

Effect of Bleaching Agents on the Nanohardness of Tooth Enamel, Composite Resin, and the Tooth-Restoration Interface

AT Abe • MN Youssef • ML Turbino

Clinical Relevance

Exposure to bleaching agents can result in changes in the tooth-restoration interface.

SUMMARY

This *in vitro* study aimed to evaluate the nanohardness of tooth enamel, composite resin, dental adhesive, and enamel hybrid layer exposed to 35% hydrogen peroxide-based bleaching agents and analyze the tooth-restoration interface using scanning electron microscopy (SEM). This study used 40 crowns of bovine incisors, which were embedded in epoxy resin. A $2 \times 2 \times 2$ -mm cavity was prepared in the medial third of the flattened buccal surface of each tooth and restored (two-step etch-and-rinse Adper Single Bond 2 + nano-composite resin Filtek Z350 XT). The speci-

mens were polished and divided into four groups ($n=10$), corresponding to each bleaching agent used (TB: Total Blanc Office, pH=7.22–6.33; HPB: Whiteness HP Blue, pH=8.89–8.85; HP: Whiteness HP, pH=6.65–6.04; PO: Pola Office, pH=3.56–3.8), applied in accordance with manufacturer protocols. The nanohardness of the substrates was measured before and immediately after the bleaching procedure and after 7-day storage in artificial saliva with an Ultra-Microhardness Tester (DUH-211S, Shimadzu). Loads used were 100 mN for tooth enamel and composite resin and 10 mN for adhesive and enamel hybrid layer. For SEM analysis, epoxy replicas were prepared through high-precision impressions of the specimens. For nanohardness, the statistical tests two-way analysis of variance and Tukey ($p<0.05$) revealed that the agent with the lowest pH value (PO) was the only one to decrease the nanohardness of enamel and the enamel hybrid layer immediately after its application; however, after 7-day storage in artificial saliva, the nanohardness levels of these substrates returned to their original values. SEM analysis revealed small gaps be-

Andrea Tami Abe, DDS, MSc, Restorative Dentistry, University of São Paulo, São Paulo, Brazil

Michel N Youssef, DDS, MSc, associate professor, Dentistry, University of São Paulo, São Paulo, Brazil

*Míriam Lacalle Turbino, DDS, MSc, associate professor, School of Dentistry, University of São Paulo, São Paulo, Brazil

*Corresponding author: School of Dentistry, University of São Paulo, Prof. Lineu Prestes Avenue 2227, São Paulo 05508-900, Brazil; e-mail: miturbin@usp.br

DOI: 10.2341/14-153-L

tween tooth enamel and adhesive after the exposure to all bleaching agents; however, the most evident gap in the tooth-restoration interface was observed immediately after application of agent PO. No bleaching agent used changed the nanohardness of the composite resin and adhesive layer.

INTRODUCTION

Hydrogen peroxide is the main agent used for dental bleaching, and it is a strong oxidizing agent with low molecular weight. It is able to disseminate through tooth enamel in the inter- and intrarod enamel regions, which are composed mainly of proteins, until it reaches the amelodentinal interface.¹⁻³ As it disseminates into the tooth structure, free radicals resulting from the decomposition of hydrogen peroxide oxidize the macromolecules of pigments, promoting the cleavage of chemical bonds; turning the long molecular chains into smaller molecular chains, which are not pigmented; making the teeth visually clearer; and thus reestablishing their optical properties.^{4,5}

Many patients undergoing dental bleaching treatments have teeth with restorations, and since the tooth-bleaching mechanism involves chemical processes, this could change the physical and mechanical properties of both dental tissues and restorative materials; therefore, it is important to study the effects of bleaching agents on these surfaces.

Nanoindentation is a method used to measure the mechanical properties of both dental tissues and restorative materials. Unlike the conventional microhardness testing procedure based on visual information, the nanoindentation test uses a load displacement curve^{6,7} to calculate the mechanical properties of a certain material. Because it uses low loads equivalent to milli-Newtons (mN), this test allows for the evaluation of mechanical properties in extremely thin regions, such as the tooth-restoration interface.

The studies analyzing the effects of bleaching agents on the adhesive interface evaluate mostly the bond strength on dental tissues previously exposed to peroxide; however, little is known about the effects of bleaching agents on existing tooth-restoration interfaces, and this study analyzed the effects of bleaching agents on this interface through the nanoindentation test.

In the attempt to contribute to a broad understanding of the effects of bleaching agents on an existing restoration, the aim of our study was to

evaluate the nanohardness of tooth enamel, composite resin, adhesive, and enamel hybrid layer by performing nanoindentation tests and to analyze the tooth-restoration interface through scanning electron microscopy (SEM) to decide whether the composite resin restorations should be replaced after the dental bleaching treatment. The null hypothesis tested was that the different bleaching agents, regardless of the bleaching technique and pH value, would not cause nanohardness alterations on the composite resin-tooth interface.

METHODS AND MATERIALS

Forty freshly extracted bovine teeth were cleaned and stored in distilled water at 4°C until use. The teeth were observed under an optical stereoscope at 50× magnification (MiView USB Digital Microscope, Chinavasion Wholesale, Guangdong, China) in order to visualize the absence of cracks in the buccal enamel surface and were cut at the cemento-enamel junction to separate the crown from the root portion. The crowns were embedded in epoxy resin and the buccal surface of each specimen was polished with 600-grit silicon carbide (SiC) paper (Buehler Ltd, Lake Bluff, IL, USA) under running water in polishing equipment (160 rpm, Buehler Ltd) in order to expose and flatten the enamel surface. The opposite surface of each specimen was also polished to obtain a parallel surface, which is essential for the correct measurement of nanohardness.

After the enamel surface was flattened, a 2 × 2 × 2-mm cavity was prepared in the medial third of each tooth using a high-speed diamond bur (No. 1090, KG Sorensen, Cotia, Brazil) and manual cutting instruments (#14/15, #26, SS White/Duflex, Rio de Janeiro, Brazil). The burs were replaced after preparation of 10 cavities, and the size of each cavity and its depth were measured using a periodontal probe.

For the restorative procedure, each cavity was etched with 37% phosphoric acid (Villevie, Joinville, Brazil) for approximately 15 seconds for enamel and 10 seconds for dentin. The cavities were washed with water spray for 15 seconds, and the excess water was removed with absorbent paper and air-dried. Next, the two-step etch-and-rinse adhesive Adper Single Bond 2 (3M ESPE, St Paul, MN, USA) was applied in two layers, according to the manufacturer's recommendation, and each layer was vigorously scrubbed in the cavity with applicator brushes for 15 seconds, permitting the solvent to evaporate.⁸ The adhesive excess was removed using a dry brush, and then the adhesive was light cured for 10 seconds (Elipar Freelight 2, 3M ESPE) with a power density of 1000

| Table 1: Manufacturer, Lot, Composition, and Application Protocol of the Materials Used | | | |
|--|--|---|--|
| Materials | Manufacturer/Lot | Composition | Application Protocol |
| Acid Gel | Villevie (Joinville, Brazil) | 37% phosphoric acid | 15 s on enamel 10 s on dentin |
| Adper Single Bond 2 | 3M ESPE (St Paul, MN, USA) #123600553 | Ethanol, Bis-GMA, HEMA, colloidal silica, 2-hydroxyethylmethacrylate, glycerol 1,3 dimethacrylate, copolymer of acrylic acids and itaconic acids, UDMA | Two layers 15 s on each layer |
| Filtek Z350 XT | 3M ESPE #1320400385 | Bis-GMA, Bis-EMA, colloidal silica, zirconia silica-oxide, silane, UDMA, polyethylene glycol dimethacrylate, TEGDMA, 2,6-di-terc-butyl-p-cresol (BHT), pigments | |
| TB | Nova DFL (Rio de Janeiro, Brazil) #13060921 | 35% hydrogen peroxide, thickener, vegetable extracts, amide, chelating agent, glycol, pigments, water | One session Two applications of 20 min each |
| HPB | FGM (Joinville, Brazil) #160913 | 35% hydrogen peroxide, thickeners, violet inactive pigment, neutralizing agents, calcium gluconate, glycol, deionized water | One session One application of 40 min each |
| HP | FGM #130213 | 35% hydrogen peroxide, thickener, red pigment, glycol, water | One session Three applications of 15 min each |
| PO | SDI Ltd (Bayswater, VIC, Australia) #122363 | Liquid: 35% hydrogen peroxide, water Powder: thickener, catalyzer, pigment, desensitizing agent | One session Three applications of 8 min each |
| Abbreviations: Bis-EMA, ethoxylated bisphenol A glycol dimethacrylate; Bis-GMA, bisphenol A diglycidyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; HP, Whiteness HP; HPB, Whiteness HP Blue; PO, Pola Office; TB, Total Blanc Office; TEGDMA, triethylene glycol dimethacrylate; UDMA, urethane dimethacrylate. | | | |

mW/cm². The intensity of the light was monitored with a radiometer (LED Radiometer, SDI Ltd, Bayswater, VIC, Australia).

The cavities were restored with nanofilled composite resin Filtek Z350 XT (A2E shade, 3M ESPE), which was incrementally placed using three layers, two oblique and one horizontal, and each layer was light cured for 20 seconds. After the placement of the last layer (horizontal layer), it was covered with a polyester matrix strip and a glass slab that was pressed using a load of 3.5 kg for 10 seconds to standardize the pressure over the glass slab and to ensure better adaptation of the composite in the cavity. Following removal of the glass slab, the composite resin was light cured for 20 seconds. The composition of the materials used is shown in Table 1. After completing the restorations, the specimens were stored in distilled water at 37°C for 7 days.

After this initial storage period, the buccal surface of each specimen was again polished to remove excess filling, if any, on the prepared enamel and to expose the tooth/adhesive/restoration line. Then a decreasing sequence of SiC paper (800, 1200, 2400, and 4000 grit) under running water was used to obtain a smooth and polished surface. Between each polishing stage, the specimens were cleaned using distilled water in an ultrasonic cleaner (Digital Ultrasonic Cleaner CD-4820, Kondortech, São Car-

los, Brazil) for 5 minutes to remove any debris. Right after polishing, the specimens were stored in artificial saliva (0.213 g/L CaCl₂*2H₂O, 0.738 g/L KH₂PO₄, 1.114 g/L KCl, 0.381 g/L NaCl, 12 g/L Tris buffer; pH adjusted to 7.0 with KOH)⁹ at 37°C.

The specimens were divided into four groups (n=10) with each group corresponding to one 35% hydrogen peroxide-based bleaching agent used: Total Blanc Office (TB, Nova DFL, Rio de Janeiro, Brazil), Whiteness HP Blue (HPB, FGM, Joinville, SC, Brazil), Whiteness HP (HP, FGM), and Pola Office (PO, SDI).

Bleaching Procedure

The bleaching gels were applied directly to the tooth enamel and restoration 24 hours after polishing, following the protocol of each manufacturer (Table 1), at room temperature without the use of light sources. During this procedure, the gel was stirred using an applicator brush to release possible oxygen bubbles formed. The specimens were washed in distilled water to remove the gel, air-dried, and stored in artificial saliva at 37°C for 7 days.

pH Measurement

The pH value of each bleaching agent was measured with a calibrated pH meter (Hanna HI 2221, Hanna Instruments, Tamboré, Brazil) on the mixture of the

bleaching gel (initial) and at the end of the first application (20 minutes for TB, 40 minutes for HPB, 15 minutes for HP, and 8 minutes for PO).

Nanoindentation Evaluation

Nanohardness measurements were performed with a Berkovich tip attached to an Ultra-Microhardness Tester (DUH-211S, Shimadzu, Tokyo, Japan), using a maximum load of 100 mN for tooth enamel and composite resin and 10 mN for adhesive and enamel hybrid layer. For each specimen, three regions were selected visually using an optical microscope coupled to the equipment. Ten indentation measurements were performed in a region (with a 10- μ m distance between measurements), in each substrate (tooth enamel, composite resin, adhesive, and enamel hybrid layer), and at three different times (before and immediately after the bleaching procedure and 7 days after the exposure to the bleaching agent and storage in artificial saliva). The average of 10 indentations for each substrate was calculated. The parametric nanohardness values were analyzed using two-way analysis of variance (ANOVA) for repeated measures and the Tukey test ($p < 0.05$).

SEM Analysis

To evaluate the tooth-restoration interface, four more specimens were prepared (one specimen for each group) in the same way that the specimens were prepared for the nanohardness test. Replicas of these specimens were created in epoxy resin (Epofix, Struers, Ballerup, Denmark) through a polyvinyl siloxane impression (Express XT, 3M ESPE) for each period of analysis. Three replicas were obtained for each specimen. The epoxy replicas were fixed on stubs and subjected to platinum plating, and the analyses were performed using a Quanta 600 FEG microscope (FEI, Hillsboro, OR, USA) and Sprit software (Bruker, Atibaia, Brazil). Observations were made in the tooth-restoration interface with 1000 \times magnification.

RESULTS

ANOVA showed a statistically significant interaction between the nanohardness values obtained between bleaching agents and measurement time in tooth enamel ($p = 0.005$) and enamel hybrid layer ($p < 0.001$).

Tukey test detected a significant decrease of tooth enamel and hybrid layer nanohardness immediately after application of the PO agent only. However, after 7 days of storage in artificial saliva, nanohard-

ness values increased, returning to values similar to those initially presented in these two substrates (Table 2). The nanohardness values of these substrates exposed to TB, HPB, and HP agents did not show statistically significant differences during the three periods of analysis.

No statistically significant difference was found between the nanohardness values obtained in the composite resin and in the adhesive ($p > 0.05$). The agent PO presented the lowest pH value (pH3.6–3.8) when compared to other agents (Table 3).

The SEM images of the tooth-restoration interface (Figures 1 and 2) revealed gaps between the enamel and adhesive after being exposed to bleaching agents; however, the most evident gap was observed immediately after the application of agent PO (Figure 2).

DISCUSSION

The hardness of dental tissues is related to mineral gain or loss,^{10,11} and in studies using resinous materials, the hardness has been applied to gain knowledge of properties regarding the composition,¹² degree of polymerization,^{13,14} and superficial degradation of the material.¹⁵ The nanoindentation test, also known as the ultra-microhardness test, has been introduced as a reliable method to study the changes in the mechanical properties of dental mineralized tissues.¹⁶⁻¹⁸ Since tooth enamel is not a homogeneous tissue, the use of extremely low loads may be related to the property analysis of isolated components of tooth enamel,¹⁹ thus not reflecting the properties of this tissue as a whole. Like the enamel tissue, the composite resin is also not a homogeneous material because it has inorganic particles immersed throughout an organic matrix. According to Masouras and others,²⁰ the increase of the indentation area guarantees the inclusion of a wider material area, which is most likely to cover resinous matrix and inorganic particles. Accordingly, a 100-mN load was used in this study, as we understood it would be able to promote a visible and significant indentation to obtain readings of tooth enamel and composite resin. This 100-mN load is within the range established by Elfallah and Swain²¹ as a low load, which is up to 500 mN. A 10-mN load was used for the nanohardness evaluation of the adhesive and the enamel hybrid layer; this value was established in order to not exceed the thickness of these thin layers.

One of the variables of this study was the bleaching agent. Hydrogen peroxide-based agents at 35% have been used because such agents at higher

| Table 2: Mean Nanohardness Values and Standard Deviations of the Tested Substrates at Different Measurement Times | | | | |
|---|------------------|-------------------|-----------------|-----------------|
| Substrates | Bleaching Agents | Measurement Times | | |
| | | Initial | Postbleaching | After 7 d |
| Tooth enamel | TB | 314.4 ± 23.65 A | 329.3 ± 29.32 A | 342.5 ± 25.93 A |
| | HPB | 341.0 ± 25.45 A | 342.8 ± 18.52 A | 344.3 ± 19.97 A |
| | HP | 327.8 ± 32.10 A | 320.0 ± 31.93 A | 325.2 ± 31.23 A |
| | PO | 343.2 ± 27.39 A | 298.6 ± 25.75 B | 347.8 ± 27.38 A |
| Enamel hybrid layer | TB | 470.0 ± 48.9 A | 454.0 ± 73.26 A | 488.9 ± 68.03 A |
| | HPB | 494.5 ± 84.16 A | 479.1 ± 64.61 A | 453.3 ± 68.68 A |
| | HP | 408.1 ± 51.37 A | 439.7 ± 67.01 A | 465.2 ± 60.17 A |
| | PO | 391.4 ± 51.24 A | 225.3 ± 30.61 B | 376.2 ± 75.64 A |
| Composite resin | TB | 71.0 ± 1.17 | 70.9 ± 2.4 | 71.6 ± 1.87 |
| | HPB | 70.3 ± 2.13 | 69.8 ± 3.5 | 70.9 ± 1.44 |
| | HP | 71.0 ± 1.07 | 71.8 ± 1.3 | 70.7 ± 1.87 |
| | PO | 70.4 ± 1.64 | 70.7 ± 2.07 | 70.5 ± 2.87 |
| Adhesive | TB | 20.6 ± 3.61 | 19.3 ± 2.14 | 21.2 ± 2.28 |
| | HPB | 21.9 ± 2.68 | 21.0 ± 3.52 | 19.9 ± 3.55 |
| | HP | 22.1 ± 3.12 | 21.9 ± 2.54 | 22.7 ± 2.36 |
| | PO | 21.8 ± 2.85 | 23.3 ± 1.92 | 23.0 ± 1.69 |
| Abbreviations: HP, Whiteness HP; HPB, Whiteness HP Blue; PO, Pola Office; TB, Total Blanc Office. Different letters in the row indicate a significant difference (p<0.05). No statistical differences were found between measurement times and bleaching agents for the substrates composite resin and adhesive. | | | | |

concentrations would cause more significant changes.^{21,22} The four bleaching agents used (TB, HPB, HP, and PO) have differences in their compositions and pH value.

The HPB bleaching agent has calcium in its composition, and this substance was added in the formulation of dental bleaching gels in order to minimize mineral loss of the tooth during the dental whitening procedure.²³⁻²⁵ The results obtained in the current study revealed that the nanohardness of tooth enamel exposed to this agent remained statistically similar to the nanohardness observed prior to its application. The above-mentioned similar nanohardness could be related to the beneficial effect of calcium addition to bleaching agents because, in fact, no decrease in tooth enamel nanohardness has been observed. However, the nanohardness values of the enamel exposed to TB and HP agents also evidenced no decrease. Since the above-mentioned agents have no calcium in their composition, it is not possible to state that the addition of calcium to the bleaching gel was able to minimize mineral loss during the bleaching procedure.

It is important to know the pH value of dental bleaching agents because it has a strong effect on hydrogen peroxide chemistry. Different oxygen-reactive molecules can be formed, depending on the solution pH value. In a basic environment, only low energy is necessary to start the hydrogen peroxide

degradation. According to Malkondu and others,²⁶ in the pH range of 9.5–10.8, the ionization of buffered hydrogen peroxide produces more perhydroxyl free radicals, resulting in 50% greater bleaching effects in the same period compared to other pH levels. Despite higher pH levels producing more oxidative free radicals, at acidic pH levels, the hydrogen peroxide is more stable, and many bleaching products are formulated at lower pH values to ensure the stability of hydrogen peroxide.^{27,28} However, more severe changes in the tooth enamel topography were observed when samples were exposed to low-pH products.^{28,29} The bleaching agent PO was the only agent that presented pH values lower than the critical pH of 5.5 (pH 3.6–3.8) and the only one to reduce the nanohardness of tooth enamel and the enamel hybrid layer immediately after its application. These findings support the theory of other

| Table 3. Initial and Final pH Values of Bleaching Agents | | |
|---|------------|----------|
| Bleaching Agents | Initial pH | Final pH |
| TB | 7.22 | 6.33 |
| HPB | 8.89 | 8.85 |
| HP | 6.65 | 6.04 |
| PO | 3.56 | 3.80 |
| Abbreviations: HP, Whiteness HP; HPB, Whiteness HP Blue; PO, Pola Office; TB, Total Blanc Office. | | |

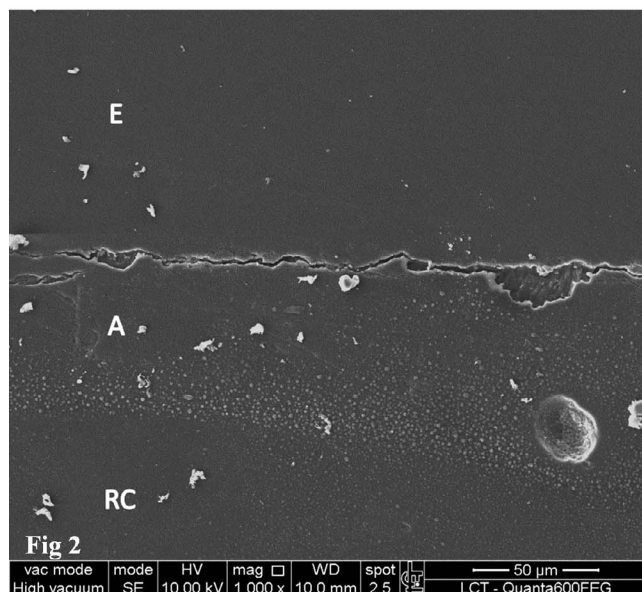
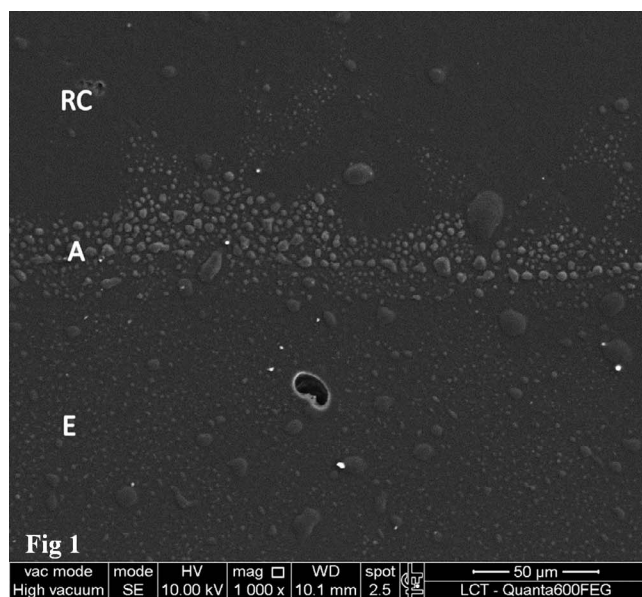


Figure 1. Scanning electron micrograph of the tooth-restoration interface before being exposed to bleaching agents. No gap was observed. E, enamel; A, adhesive; RC, composite resin. 1000× magnification.

Figure 2. Scanning electron micrograph of the tooth-restoration interface immediately after being exposed to agent Pola Office. A well-defined gap may be observed between tooth enamel and adhesive. E, enamel; A, adhesive; RC, composite resin. 1000× magnification.

authors^{27,29-31} that the demineralization of tooth structure is related more to dental erosion due to low pH values of the bleaching solutions than to the effect of peroxide itself. So the null hypothesis tested in the current study was rejected.

Other studies that used the nanoindentation test to evaluate the mechanical properties of tooth

enamel exposed to hydrogen or carbamide peroxide presented a decrease of superficial nanohardness of tooth enamel after being exposed to bleaching agents.^{17,18,32,33} These results were different from those observed in this study because no changes were observed after the use of the three bleaching agents TB, HPB, and HP. The fact that these studies identified a decrease in nanohardness with the use of all bleaching agents can be explained by the loads used in nanohardness measurement: these were much lower (up to 20 mN) than the load used in this study (100 mN). Thus, lower loads may have evidenced changes that were restricted solely to the surface and that may be less clinically significant.

Although PO caused a significant nanohardness reduction of tooth enamel and enamel hybrid layer, after the 7-day storage in artificial saliva, the superficial hardness value of these substrates increased significantly and returned to the original value. The hardness recovery can be attributed to the storage in artificial saliva because according to Sa and others,²⁸ artificial saliva is able to promote a certain level of mineralization of tooth enamel surface due to the high level of calcium and phosphate in this solution, which can interact with enamel during the storage and induce mineralization. However, in other studies,^{33,34} the storage of specimens in artificial saliva did not increase the hardness of tooth enamel exposed to bleaching agents; therefore, there is still no consensus on the beneficial action of storage in artificial saliva.

The tooth enamel and enamel hybrid layer presented similar nanohardness behaviors in relation to the action of bleaching agents. However, the nanohardness values obtained were different, being higher in the hybrid layer than in enamel. This difference in hardness values can be related to the size of indentation. In the enamel hybrid layer, a 10-mN load was applied, and the indentation depth in this substrate was lower than the indentation depth observed in tooth enamel. According to Zhou and Hsiung,³⁵ there is a relationship between hardness and indentation depth of crystalline tissues, and hardness level decreases as indentation depth increases.

The results showed that in-office bleaching agents did not change the superficial nanohardness of nanofilled composite resin. The literature has no studies evaluating the superficial changes in this type of composite through the nanoindentation test. The studies found in the literature are related to the superficial microhardness of these composite resins, and the effects of bleaching agents on such restor-

ative material are still controversial. Most studies did not present changes in the superficial microhardness of nanofilled composite resins after exposure to hydrogen and carbamide peroxide *in vitro*³⁶ and *in situ*.³⁷ However, a microhardness decrease of this material was also presented in other studies.^{26,38} This divergence of results can be explained by the presence of different components in both the organic and the inorganic matrix of composite resins; thus, even materials of the same classification can react differently to a bleaching agent.³⁹

Better mechanical properties of the adhesive interface can be related to better adhesion stability and longevity of restorations.⁴⁰⁻⁴² However, the adhesive interface is the most susceptible to degradation, and its exposure to bleaching agents may affect the longevity of the restoration.^{43,44} Aiming to evaluate the mechanical properties of the tooth-restoration interface after being exposed to different bleaching agents, this study was the first to evaluate the effects of bleaching agents on this interface through the nanoindentation test.

The behavior of the two-step etch-and-rinse adhesive Adper Single Bond 2 versus the action of bleaching agents was similar to the behavior of the composite resin because no hardness changes were found. Adhesive hardness is directly related to the bond strength.⁴² Lower bond strength is related to a higher susceptibility to adhesive degradation, which depends on the composition and application of the adhesive system.⁴⁴ Accordingly, etch-and-rinse adhesives are less susceptible to degradation due to peroxide than self-etch adhesives.⁴⁵ Since there were no changes in the adhesive hardness, we can add that there were no demonstrated changes in its mechanical properties; thus, the exposure to 35% hydrogen peroxide-based bleaching agents did not cause adhesive degradation and did not compromise the longevity of the restoration.

However, by analyzing the SEM images of the epoxy replicas, it is possible to visualize the formation of small cracks between tooth enamel and adhesive in the samples exposed to the four bleaching agents used. These results may be related to the results of Dudek and others,⁴⁴ who found imperfections mostly between tooth enamel and adhesive by analyzing crack patterns of the specimens subject to shear bond strength testing. Through SEM images, it is possible to evaluate the marginal quality of restorations, which is one of the factors responsible for its clinical success. The presence of cracks in tooth enamel and the adhesive interface can be related to the penetration of

bacteria, fluids, and other liquids that can cause sensitivity, marginal staining, restoration detachment, and even secondary caries.⁴⁶

The most evident gap was observed immediately after exposure to the agent with the lowest pH value (PO). Despite the observation of small gaps between enamel and adhesive, it is still not possible to state that the bleaching agents cause deterioration of the marginal integrity of restorations. In clinical practice, it is common to perform dental bleaching treatments on teeth with restorations. The nano-hardness evaluation showed that despite the decrease of values on dental tissues, there was a recovery after the storage period. Accordingly, it would be possible to infer that restorations were not compromised after bleaching procedures. However, the observation of small cracks between enamel and adhesive after bleaching procedures shows that there has been some change in this interface; thus, clinical studies are necessary to evaluate possible deleterious effects in teeth restored with composite resin and subject to bleaching treatment; that is, it is not yet possible to predict how the presence of these small gaps can affect the longevity of these restorations.

CONCLUSION

Under the conditions of this study, the bleaching agent with lower pH value was the only one to decrease tooth enamel and enamel hybrid layer nanohardness immediately after its application and the one to promote the most evident gap in the tooth-restoration interface. However, the nanohardness values returned to their original values after 7 days of storage in artificial saliva. None of the bleaching agents used changed the composite resin and adhesive nanohardness.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local ethics oversight committee guidelines and policies of the University of São Paulo, São Paulo, Brazil.

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.

(Accepted 29 January 2015)

REFERENCES

1. Hegedüs C, Bistey T, Flóra-Nagy E, Keszthelyi G, & Jenei A (1999) An atomic force microscopy study on the effect of

- bleaching agents on enamel surface *Journal of Dentistry* **27(7)** 509-515.
2. Park HJ, Kwon TY, Nam SH, Kim HJ, & Kim KH (2004) Changes in bovine enamel after treatment with 30% hydrogen peroxide bleaching agent *Dental Materials Journal* **23(4)** 517-521.
 3. Ubaldini ALM, Baesso ML, Medina Neto A, Sato F, Bento AC, & Pascotto RC (2013) Hydrogen peroxide diffusion dynamics in dental tissues *Journal of Dental Research* **92(7)** 661-665.
 4. Dahl JE, & Pallesen U (2003) Tooth bleaching—A critical review of the biological aspects *Critical Reviews in Oral Biology and Medicine* **14(4)** 292-304.
 5. Joiner A (2006) The bleaching of teeth: A review of the literature *Journal of Dentistry* **34(7)** 412-419.
 6. Oliver WC, & Pharr GM (1992) An improved technique for determining hardness and elastic modulus using load and displacement sensing indentation experiments *Journal of Materials Research* **7(6)** 1564-1583.
 7. Mahoney E, Holt A, Swain M, & Kilpatrick N (2000) The hardness and modulus of elasticity of primary molar teeth: An ultra-micro-indentation study *Journal of Dentistry* **28(8)** 589-594.
 8. Reis A, Carrilho M, Breschi L, & Loguercio AD (2013) Overview of clinical alternatives to minimize the degradation of the resin-dentin bonds *Operative Dentistry* **38(4)** e103-e127.
 9. Scaramucci T, Borges AB, Lippert F, Frank NE, & Hara AT (2013) Sodium fluoride effect on erosion-abrasion under hyposalivatory simulating conditions *Archives of Oral Biology* **58(10)** 1457-1463.
 10. Kodaka T, Debari K, Yamada M, & Kuroiwa M (1992) Correlation between microhardness and mineral content in sound human enamel *Caries Research* **26(2)** 139-141.
 11. Attin T, Betke H, Schippan F, & Wiegand A (2007) Potential of fluoridated carbamide peroxide gels to support post-bleaching enamel re-hardening. *Journal of Dentistry* **35(9)** 755-759.
 12. Chung KH (1990) The relationship between composition and properties of posterior resin composites *Journal of Dental Research* **69(3)** 852-856.
 13. Rueggeberg FA, & Craig RG (1998) Correlation of parameters used to estimate monomer conversion in a light-cured composite *Journal of Dental Research* **67(6)** 932-937.
 14. Rode KM, Kawano Y, & Turbino ML (2007) Evaluation of curing light distance on composite resin microhardness and polymerization *Operative Dentistry* **32(6)** 571-578.
 15. Yap AU, Tan SH, Wee SS, Lee CW, Lim EL, & Zeng KY (2011) Chemical degradation of composite restoratives *Journal of Oral Rehabilitation* **28(11)** 1015-1021.
 16. Habelitz S, Marshall GW Jr, Balooch M, & Marshall SJ (2002) Nanoindentation and storage of teeth *Journal of Biomechanics* **35(7)** 995-998.
 17. Hairul Nizam BR, Lim CT, Chng HK, & Yap AUJ (2005) Nanoindentation study of human premolars subjected to bleaching agents *Journal of Biomechanics* **38(11)** 2204-2211.
 18. Azer SS, Machado C, Sanchez E, & Rashid R (2009) Effect of bleaching systems on enamel nanohardness and elastic modulus *Journal of Dentistry* **37(3)** 185-190.
 19. Ge J, Cui FZ, Wang XM, & Feng HL (2005) Property variations in the prism and the organic sheath within enamel by nanoindentation *Biomaterials* **26(16)** 3333-3339.
 20. Masouras K, Akhtar R, Watts DC, & Silikas N (2008) Effect of filler size and shape on local nanoindentation modulus of resin-composites *Journal of Materials Science: Materials in Medicine* **19(2)** 3561-3566.
 21. Elfallah HM, & Swain MV (2013) A review of the effect of vital teeth bleaching on the mechanical properties of tooth enamel *New Zealand Dental Journal* **109(3)** 87-96.
 22. Jiang T, Ma X, Wang Y, Tong H, Shen X, Hu Y, & Hu J (2008) Investigation of the effects of 30% hydrogen peroxide on human tooth enamel by Raman scattering and laser-induced fluorescence *Journal of Biomedical Optics* **13(1)** 14-19.
 23. Joiner A, & Thakker G (2004) In vitro evaluation of a novel 6% hydrogen peroxide tooth whitening product *Journal of Dentistry* **32(Supplement 1)** 19-25.
 24. Borges AB, Samezina LY, Fonseca LP, Yui KCK, Borges ALS, & Torres CRG (2009) Influence of potentially remineralizing agents on bleached enamel microhardness *Operative Dentistry* **34(5)** 593-597.
 25. Cavalli V, Rodrigues LK, Paes-Leme AF, Soares LE, Martin AA, Berger SB, & Giannini M (2011) Effects of the addition of fluoride and calcium to low-concentrated carbamide peroxide agents on the enamel surface and subsurface *Photomedicine and Laser Surgery* **29(5)** 319-325.
 26. Malkondu Ö, Yurdagüven H, Say EC, Kazazoglu E, & Soyman M (2011) Effect of bleaching on microhardness of esthetic restorative materials *Operative Dentistry* **36(2)** 177-186.
 27. Xu B, Li Q, & Wang Y (2011) Effects of pH values of hydrogen peroxide bleaching agents on enamel surface properties *Operative Dentistry* **36(5)** 554-562.
 28. Sa Y, Sun L, Wang Z, Ma X, Liang S, Xing W, Jiang T, & Wang Y (2013) Effects of two in-office bleaching agents with different pH on the structure of human enamel: An *in situ* and *in vitro* study *Operative Dentistry* **38(1)** 100-110.
 29. Sun L, Liang S, Sa Y, Wang Z, Ma X, Jiang M, & Wang Y (2011) Surface alteration of human tooth enamel subjected to acidic and neutral 30% hydrogen peroxide *Journal of Dentistry* **39(10)** 686-692.
 30. Sulieman M, Addy M, Macdonald E, & Rees JD (2004) A safety study *in vitro* for the effects of an in-office bleaching system on the integrity of enamel and dentine *Journal of Dentistry* **32(7)** 581-590.
 31. Joiner A (2007) Review of the effects of peroxide on enamel and dentine properties *Journal of Dentistry* **35(12)** 889-896.
 32. Ushigome T, Takemoto S, Hattori M, Yoshinari M, Kawada E, & Oda Y (2009) Influence of peroxide treatment on bovine enamel surface—Cross-sectional analysis *Dental Materials Journal* **28(3)** 315-323.

33. Garrido MA, Giráldez I, Ceballos L, Gómes del Río MT, & Rodríguez J (2011) Nanotribological behavior of tooth enamel rod affected by bleaching treatment *Wear* **271(9-10)** 2334-2339.
34. da Costa Soares MU, Araújo NC, Borges BC, Sales Wda S, & Sobral AP (2013) Impact of remineralizing agents on enamel microhardness recovery after in-office tooth bleaching therapies *Acta Odontologica Scandinavica* **71(2)** 343-348.
35. Zhou J, & Hsiung LL (2007) Depth-dependent mechanical properties of enamel by nanoindentation *Journal of Biomedical Materials Research Part A* **81(1)** 66-74.
36. Silva Costa SX, Becker AB, Rastelli ANS, de Castro Monteiro Loffredo L, de Andrade MF, & Bagnato VS (2009) Effect of four bleaching regimens on color changes and microhardness of dental nanofilled composite *International Journal of Dentistry* **2009** 313845.
37. Yu H, Li Q, Hussain M, & Wang Y (2008) Effects of bleaching gels on the surface microhardness of tooth-colored restorative materials *in situ Journal of Dentistry* **36(4)** 261-267.
38. AlQahtani MQ (2013) The effect of a 10% carbamide peroxide bleaching agent on the microhardness of four types of direct resin-based restorative materials *Operative Dentistry* **38(3)** 316-323.
39. Wang L, Francisconi LF, Atta MT, dos Santos JR, Del Padre NC, Gonini A Jr, & Fernandes KBP (2011) Effect of bleaching gels on surface roughness of nanofilled composite resins *European Journal of Dentistry* **5(2)** 173-179.
40. Takahashi A, Sato Y, Uno S, Pereira PNR, & Sano H (2002) Effects of mechanical properties of adhesives on bond strength to dentin *Dental Materials* **18(3)** 263-268.
41. Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, & De Stefano Dorigo E (2008) Dental adhesion review: Aging and stability of the bonded interface *Dental Materials* **24(1)** 90-101.
42. Tagami J, Nikaido T, Nakajima M, & Shimada Y (2010) Relationship between bond strength tests and other *in vitro* phenomena *Dental Materials* **26(2)** e94-e99.
43. Barcellos DC, Benetti P, Fernandes VV Jr, & Valera MC (2010) Effect of carbamide peroxide bleaching gel concentration on the bond strength of dental substrates and resin composite *Operative Dentistry* **35(4)** 463-469.
44. Dudek M, Roubickova A, Comba L, Housova D, & Bradna P (2013) Effect of postoperative peroxide bleaching on the stability of composite to enamel and dentin bonds *Operative Dentistry* **38(4)** 394-407.
45. Roubickova A, Dudek M, Comba L, Housova D, & Bradna P (2013) Effect of postoperative peroxide bleaching on the marginal seal of composite restorations bonded with self-etch adhesives *Operative Dentistry* **38(6)** 644-654.
46. Heintze SD (2013) Clinical relevance of tests on bond strength, microleakage and marginal adaptation *Dental Materials* **29(1)** 59-84.