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Effects of Light Activated In-office Bleaching on Permeability, Microhardness, and Mineral Content of Enamel

SO Parreiras • P Vianna • S Kossatz
AD Loguercio • A Reis

Clinical Relevance

The use of LED/laser with in-office bleaching gel does not produce severe alterations in dental enamel.

SUMMARY

The aim of this study was to evaluate the permeability (PE), microhardness (KHN), and mineral change in enamel after LED/laser activated in-office bleaching. For PE, the coronal portion of premolars (n=51) was subjected to bleaching with 35% hydrogen peroxide (Whiteness HP Maxx, FGM Dental Products, Joinville, SC, Brazil). The samples were stained via the histochemical method, which involves a copper sulphate solution and rubeanic acid. The penetration of dye into the enamel was measured. The KHN of enamel was assessed before treatment, immediately after the bleaching treatment, and again after one week. The calcium and phosphorus content were analyzed with a scanning electron microscope with energy-dispersive X-ray (JSM 6360LV, Jeol Ltd, Tokyo, Japan). The data set from each test was subjected to appropriate parametric statistical analysis (α=0.05). No significant differences were observed for PE in NLA and LA compared to the control group (p=0.98), as well as for calcium (p=0.16) and phosphorus (p=0.80) content. Significant reduction of KHN after bleaching occurred for both groups ($p<0.001$). After immersion in artificial saliva, the KHN of the enamel for all groups was similar to that seen before bleaching. Light activation during in-office bleaching does not produce significant changes in the enamel compared to a non–light-activated technique.

Sibelli Olivieri Parreiras, DDS, MS student, Department of Restorative Dentistry, School of Dentistry, State University of Ponta Grossa, Uvaranas, PR, Brazil

Priscilla Vianna, DDS, School of Dentistry, Department of Restorative Dentistry, State University of Ponta Grossa, Uvaranas, PR, Brazil

Stella Kossatz, DDS, MS, PhD, associate professor, Department of Restorative Dentistry, State University of Ponta Grossa, Uvaranas, PR, Brazil

Alessandro D Loguercio, DDS, MS, PhD, adjunctive professor, Department of Restorative Dentistry, State University of Ponta Grossa, Uvaranas, PR, Brazil

*Alessandra Reis, DDS, PhD, adjunctive professor, Department of Restorative Dentistry, State University of Ponta Grossa, Uvaranas, PR, Brazil

*Corresponding author: Rua Carlos Cavalcanti, 4748, Bloco M, Sala 64A, Uvaranas, Ponta Grossa, PR 84030-900, Brazil; e-mail: reis_ale@hotmail.com

DOI: 10.2341/13-031-L

INTRODUCTION

With a growing awareness of dental esthetic options, there is subsequently a greater demand for cosmetic solutions. Within this context, vital tooth bleaching is one of the cosmetic dental procedures requested most often by patients who want a more pleasant smile. At-home bleaching systems are the most frequently recommended treatment for vital teeth.[1] However, as reported by Marson and others,[2] some patients do not adapt to the technique, mainly because they prefer not to use a bleaching tray or do not like to wait two to three weeks to see the results of their treatment. These patients might request a method that produces more immediate results, such as the in-office bleaching technique.

Since the introduction of in-office bleaching protocols, the use of curing lights has been recommended to accelerate the action of the bleaching gel.[3] The theoretical advantage of a light source is its ability to heat the hydrogen peroxide (HP), thereby increasing the kinetic energy of the molecules and the rate of decomposition of oxygen to form oxygen-free radicals and enhance the bleaching outcome.[4,5]

Even though the body of research is not definitive on the use of light-enhanced bleaching, with many conflicting results having been published,[2,6-14] patients often demand its use due to media coverage. Many clinicians look upon light-activated bleaching as an important factor for patient satisfaction and recognize that many current systems use light activation in the process of tooth whitening.

Thus, it is relevant to compare the side effects of light-activated vs non–light-activated bleaching in order to discover whether the benefits can be said to outweigh the side effects. For instance, it has been consistently reported that light-activated bleaching is associated with increased tooth sensitivity.[9-11,13,14] Although this can be due to significant increases in pulpal temperatures produced by light activation,[15-18] one cannot rule out the fact that modifications to the enamel structure caused by the association of light activation and HP might be responsible for this increased sensitivity. Therefore, the aim of this study was to evaluate the effects of in-office bleaching associated with light emitting diode (LED)/laser light activation on the permeability, microhardness, and mineral content of enamel.

METHODS & MATERIALS

Seventy-seven caries-free premolars, stored in distilled water and with no coronal cracks or enamel malformations, were randomly selected from a pool of extracted teeth of unknown origin. The use of extracted human teeth followed a protocol that was reviewed and approved by the local ethics committee of the local university under protocol 82/2009.

Enamel Permeability

Fifty-one premolars were scraped of any remaining soft tissues and polished with a pumice slurry. Subsequently, a 7.0 mm^2 circular area was isolated on the labial surface of each tooth by applying cyanoacrylate resin (Super Bonder, Loctite, São Paulo, SP, Brazil) and three layers of nail varnish (Nati Esmaltes, Nati, Moóca, SP, Brazil) to the remaining surfaces of the tooth. Afterwards, specimens were randomly divided in three groups and kept in distilled water at room temperature until exposure to the bleaching agent.

In the non–light-activated (NLA) group, the 35% HP gel (Whiteness HP Maxx, FGM Dental Products, Joinville, SC, Brazil) with a pH of 6.5 was applied in the exposed enamel area following the manufacturer's directions. The pH of the gel was measured in triplicate with a pH meter (pHmetro Nova Técnica NT-PHM, Piracicaba, SP, Brazil). Three 15-minute applications were performed in each bleaching session. Two consecutive bleaching sessions with no interval were performed. In the light-activated (LA) group, the same procedure was repeated; however, LED/laser energy (Whitening Lase Light Plus, DMC Odontológica, São Carlos, SP, Brazil) was used following the manufacturer's directions. This light source was made of a matrix of LEDs with a wavelength of 470 nm and three infrared laser diodes with a wavelength of 830 nm and a radiant energy of 200 mW. The tooth surfaces were activated for one minute. The device was turned off for two minutes. This procedure was repeated three times for each 15-minute application of the gel. Specimens from the control group were not submitted to any bleaching protocol and were maintained in distilled water until immersion in the dye.

After the aforementioned procedures, specimens of all groups were immersed in 35 mL of 10% copper sulfate aqueous solution and kept in a vacuum (Bomba de Vácuo e Compressor de Ar, Pump37, Exipump, Paulinia, SP, Brazil) for five minutes under 20 psi. This procedure was done to remove air from the dental substrate and allow for the highest dye penetration. Specimens were soaked in 35 mL of the solution for an additional 10 days. After being dried with absorbent paper, specimens were immersed in a 1% dithiooxamide acid alcoholic solution with a pH of 4.6, following the same protocol

as outlined for the copper sulfate solution. Afterwards, specimens were rinsed with distilled water for 15 seconds, dried, and stored in a covered vial containing ammonia vapor for 7 days. Copper ions were revealed by the dithiooxamide acid, resulting in specific colorations that ranged from dark blue to black, depending on the amount of copper ion penetration.[19] Each specimen was embedded in self-curing acrylic resin and sticky wax and three buccolingual tooth sections of 700 μm were cut longitudinally at the middle of the exposed area using a low-speed diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA). Slabs were then slightly abraded and polished by hand with silicon carbide abrasive papers of decreasing coarseness up to approximately 300 μm.

The sections were examined at 40× magnification with a light microscope (Olympus model BX 51, Olympus Optical Co, Tokyo, Japan). Digital images of the sections were recorded, and dye penetration along the enamel thickness was evaluated with an image-analyzing system (Image Tool 3.0 software, University of Texas Health Science Center, San Antonio, TX, USA) by one trained evaluator. Permeability was quantitatively analyzed as the percentage of copper ion penetration over the total enamel thickness. Three measurements of dye penetration were performed per tooth. The average of these measurements was the outcome value for each tooth. The data were submitted to a one-way analysis of variance (ANOVA). Multiple comparisons were made utilizing the Tukey test at a significance level of 0.05.

Enamel Microhardness

Twenty human premolars had their roots cut with a diamond saw (Isomet 1000, Buehler) under water cooling. Each tooth crown was embedded in a self-curing methylmethacrylate resin (AutoClear, Dentbras, Pirassununga, SP, Brazil). Enamel buccal surfaces were flattened with wet 600-, 1000-, 1200-, 2000-, and 2200-grit aluminum oxide abrasive papers (3M, Sumaré, SP, Brazil). They were then polished with 1-μm grit and 0.25-μm grit diamond pastes with a polishing machine (Aropol E, Arotec SA, Cotia, SP, Brazil) until a 3-mm long by 3-mm wide flat enamel surface was achieved.

The specimens were kept in artificial saliva (0.0625% KCl, 0.0865% NaCl, 0.0056% $MgCl_2$, 0.0166% $CaCl_2$, 0.0804% Na_2HPO_4, 0.0326% KH_2PO_4, 4.274% sorbitol, 0.0004% NaF, 0.1% C_6H_5COONa, 2% carboxymethylcellulose, and distilled water)[20] at room temperature for one week and were then randomly divided into two groups (NLA and LA groups; n=10). These enamel specimens were submitted to the same bleaching procedure described in the enamel-permeability section. Microhardness measurements were taken before initial exposure to the bleaching agents (baseline), immediately posttreatment, and one week after immersion in artificial saliva. Specimens were positioned to record the Knoop hardness (KHN) with a load of 25 g during five seconds in the microhardness tester HMV (HMV2, Shimadzu Co, Tokyo, Japan). Three measurements were performed on each specimen in each evaluation period, and these values were averaged to determine the KHN of each specimen at each evaluation period. The data from the microhardness tester were analyzed by repeated measures via two-way ANOVA and Tukey test at a significance level of 0.05, considering treatment and evaluation time (baseline, after treatment, and one week in artificial saliva) as the main factors. The repeated measure was the evaluation time.

Mineral Evaluation by Scanning Electron Microscope

Six premolars had their roots removed from the crowns with a diamond saw (Isomet 1000, Buehler) under water cooling. Three buccal to lingual tooth sections were cut longitudinally using a low-speed diamond saw. The fragments were isolated with cyanoacrylate resin (Super Bonder, Loctite) with the exception of the buccal areas, and each fragment was assigned to a different treatment. No bleaching procedure was performed on one fragment (control), while the two other fragments were submitted to LA and NLA bleaching as described in the enamel permeability test. Six fragments from different teeth were used for each group.

Specimens were dried in a desiccator containing colloidal silica for 24 hours at 37°C. Specimens were then mounted on stubs and sputter-coated with a 10-nm gold layer to be analyzed in SEM (JSM 6360LV, Jeol Ltd, Tokyo, Japan) in the secondary electron mode with dispersive X-ray spectrometry energy (EDX). The ratings of EDX quantified the levels of calcium and phosphorus of the enamel surfaces. The levels of calcium and phosphorus were evaluated by a one-way ANOVA and Tukey test for multiple comparisons at a significance level of 0.05.

RESULTS

The means and standard deviations of the dye penetration (%), calcium (%), and phosphorus (%) are reported in Table 1. The one-way ANOVA did not

Table 1: *Means and Standard Deviations of Dye Penetration (%), Calcium (%), and Phosphorus (%) in Enamel Surfaces for the Experimental Conditions*[*]

Groups	Dye Penetration	Calcium	Phosphorus
Control	9.9 ± 4.2[a]	21.4 ± 4.2[a]	14.7 ± 2.53[a]
NLA	9.3 ± 2.9[a]	24.5 ± 3.0[a]	14.0 ± 2.9[a]
LA	9.4 ± 2.9[a]	24.2 ± 4.2[a]	14.1 ± 2.1[a]

Abbreviations: LA, light-activated; NLA, non–light-activated.
[*] *Comparisons are valid only within columns. Identical letters indicate statistically similar means (p>0.05).*

Table 2: *Means and Standard Deviations of Enamel Microhardness for the Experimental Conditions*[*]

Groups	Baseline (Control)	Posttreatment	One Week After
NLA	359.5 ± 35.0[Aa]	341.6 ± 25.5[Bb]	375.2 ± 31.9[Ac]
LA	358.3 ± 30.1[Aa]	319.8 ± 53.0[Bb]	372.5 ± 26.6[Ac]

Abbreviations: LA, light-activated; NLA, non–light-activated.
[*] *Uppercase letters indicate comparisons within rows. Lowercase letters indicate comparisons within columns. Identical letters indicate statistically similar means (p>0.05).*

show any statistical difference among groups with regard to the dye penetration (p=0.98), calcium (p=0.16), and phosphorus (p=0.80). Representative images of enamel after permeability evaluation are seen in Figure 1.

With regard to enamel microhardness data (Table 2), the two-way ANOVA detected that the interaction treatment vs the evaluation period (p=0.25) and the main factor treatment (p=0.514) were not statistically significant. On the other hand, the main factor evaluation period was significant (p<0.001). Both groups showed reductions in enamel microhardness after bleaching, which recovered to baseline values after 1 week of immersion in artificial saliva (Table 2).

DISCUSSION

It has been reported that HP and its byproducts can easily pass through enamel and dentin and reach the pulp tissue. This can occur in approximately five to 15 minutes.[21] Further proof of this rapid passage is that dentin changes color next to the pulp as fast as it does next to the dentin-enamel junction.[22] Although there is some acceptance that such fast passage is due to an increase in enamel permeability produced by the oxidizing HP agent,[19,23] the results of this study do not support such a concept.

We did not detect significant differences in the enamel permeability specimens among the three groups. Although enamel is both extremely hard and dense, it also has a porosity[24] that allows HP penetration. It is likely that HP and its byproducts penetrate into the enamel through these porosities by a diffusion process, similar to what occurs after fluoride application.[25]

So far, only a few studies in the literature have attempted to investigate enamel permeability after bleaching using the histochemical coloring method herein employed.[19,23] Although earlier studies demonstrated that this methodology was capable of detecting differences between the enamel permeability of bleached and unbleached specimens, the clinical relevance of these results is yet to be addressed.

The slightly acidic pH of the gel employed in this study, around 6.5, may also explain why decreases in the calcium and phosphorus levels were not detected. Although this finding is in agreement with other studies that evaluated mineral content by EDX and Fourier transform infrared spectroscopy,[26,27] it is contrary to others that detected reductions in the mineral levels after bleaching.[28-30] It is likely that

Figure 1. *Dye penetration in enamel in (A): control group; (B) light-activated group; and (C) non–light-activated group. (D): Dentin. (E): Enamel. * represents the penetration of the dye.*

this controversy may be due to differences in the bleaching protocol, number of sessions, period of evaluation, and bleaching product employed. More pronounced morphologic alterations might be observed if bleaching gels with a lower pH were employed.[31]

With regard to the microhardness measurement, a significant decrease in enamel microhardness was observed immediately after bleaching for both groups. Although literature on this topic is controversial,[32] *in vitro* studies frequently report drops in enamel microhardness when measurement is done soon after bleaching.[33-35]

When enamel microhardness was evaluated one week after immersion in artificial saliva, no significant alteration was observed for either bleached group. The hardness reductions induced by bleaching under *in vitro* conditions usually resolve themselves due to the remineralizing impact of saliva.[33] The closer we get to the oral conditions, the less likely it is that there will be any loss of microhardness. For instance, the only *in vivo* study that evaluated microhardness after bleaching did not report any loss of microhardness.[36,37]

Despite the fact that the benefits of light activation on bleaching effectiveness are still controversial in the literature,[2,6-14,18] many clinicians still perform this procedure due to media coverage and patient request. Based on the findings of this study, we cannot consider light-activated bleaching to be more detrimental to enamel than the chemical bleaching alone. It seems reasonable to conclude that either bleaching alone or associated with light activation produce similar effects on the enamel surface.

CONCLUSION

Although the benefit of light-activated bleaching is still doubtful in terms of color change, light-activated bleaching did not increase enamel permeability and did not reduce the mineral content and microhardness of enamel.

Acknowledgements

The authors would like to thank FGM Dental Products for the generous donation of the products employed in this study. This study was partially supported by the National Council for Scientific and Technological Development (CNPq) under grants 301937/2009-5 and 301891/2010-9.

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.

(Accepted 9 December 2013)

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