Objectives. The aim of this study was to assess the surface microhardness (SMH) of intracoronal dentin exposed to 38% hydrogen peroxide (HP) light-activated or not and to 2% sodium fluoride gel (F2%) or 5% varnish (F5%).

Study design. Intracoronal dentin specimens were exposed to bleaching (B), bleaching and light activation (BL), or no bleaching (NB), followed by F2%, F5%, or no exposure (NF). SMH test was performed. Four specimens of each group were analyzed by scanning electron microscopy (SEM).

Results. Analysis of variance and Tukey test showed higher SMH of NB than BL or B. Specimens exposed to F5% presented the highest SMH and differed from F2% and NF. BL/BL yielded inferior SMH and was similar to B/NF, B/F2%, B/F5%, and NB/NF. NB/F5% showed superior values and did not differ from NB/F2%, B/F5%, and NB/NF.

Conclusions. Bleaching with 38% HP, light-activated or not, reduced the SMH of intracoronal dentin. F5% provided SMH to the level of unbleached specimens. (Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;112:e1-e5)

Because the interest of patients in the esthetics in cosmetic dentistry is growing, more emphasis is given to establish strategies to meet this expectation. Techniques combining preservation of the dental structures with satisfactory esthetic have been required.

Dental discoloration is a common condition that leads patients to seek esthetic treatment. The color alteration is a result of changes in the structure of enamel, dentin, or coronal pulp that interfere with the interaction between light and dental surface. This phenomenon may result from extrinsic or intrinsic causes. For intrinsic staining, endodontically treated teeth may discolor as a consequence of tissue decomposition, intrapulpal hemorrhage, pulp tissue remaining after endodontic therapy, and/or presence of sealer material in the pulp chamber.1,2

Among the methods to recover the compromised esthetics, dental bleaching is a conservative, simple, and cost-effective means to lighten discolored teeth3,4 rather than veneers or total crowns that require extensive wear of sound dental tissue.5

The materials used to lighten root-filled teeth are based on hydrogen peroxide, carbamide peroxide, or sodium perborate (pure or diluted), which eventually can be associated.7,8 The action mechanism of bleaching agents consists of an oxidation reaction with release of free radicals that reaches the dark tissue found within tooth structures, leading to a lighter shade.2,9 To accelerate the chemical bleaching process, a sudden temperature rise in the concentrated agents can be promoted by light sources, such as blue halogen lamps, plasma arch lamp, light-emitting diode (LED), CO2 laser, GaAlAs laser, and ultraviolet irradiation.10-12 The use of light activation allows applying the bleaching agents several times in the same clinic session.3,13

Although the internal bleaching process may provide satisfactory esthetic results, investigations have pointed out that the agents applied in the pulp chamber may have some implications for dental hard tissues.2,8,14-16 Reduction of the dentin microhardness has been described as a consequence of the bleaching procedure.17

Efforts have been devoted to decrease the deleterious effects of the whitening treatment on the properties of dental tissues. Changes to microhardness of the bleached dental surfaces might be minimized with the application of fluoride after whitening.2,18,19 Such effect could be attributed mainly to the potential of fluoride to influence the events of demineralization.20,21

There is a lack of studies investigating whether sodium fluoride at different concentrations would interfere with microhardness changes of the dentin submitted to internal bleaching treatment. Therefore, the aim of the present in vitro study was to assess the influence of the bleaching agent with 38% hydrogen peroxide followed by the application of different sodium fluo-
ride–based products on microhardness of the intracoronal dentin. The first null hypothesis formulated was that there was no difference between the microhardness of the bleached intracoronal dentin when submitted to light activation and fluoride exposition.

**MATERIALS AND METHODS**

This study was independently reviewed and approved by the Ethics Committee in Research of the University of Ribeirão Preto.

Forty-five human permanent maxillary canines, stored in 0.1% thymol solution at 9°C, were selected and rinsed with running tap water for 6 hours to remove any residues of the storage product. Subsequently, teeth were radiographically examined to verify absence of calcification or resorptions in the canals and inspected under ×20 stereomicroscope magnification (Leica Microsystems, Wetzlar, Germany) to exclude those with fracture lines or fissures.

Teeth were cut transversally at the cementoenamel junction to separate crowns and roots using a diamond disk (KG Sorensen, Barueri, Brazil). Each crown was further cut in the buccal-lingual direction. These sections were worn with a diamond cylinder bur (KG Sorensen), obtaining 90 slabs of intracoronal dentin measuring 5 × 5 × 3 mm. The specimens were embedded in autopolymerized acrylic resin (JET-Clássico, São Paulo, Brazil), ground with 280-, 400-, and 1,200-grit silicon carbide papers (3M, Sumaré, Brazil) and polished on cloths with an aluminum oxide suspension (Profilt; S.S. White, Rio de Janeiro, Brazil). Then samples were stored at 37°C in 95% relative humidity.

The samples were randomly assigned to groups: no bleaching (NB; control); bleaching (B); and bleaching with LED-laser light activation (BL). Each group was divided into 3 subgroups (n = 10), according to the fluoride treatment: no fluoride (NF); 2% sodium fluoride gel (DFL; Rio de Janeiro, Brazil) (F2); and 5% sodium fluoride varnish (Duraphat; Colgate, Rio de Janeiro, Brazil) (F5).

The bleaching agent was composed of 38% hydrogen peroxide (Opalescence Xtra Boost; Ultradent Products, South Jordan, UT), and it was applied following the manufacturer’s instructions for endodontically treated teeth. A layer of ~2 mm bleaching gel was applied on the dentin surface for 10 minutes. In the BL group, the gel was activated by LED-laser system (Brightness; Kondortech, São Carlos, Brazil) for 45 seconds. An apparatus to standardize the distance of 10 mm between the light and specimen was used. Subsequently, the gel was aspirated and the surfaces rinsed with 5 mL distilled water. This sequence of procedures was performed 3 times.

On completion of the whitening process, specimens were washed with distilled water for 10 minutes to remove traces of the bleaching agent and dried with sterile gauze. In the subgroups that received sodium fluoride treatment, a 2 mm layer of the allocated fluoridated product was applied on the dentin surfaces for 4 minutes. In the F2 group, specimens were rinsed with 5 mL distilled water followed by aspiration. In the F5 group, the excess product was removed with gauze, followed by rinsing with 5 mL distilled water and aspiration.

Subsequently, specimens were tested for surface microhardness (HMV2; Shimadzu, Tokyo, Japan). Three indentations spaced 200 μm apart were made 250 μm from the edge of each slab. A 25 g indentation load was applied for 10 seconds. The triplicates were averaged and expressed as a mean for each specimen.

Four specimens of each subgroup were prepared for morphologic analysis under scanning electron microscopy (SEM). The samples were fixed in 2.5% glutaraldehyde (KGaA, Merck, Darmstadt, Germany) buffered with 0.1 mol/L sodium cacodylate buffer at pH 7.4 for 12 hours at 4°C. After fixation, the sections were rinsed with distilled water. They were then sequentially dehydrated in ascending grades of ethanol (25%, 50%, 75%, and 95%) for 20 minutes each and 100% for 60 minutes. All samples were dried, mounted on aluminum stubs (Ted Pella), placed in a vacuum chamber and sputter-coated with a gold layer of approximately 300 Å (Bal-Tec SCD 005, Bal-Tec CO, USA). They were examined under a field-emission scanning electron microscope (JSM 5410; Jeol, Tokyo, Japan) operating at 10.0-20.0 kV. The most representative areas were photographed, at ×2,000 magnification.

After the assumptions of equality of variances and normal distribution had been checked, the data were analyzed using two-way analysis of variance (ANOVA) followed by the Tukey test at a significance level of 5% (InStat, GraphPad Software, San Diego, CA).

**RESULTS**

**Microhardness analysis**

Two-way ANOVA revealed significant difference for the bleaching procedure (P < .05), fluoride treatment (P < .05), and interaction between these factors (P < .05).

Considering the bleaching factor, Tukey test showed that the unbleached specimens (NB) presented statistically higher values of microhardness (P < .05) than those bleached with (BL) or without (B) light activation by the LED-laser, which did not differ from each other (P > .05).
Table 1. Microhardness data [mean (SD)] for the different treatments, expressed in Knoop hardness number

<table>
<thead>
<tr>
<th>Fluoride treatment</th>
<th>No fluoride</th>
<th>2% fluoride gel</th>
<th>5% fluoride varnish</th>
</tr>
</thead>
<tbody>
<tr>
<td>No bleaching</td>
<td>62.02 (7.86)abcd</td>
<td>69.04 (9.25)pab</td>
<td>75.97 (8.92)abcd</td>
</tr>
<tr>
<td>Bleaching</td>
<td>53.56 (12.32)ab</td>
<td>55.56 (8.99)pab</td>
<td>65.01 (10.15)pabcd</td>
</tr>
<tr>
<td>Bleaching with light activation</td>
<td>49.7 (11.05)a</td>
<td>53.57 (12.8)pab</td>
<td>61.15 (7.94)pabcd</td>
</tr>
</tbody>
</table>

Different letters indicate statistically significance difference at 5% from each other.

For the factor of fluoride treatment, the application of 5% sodium fluoride varnish in the dentin samples yielded the highest microhardness values ($P < .05$) compared with the specimens that did not receive fluoride treatment or were exposed to 2% sodium fluoride gel, which were similar to each other ($P > .05$).

Tukey test applied for interaction between the factors (Table 1) showed that BL + NF provided inferior microhardness and was statistically similar to B + NF, BL + F2, B + F2, BL + F5, and NB + NF, which did not differ from each other ($P > .05$). The samples submitted to NB + F5 presented superior microhardness values and were similar to NB + F2, B + F5, and NB + NF, which were not statistically different from each other ($P > .05$). NB + F2, B + F5, NB + NF, BL + F5, B + F2, BL + F2, and B + NF showed intermediate values.

**SEM analysis**

SEM analysis showed that, regardless of the fluoride treatment, the samples not subjected to the bleaching presented areas of remaining smear layer and occluded dentinal tubules (Fig. 1, A-C). On the other hand, when the bleaching process was carried out, regardless of the light activation, the dentinal tubules were found to be partially exposed (Figs. 1, D-F). In addition, the bleached specimens treated with fluoride showed reduced smear layer, and those exposed to varnish exhibited partially obliterated tubules (Fig. 1, F).

**DISCUSSION**

Internal bleaching is commonly used in discolored endodontically treated anterior teeth. Although the bleaching agents used are effective in this process, chemical alterations in dentin structure with subsequent changes in biomechanical properties have been reported, especially the decrease in its microhardness.$^{2,4,17}$ Such changes can be related to the reduction of the mineral content of enamel$^{22}$ and organic components of dentin,$^{1,23}$ as well as alterations in the morphology of the dental substrate.$^{15}$

The outcome of the present study demonstrated that the whitening treatment, regardless of light activation, resulted in significantly lower dentin microhardness, corroborating other studies.$^{4,18,23}$ A feasible explanation is that 38% hydrogen peroxide can lead to demineralization and reduction of the organic components, as a result of protein oxidation.$^{2,23}$ On the other hand, light activation by the LED-laser system did not influence the dentin microhardness, a result similar to that found by Gomes et al. (2009).$^{24}$ Although it has been reported that some degree of tooth degradation and demineralization may occur as a consequence of the photothermal effect,$^{24}$ it is speculated that the increase of temperature provided by the LED-laser system was not enough to alter the microhardness.

The application of 5% sodium fluoride varnish after the bleaching procedure effectively protected the decrease in dentin microhardness, differing from the groups that were not exposed to fluoride or were treated with 2% gel. Studies have shown that fluoride enhances the resistance to demineralization and favors the remineralization of the softened dental tissue.$^{21,26}$ Especially by forming a calcium fluoride (CaF$_2$)–like layer on the tooth surface.$^{21}$ The highly fluoridated products may lead to a more stable CaF$_2$–like layer$^{27,28}$ and better protect the dental surface. This aspect may explain the highest values of microhardness provided by 5% varnish. An additional benefit of fluoride varnish is the mechanical protection yielded by the persistence of the product on the tooth surface and within the dentinal tubules.$^{29}$

The fact that the microhardness values of specimens treated with 2% gel and not exposed to fluoride were statistically similar to each other may be related to the single fluoride gel exposition for 4 minutes, whereas the literature recommends 4 sessions of 4 minutes each.$^{27}$ The exposure time has been shown to be another factor related to the amount and density of CaF$_2$–like layer.$^{30,31}$ Therefore, the length of exposure to 2% fluoride performed in the present study may not have been enough to control the decrease in microhardness.

Considering that the remineralization potential of saliva can be increased by fluoride,$^{32}$ it is speculated that the fluoride benefits could be more evident in dental surfaces exposed to saliva. Nevertheless, in the experimental protocol adopted, specimens were not exposed to artificial saliva. This conduct aimed to mimic the clinical condition of internal dental bleaching, in which the contact of the pulp chamber with the oral environment is not allowed.

Analyzing the interaction between the factors of bleaching procedure and fluoride treatment, it was ver-
ified that the application of 5% fluoride varnish subsequent to the whitening process reestablished the dentin microhardness to values similar to those of the control specimens not bleached and not treated with fluoride. Investigations have demonstrated the potential of fluoride in remineralizing the bleached surface, even in low concentration.18,19,33 The lack of significant protection of the 2% fluoride gel against the reduction of dentin microhardness could be attributed to the methodologic differences between the cited studies and the present experiment, such as protocols of bleaching and fluoride application, substrate, and method of storage.

The qualitative SEM analysis showed the morphologic effects of the bleaching procedure on dentin, with presence of open dentinal tubules and reduction of the smear layer. In the bleached specimens treated with 5% sodium fluoride varnish, the dentinal tubules were found partially obliterated, probably due to the remaining varnish deposited on the surface.

This in vitro study suggested that fluoride varnish might be recommended to decrease the changes on surface microhardness of the intracoronal dentin submitted to bleaching with 38% hydrogen peroxide. Nevertheless, further laboratory and clinical investigations

Fig. 1. Photomicrographs of intracoronal dentin (×2000). Unbleached dentin (A) untreated with fluoride and exposed to (C) fluoride gel and (E) fluoride varnish: presence of remaining smear layer and closed dentinal tubules. Bleached dentin followed by (B) no fluoride treatment and application of (D) fluoride gel and (F) fluoride varnish: exposed tubules and reduction of the smear layer.
are required to ascertain the validity of this finding and to assess the role of different products and protocols of whitening and fluoride treatments on dentin properties.

It can be concluded that dental bleaching with 38% hydrogen peroxide, light-activated by the LED-laser system or not, decreased the microhardness of the intracoronal dentin. The application of 5% sodium fluoride varnish on the bleached dentin reestablished the microhardness values to the level of unbleached dentin.

REFERENCES


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