A similar appearance between the two sides may be observed, although a few irregular areas are observed on the surface (arrows). Abbreviations: SE, sound enamel; WSL, white spot lesion.*
Figure 2. *SEM image from G2. Right side represents the white spot lesion after resin infiltrant application. Left side is sound enamel. Irregularities in the test surface were observed when compared with sound enamel (arrows), and areas with resin infiltrant may be observed (asterisk). Abbreviations: SE, sound enamel; WSLI, white spot lesion infiltrated.*
Figure 3. *SEM image from G3. Right side represents the white spot lesion after resin infiltrant application and brushing. Left side is sound enamel. Large areas with resin infiltrant may be observed (asterisk) associated with areas of cracks (arrows). Abbreviations: SE, sound enamel; WSLI + TB, white spot lesion infiltrated and toothbrushing.*
Figure 4. *SEM image from G4. Right side presents the white spot lesion after resin infiltrant application and pH cycling. Sound enamel is observed on the left side. A great difference between sound enamel and the test side is seen. Islands of infiltrant material can be observed in darker areas (asterisk). Modified areas of enamel surface may be observed, evidence of an aggressive challenge. Abbreviations: SE, sound enamel; WSLI + PH, white spot lesion infiltrated and pH cycling.*
Figure 5. *SEM image from G5. Right side presents the white spot lesion after resin infiltrant application and artificial aging. Left side is sound enamel. Many cracks are seen on the test side compared with sound enamel (arrows). Abbreviations: SE, sound enamel; WSLI + AA, white spot lesion infiltrated and artificial aging.*

are in line with previous studies reporting lower microhardness values in artificial white spot lesions treated with a resin infiltrant compared with sound enamel.[13-15] This fact may be associated with characteristics of the resin infiltrant organic matrix, mainly composed of triethylene glycol dimethacrylate (TEGDMA).[6,12,13,16] This monomer has a high degree of conversion; however, formation of the polymer chain does not always occur.[17] Moreover, the absence of strong intermolecular secondary bonds, as well as aromatic rings, results in mechanical properties inferior to those of other monomers.[17] Furthermore, material shrinkage during polymerization leaves some regions of noninfiltrated demineralized enamel, which may also contribute to the decrease in surface microhardness compared with sound enamel.[14,15]

Despite the aforementioned disadvantages, the great penetration capacity of resin infiltrant may be due to the low viscosity presented by TEGDMA as well as its low molecular weight, allowing greater penetration of infiltrant compared with other materials, such as sealants and adhesives.[12,15,18,19] In this sense, although the mechanical properties of TEGDMA-infiltrated and sound enamel are different, significantly higher microhardness values were observed for groups with white spot lesions treated with resin infiltrant (G2, G3, and G5) compared with artificial white spot lesions (G1), demonstrating that the resin infiltrant was able to penetrate the lesion body. A confocal microscopic study showed higher penetration of Icon resin infiltrant than other resin-based materials into initial erosion lesions.[20]

An increase in surface roughness of infiltrated groups was observed in the present study compared with sound enamel. Demineralized prismatic areas not filled by the infiltrant, either by polymerization shrinkage or by interference of ethanol in the

polymerization, could contribute to this increase in roughness.[15,21] Conditioning with hydrochloric acid may also have contributed to this phenomenon, since Yim and others,[22] when comparing the effectiveness of enamel conditioning with 15% hydrochloric acid (120 seconds) and 37% phosphoric acid (5 seconds), observed that hydrochloric acid produced grooves and cracks in enamel, which would result in higher surface roughness, even though it promoted better penetration of the resin infiltrant due to its high demineralizing capacity. Irregularities in test surfaces were observed compared with sound enamel in Figures 2, through 5, in which resin infiltrant was used.

The protective capacity of resin was also shown to significantly increase Vickers microhardness when the infiltrant was applied in two layers instead of a single application, even after a new cariogenic challenge.[13] The protective effect of this infiltrant was also assessed by transverse microradiography and confocal microscopy, demonstrating that the infiltrant was able to resist to new cariogenic challenges, especially when used in combination with a fluoride gel.[23] Thereby, the resin material acts as a physical barrier to new acidic attacks and fluoride catalyzes calcium, allowing enamel remineralization by saliva.[23] Moreover, the same resin infiltrant was also able to protect the enamel surface from erosive challenges, regardless of etching with 15% HCl gel.[19] It has been suggested that the presence of resin infiltrant in the lesion subsurface has a protective effect on enamel because it acts as a barrier, occluding the pores formed by a white spot lesion, which then becomes more resistant to new cariogenic attacks.[14] Based on this information, the results of the present study were somehow unexpected, since the resin infiltrant was unable to protect enamel from pH cycling. The discrepant results may be due to differences in pH cycling models for *in vitro* evaluations.[8] In this sense, it is noteworthy that the majority of studies cited earlier used long-term (50 day) pH cycling models under weaker challenge conditions,[13,23] which is very different from the short-term protocol used in the present study. Another reason might be due to the excess removal done during the polishing procedure after infiltrant application, since it was found that resin-based materials are able to protect enamel against erosion only when they are present over enamel as a physical barrier.[24] Nonetheless, the different results obtained in this study and those previously reported indicate that further investigations are still needed in this field.

Increased porosity of the resin infiltrant due to its low wear resistance against acid challenge has also been shown.[13] This low wear resistance may be related to the polymer chain conformation and weak secondary bonds present in the TEGDMA molecule, as previously described.[17] Furthermore, TEGDMA may be released from homopolymers or copolymers, forming a polymer chain prone to chemical degradation, especially in acidic environments.[15] After pH cycling, there were perceptible changes on the specimen surface (Figure 4), being heterogeneous with the majority of the resin infiltrant removed by the acid attack and revealing only small islands of infiltrants that resisted the acidic stress. These observations were also noted in an *in vitro* study on the resistance of light-cured sealants to acidic soft drinks.[7]

Regarding resistance to abrasion, the challenge adopted in the present study (10,000 brushing cycles) was unable to remove the infiltrant (Figure 3), as noted in previous studies in which the resin infiltrant resisted up to 20,000 cycles, as shown by profilometric and confocal microscopy data.[6,19] Abrasion resistance has been related to the thick layer of material, since two layers of resin infiltrant are necessary according to the manufacturer's instructions.[19] However, resin infiltrant was not resistant when submitted to erosive and abrasive challenges by 15 cycles of acidic beverages and toothbrushing.[25]

As for the accelerated aging assay, similar results were obtained for groups in which this challenge was used. This accelerated aging system simulates the destructive environmental capacity and predicts the relative durability of materials, simulating the chemical and physical challenges. Saliva is simulated by conditions of 100% humidity and a condensation process using distilled water saturated with oxygen. Light is simulated by sources of UV-B light. The specimens were positioned on the machine's fixing plates for automatically repeated and alternating cycles of UV-B light and condensation.[9] This type of irradiation has the potential for photo-oxidation that induces breaks of single or double carbon bonds.[9] These chemical bonds have an important function in the configuration of polymer chains present in the organic matrix of resin-based materials.[9] No influence in microhardness and roughness was observed on the resin infiltrant surface after this challenge, although many cracks might be seen on the test side under SEM evaluation (Figure 5). This type of challenge might impact other characteristics, such as color alteration.

Despite the limitations of an *in vitro* study, the objective was to simulate some of the clinical conditions to which the material would be subjected. In the present study, the infiltrant was not effective against a new acid challenge, which may impose some limitations regarding individuals at high caries risk because some aspects of caries progress may not be under control. However, further clinical studies are needed since the conditions described in this present study differ from *in vivo* conditions, considering that some clinical circumstances, such as the roles of saliva and resin expansion or shrinkage by thermal cycling, were not evaluated.

## CONCLUSION

The resin infiltrant was not able to reestablish sound enamel properties and was not resistant to a new cariogenic challenge. However, it presented superiority over unprotected white spot lesions, resisting the challenges of mechanical abrasion and artificial accelerated aging.

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#### Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the FOA UNESP animal ethical committee. The approval code for this study is protocol #2014-01115.

#### 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.

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