Microstructural effect of a laser-activated bleaching agent containing titanium dioxide on human enamel

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Abstract

Aim: This study aimed to evaluate the effect of a laser-activated bleaching agent containing titanium dioxide (TiO₂) nanoparticles on enamel roughness and hardness.

Materials and Methods: Twenty human premolars were randomized into two groups according to the bleaching treatments performed: HP – 35% hydrogen peroxide and HP + TiO₂ – 30% hydrogen peroxide containing TiO₂ light-activated by diode laser (980 nm). It was performed two bleaching sessions with an interval of 7 days. Microhardness and roughness of the enamel were assessed at three times: T0 – Before 1st appointment, T1 – after 2nd appointment, and T2 – 7 days after 2nd appointment.

Results: The HP + TiO₂ did not cause changes on enamel roughness and hardness and presented the same effects of the HP.

Conclusions: Both bleaching agents showed no difference between them. Then, it is possible to conclude that both are viable for clinical use during in-office dental bleaching technique regarding the microstructural changes that they might cause.

Keywords: Hardness; hydrogen peroxide; laser; roughness; titanium dioxide

INTRODUCTION

Smile esthetics' and tooth color are considered of great importance for patients. Therefore, the desire for white teeth among the population is one of the responsible factors for the increase in patients’ number in dental offices looking for dental bleaching procedures.

Under proper conditions, dental bleaching is a low-cost and effective procedure for the treatment of discolored teeth and it is also possible to apply it for a short period of time. Bleaching agents are mostly based on hydrogen peroxide (H₂O₂) and can be used in different techniques by professionals and/or by patients, at dental offices and/or at home.

Considering the technological advance, a new in-office bleaching agent, containing titanium dioxide nanoparticles (TiO₂), have been introduced into the market. Its use has the premise of enhance H₂O₂ action, reduce the operative time, and the number of bleaching sessions. These features may decrease the dental structure damage and the occurrence of tooth sensitivity during and after bleaching procedures. It is important to note that the use of a light source is necessary to activate the TiO₂ photocatalytic oxidation reaction. Diode laser (light amplification by stimulated emission of radiation), represents an example of light source that can be used. Despite the advantages, there still are hesitations in the use of H₂O₂ containing TiO₂ regarding the structural changes of the dental tissue. Moreover, the use of light units and their consequences is not clearly elucidated.

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Thus, this study aimed to evaluate the effects of a laser-activated bleaching agent containing TiO$_2$ nanoparticles on hardness and surface roughness of the human enamel. The null hypothesis investigated was that both bleaching agents tested do not provide changes on the human enamel.

MATERIALS AND METHODS

Experimental design
This study was single-blind (statistician). The teeth were randomized into two groups, with a similar allocation rate, according to the type of enamel bleaching treatments [Table 1].

Samples’ preparation
Twenty ($n = 20$) human premolars were used. These teeth did not have enamel defects or cracks visible in a stereoscopic magnifying lens ($\times 10$). The premolars were extracted because of periodontal disease or orthodontic treatment. This study received approval from the Research Ethics Committee in Involving Human Beings of State University of Londrina (CEP UEL 1.730.528).

Premolars were cleaned using periodontal curettes (Trinity Indústria e Comércio Ltda., Jaraguá, São Paulo, Brazil) and disinfected by 0.1% thymol solution (Dinâmica, Piracicaba, São Paulo, Brazil). The teeth were sectioned at 1–2 mm below the enamel/cementum junction using flexible diamond disks (7020, KG Sorensen, São Paulo, Brazil). In sequence, pulp chamber of each tooth was cleaned with dentin excavators (Nº 5, Duflex, S.S. White, Rio de Janeiro, Brazil) and endodontics files (K, Maillefer instruments AS-CH 1338, Ballaigues, Switzerland). After this, a pulp chamber irrigation was performed with 1% sodium hypochlorite solution (Ciclo Farma Indústria Química, São Paulo, Brazil) and 0.9% sodium chloride (Fresenius Kabi Indústria e Comércio Ltda., Jaraguá, São Paulo, Brazil) at 37ºC, which was changed every 2 days.

In both groups, HP and HP + TiO$_2$, two bleaching sessions were performed, with an interval of 7 days. After each session, the samples were immersed in artificial saliva (Odontofarma Farmácia de Manipulação, Londrina, PR, Brazil) at 37ºC, which was changed every 2 days.

Roughness and microhardness tests
On the buccal surface of the samples, the enamel roughness (left half) and the enamel hardness (right half) were evaluated. For each test, three measurements were performed at different times: T0 (before 1st bleaching session—control group), T1 (after 2nd bleaching session), T2 (7 days after 2nd bleaching session). All the measurements were performed by the same calibrated operator.

Roughness test
Samples were submitted for roughness evaluation using a portable profilometer (Mitutoyo Sul América Ltda., Santo Amaro, SP, Brazil), which had been calibrated before the readings. The standard roughness (Ra) was measured using a cut off value of 0.25 mm and a speed of 0.1 mm/s. Three occlusal-cervical equidistant readings were taken, in areas visibly more regular, at each time reading.

Bleaching treatment
In HP + TiO$_2$ samples, the bleaching agent was applied and activated, for three times, using a diode laser for 15 s (DCLase, DC International LLC, Wellington, FL, USA; 980 nm wavelength). The bleaching agent remained in contact with the tooth surface for 5 min, according to the manufacturer’s instructions.

In HP group, only one bleaching agent application was performed, which remained in contact with the tooth surface for 40 min. Each 10 min, a microbrush was used to move the bleaching agent, release any oxygen bubbles, and renew the contact of the gel with the teeth.

After, the enamel surface was planned with sandpaper of increasing granulation, #400, #600 and #1200 using a politrix machine (Teclag – Tecnologia em Máquinas Metalográficas, Vargem Grande Paulista, São Paulo, Brazil). Surface polishing was performed using diamond pastes and felt discs (Top, Gold, e Ram, Arotec Ind e Com Ltda). The samples were washed for 10 min in an ultrasonic cleaner using distilled water, to remove any residue from the abrasives.

After that process, all the samples were submitted to surface hardness assessment in order to standardize the samples and select those with $270 \pm 5$ Vickers hardness number for further experiments. Samples were stored in distilled water at 37ºC until the beginning of the experimental procedures.

The samples were covered, except for the enamel surface, by an acrylic resin, inside of polyvinyl chloride tubes.

Table 1: Experimental design

| Group        | Treatment                                                                 | Product (manufacturer)                  | $n$ | Tests                           |
|--------------|---------------------------------------------------------------------------|-----------------------------------------|-----|---------------------------------|
| HP           | Dental bleaching with 35% H$_2$O$_2$ on a light activated by diode laser  | Whiteness HP (FGM Produtos)            | 10  | Roughness and hardness           |
| HP + TiO$_2$ | Dental bleaching with 30% H$_2$O$_2$, containing TiO$_2$, and light       | Odontológicos Ltda, Joinville, SC,     |     |                                 |
|              | activated by diode laser (980 nm wavelength)                              | Brazil)                                 |     |                                 |

H$_2$O$_2$: Hydrogen peroxide, TiO$_2$: Titanium dioxide
Microhardness test
Samples were submitted to Vickers microhardness evaluation (Mitutoyo Sul América Ltda., Santo Amaro-SP-Brasil) using a static load of 25 g for 10 s. Three indentations were performed in the right region of the sample surface with 100 µm between each indentation, at each time reading.

Statistical analysis
The normal distribution was verified by Shapiro–Wilk test ($P > 0.05$). After that, all groups presented normal distribution allowing the use of a parametric analysis. ANOVA two-way followed by Bonferroni test was used. All analyzes were performed at a significance level of 5% using the statistic software SPSS Statistics version 22 (International Business Machines Corp., NY, USA).

RESULTS
The dental bleaching performed did not change roughness [Table 2] and microhardness [Table 3] of the enamel for both the groups (GPHTiO$_2$ and GPH). Moreover, there was no statistical difference between the time readings ($P > 0.05$), except for microhardness between T0 and T1 ($P = 0.028$).

DISCUSSION
In this study, the null hypothesis was accepted showing that there were no changes on roughness and microhardness after dental bleaching procedures using both bleaching agents (HP + TiO$_2$ and HP) for T1 and T2 when compared to the control group (T0).

These results may have been favored by the fact that all the samples were immersed in artificial saliva after the dental bleaching sessions, with systematic refreshing at 2-day intervals to simulate the oral conditions, the human saliva buffering and remineralization capacity. As a result, a neutralization of the bleaching agents’ adverse effects could be provided by the saliva.$^{[11-15]}$

No significant differences were observed in surface roughness of the enamel after dental bleaching sessions, regardless of the bleaching agent used, according to other findings in the literature.$^{[12,16,17]}$ Therefore, it may be inferred that the proper use of bleaching agents, according to the manufacturer’s guidelines, do not cause deleterious effects to the enamel surface micromorphology.$^{[12]}$ and make them safe for clinical practice.

Regarding the enamel microhardness, no differences were observed before and after dental bleaching procedures, because both evaluated agents were not able to provide a significant mineral loss to provide structural changes. These results corroborate with other studies, in which the application of 35% H$_2$O$_2$ also did not decreased the enamel surface microhardness.$^{[13,16,18]}$

Currently, the modernization of procedures has been aimed to simplify techniques. Therefore, semiconductors such as TiO$_2$ have been added to the hydrogen peroxide bleaching agents to accelerate the bleaching process, favoring the biocompatibility of the final product, avoiding or reducing the side effects, such as sensitivity after dental bleaching and damages to the dental structure. Thus, these new bleaching agents may be more effective and safer than traditional formulations.$^{[6,9,19]}$ Despite the evidence of the effectiveness of 35% H$_2$O$_2$ as bleaching agents in clinical and laboratory studies, there is still a lack of studies on bleaching agents containing TiO$_2$ nanoparticles.

The use of a light source is necessary for the photocatalytic oxidation reaction of TiO$_2$. Usually, TiO$_2$ has been known as the most important semiconductor photocatalyst reacting to ultraviolet light.$^{[20]}$ However, according to a preview study, TiO$_2$ nanoparticles (3–30 nm) can give scatter but also absorption radiation of approximately 800 nm to 1100 nm, with the effect that the laser energy remains within the gel and is minimally transmitted.$^{[21]}$ Therefore, in this study, the bleaching agent containing TiO$_2$ was light-activated by a diode laser (980 nm), according to the manufacturer’s instructions.

HP + TiO$_2$ samples showed no structural alterations after dental bleaching. Therefore, it is possible to show that the bleaching agents containing TiO$_2$ and the use of the diode laser as a photocatalytic light source are safe options regarding the enamel microhardness and enamel surface roughness properties and can decrease the clinical time. In our study, all the HP + TiO$_2$ bleaching procedures were around 15 min time-consuming, proving to be faster than

### Table 2: Mean±standard deviation of Vickers hardness number of the enamel surface before and after the bleaching treatments (n=10)

| Bleaching agent | Time | T0        | T1        | T2        |
|-----------------|------|-----------|-----------|-----------|
| HP              | T0   | 272.80±26.74ab | 247.30±22.43ab | 273.40±32.22ab |
| HP + TiO$_2$    | T0   | 279.30±46.82ab | 267.00±41.56ab | 273.40±32.22ab |
| HP + TiO$_2$    | T1   | 279.30±46.82ab | 267.00±41.56ab | 273.40±32.22ab |
| HP + TiO$_2$    | T2   | 279.30±46.82ab | 267.00±41.56ab | 273.40±32.22ab |

Different lowercase letters on the same line denote the presence of a statistically significant difference ($P<0.028$), equal capital letters in the same column denote absence of statistically significant difference ($P>0.18$). TiO$_2$: Titanium dioxide

### Table 3: Mean±standard deviation of roughness (µm) of the enamel surface before and after the bleaching treatments (n=10)

| Bleaching agent | Time | T0        | T1        | T2        |
|-----------------|------|-----------|-----------|-----------|
| HP              | T0   | 0.21±0.04ab | 0.20±0.06ab | 0.21±0.05ab |
| HP + TiO$_2$    | T0   | 0.22±0.04ab | 0.18±0.03ab | 0.20±0.04ab |
| HP + TiO$_2$    | T1   | 0.22±0.04ab | 0.18±0.03ab | 0.20±0.04ab |
| HP + TiO$_2$    | T2   | 0.22±0.04ab | 0.18±0.03ab | 0.20±0.04ab |

Equal capital letters in the column and lowercase letters in the line denote absence of significant statistical difference ($P<0.027$). TiO$_2$: Titanium dioxide

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HP (40 min). However, further studies are needed to better understand the behavior of this light source and to evaluate the influence of its use on the dental bleaching efficacy and the temperature increase of the pulp chamber.

The results of the present study diverge from other findings, in which the reduction of microhardness values and increase of surface roughness after bleaching treatments are reported.[14,15,21] These alterations may be associated with the pH of the bleaching agent used due to acidic agents are more likely to cause enamel demineralization;[15,22,24] can be altered because of the exposure time of the agent in contact with the dental structure[15] and to the morphological characteristics related to the structure itself, because the surface variations and enamel permeability can occur between different teeth and individuals. However, these adverse effects can be eliminated by human saliva.[23]

This study also demonstrated the importance of the experimental design in assessing the adverse effects of bleaching agents[26] and in the clinical extrapolation of the results. Therefore, in situ and in vivo studies, which reliably simulate oral conditions, are ideal and necessary. Moreover, the long-term assessment is very important to provide information on the longevity of the treatments and their adverse effects.

According to the results and considering the limitations of this study, both bleaching treatments (HP and HP + TiO₂) were similar in their effect on enamel microstructural changes. Nevertheless, the required devices (light source) to enable the clinical application of the bleaching agents containing TiO₂, ends up increasing its cost in the market and consequently the cost paid by patients. Thus, conventional bleaching with 35% H₂O₂, which exhibited the same effect compared to HP + TiO₂, is a safe option, established by the frequent use and low cost.

CONCLUSIONS

Within the limitations of this study, the bleaching agents did not provide significant changes on the roughness and microhardness of the dental enamel surface, regardless of the analysis time. Moreover, both bleaching agents showed no difference between them. Then, it was possible to conclude that both bleaching agents are viable for clinical use in-office dental bleaching technique.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Joiner A. The bleaching of teeth: A review of the literature. J Dent 2006;34:412-9.
2. Spalding M, Taveira LA, de Assis GF. Scanning electron microscopy study of dental enamel surface exposed to 35% hydrogen peroxide: Alone, with salvia, and with 10% carbamide peroxide. J Esthet Restor Dent 2003;15:154-64.
3. Suleiman M, Addy M, Macdonald E, Rees JS. A safety study in vitro for the effects of an in-office bleaching system on the integrity of enamel and dentine. J Dent 2004;32:581-90.
4. Bortolatto JF, Pretel H, Fierro MC, Luizzi AC, Dantas AA, Fernandes E et al. Low concentration H₂O₂/TiO₂ in office bleaching: A randomized clinical trial. J Dent Res 2014;93:665-71S.
5. Tano E, Otsuki M, Kato J, Sadr A, Ikeda M, Tagami J. Effects of 405 nm diode laser on titanium oxide bleaching activation. Photomed Laser Surg 2012;30:648-54.
6. Bortolatto JF, Trevisan TC, Bernardi PS, Fernandes E, Dovigo LN, Loguercio AD, et al. A novel approach for in-office tooth bleaching with 6% H₂O₂/TiO₂ and LED/laser system-a controlled, triple-blinded, randomized clinical trial. Lasers Med Sci 2016;31:437-44.
7. Cintra LT, Benetti F, da Silva Facundo AC, Ferreira LL, Gomes-Filho JE, Ervolino E, et al. The number of bleaching sessions influences pulp tissue damage in rat teeth. J Endod 2013;39:1576-80.
8. Lee MC, Yoshino F, Shoji H, Takahashi S, Todoki K, Shimada S, et al. Characterization by electron spin resonance spectroscopy of reactive oxygen species generated by titanium dioxide and hydrogen peroxide. J Dent Res 2005;84:179-85.
9. Kishi A, Otsuki M, Sadr A, Ikeda M, Tagami J. Effect of light units on tooth bleaching with visible-light activating titanium dioxide photocatalyst. Dent Mater J 2011;30:723-9.
10. Suemori T, Kato J, Nakazawa T, Akashi O, Igarashi A, Hirai Y, et al. Effects of light irradiation on bleaching by a 3.5% hydrogen peroxide solution containing titanium dioxide. Laser Phys Lett 2005;5:379-83.
11. Borges AB, Guimaraes CA, Bresciani E, Ramos CJ, Borges AL, Rocha Gomes Torres C. Effect of incorporation of remineralizing agents into bleaching gels on the microhardness of bovine enamel in situ. J Contemp Dent Pract 2014;15:195-201.
12. Cadarano M, Breschi L,ucci C, Antonioli F, Visintini E, Prati C, et al. Effect of two in-office whitening agents on the enamel surface in vivo: A morphological and non-contact profilometric study. Oper Dent 2008;33:127-34.
13. Justino LM, Tames DR, Demarco FF. In situ and in vitro effects of bleaching with carbamide peroxide on human enamel. Oper Dent 2004;29:219-25.
14. Mirzaie M, Yassini E, Ganji S, Moradi Z, Chinarf oursh N. A comparative study of enamel surface roughness after bleaching with diode laser and Nd: YAG laser. J Lasers Med Sci 2016;7:197-200.
15. Pimenta-Dutra AC, Albuquerque RC, Morgan LS, Pereira GM, Nunes E, Horta MC, et al. Effect of bleaching agents on enamel surface of bovine teeth: A SEM study. J Clin Exp Dent 2017;9:446-50.
16. Franco LM, Machado LS, Salomão FM, Dos Santos PH, Briso AL, Sundefeld RH. Surface effects after a combination of dental bleaching and enamel microabrasion: An in vitro and in situ study. Dent Mater J 2016;35:13-20.
17. Machado LS, Anchieta RB, dos Santos PH, Briso AL, Tovar N, Janal MN, et al. Clinical comparison of at-home and in-office dental bleaching procedures: A randomized trial of a split-mouth design. Int J Periodontics Restorative Dent 2016;36:251-60.
18. Basting RT, Rodrigues AL, Serra MC. Micromorphometry and surface roughness of sound and demineralized enamel and dentin bleached with a 10% carbamide peroxide bleaching agent. Am J Dent 2007;20:97-102.
19. Sakai K, Kato J, Kurata H, Nakazawa T, Akashi G, Kameyama A, Hirai Y. The amounts of hydroxyl radicals generated by titanium dioxide and 3.5% hydrogen peroxide under 405-nm diode laser irradiation. Laser Phys 2007;17:1062-6.
20. Fujishima A, Honda K. Electrochemical photolysis of water at a semiconductor electrode. Nature 1972;238:37-8.
21. Goharkhay K, Schoop U, Wernisch J, Hart S, De Moor R, Moritz A. Frequency doubled neodymium:yttrium-aluminum-garnet and diode laser-activated power bleaching—pH, environmental scanning electron microscopy, and colorimetric in vitro evaluations. Lasers Med Sci 2009;24:339-46.
22. Pinto CF, Oliveira Rd, Cavalli V, Giannini M. Peroxide bleaching agent effects on enamel surface microhardness, roughness and morphology. Braz Oral Res 2004;18:306-11.

23. Sa Y, Chen D, Liu Y, Wen W, Xu M, Jiang T, et al. Effects of two in-office bleaching agents with different pH values on enamel surface structure and color: An in situ vs. in vitro study. J Dent 2012;40 Suppl 1:e26-34.

24. Sun L, Liang S, Sa Y, Wang Z, Ma X, Jiang T, et al. Surface alteration of human tooth enamel subjected to acidic and neutral 30% hydrogen peroxide. J Dent 2011;39:686-92.

25. Henn-Donassollo S, Fabris C, Gagiolla M, Kerber I, Caetano V, Carboni V, et al. In situ and in vitro effects of two bleaching treatments on human enamel hardness. Braz Dent J 2016;27:56-9.