

William Kabbach

**CLAREAMENTO COM PERÓXIDO DE HIDROGÊNIO A
35% ATIVADO POR DIVERSAS FONTES DE ENERGIA.
ANÁLISE TÉRMICA E MICRODUREZA DO ESMALTE**

Dissertação apresentada ao Programa de Pós-Graduação em Dentística Restauradora, da Faculdade de Odontologia de Araraquara, da Universidade Estadual Paulista para obtenção do título de Mestre em Dentística Restauradora.

Orientador: *Prof. Dr. Marcelo Ferrarezi de Andrade*

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Araraquara, 01 de abril de 2008.

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Resumo e Abstract

Kabbach W. Clareamento com peróxido de hidrogênio a 35% ativado por diversas fontes de energia. Análise térmica e microdureza do esmalte. [Dissertação de Mestrado]. Araraquara: Faculdade de Odontologia da UNESP; 2008.

RESUMO

O objetivo do presente estudo foi avaliar in vitro a variação de temperatura e a microdureza *Knoop* superficial do esmalte durante o processo de clareamento dental com peróxido de hidrogênio a 35% sob irradiação da luz halógena com o comprimento de onda de 400 a 500nm (HL), luz emitida por diodo com comprimento de onda de 460 de 480 (LED) e laser de diodo (DL) de alta intensidade e comprimento de onda de 810nm, em dentes humanos extraídos. Inicialmente investigou-se a variação de temperatura superficial através da termografia de infravermelho e a temperatura do interior da câmara pulpar por meio de termopares em incisivos inferiores humanos, quando submetidos ao clareamento dental com peróxido de hidrogênio a 35% nas cores vermelha (HP) e verde (HPM) irradiados por: HL e LED. Quatro grupos (n=10) foram divididos de acordo com o gel clareador e a fonte de luz. Os resultados foram submetidos à análise de variância e teste de *Tukey* ($p < 0,05$). Os valores médios e desvios padrão do aumento de temperatura dentro da câmara pulpar com a HL foram de 4.4 ± 2.1 °C para HP, e 4.5 ± 1.2 °C para HPM; enquanto que nos grupos usando LED, foram 1.4 ± 0.3 °C para HP, e 1.5 ± 0.2 °C para HPM. Para todas as temperaturas superficiais a variação máxima dos grupos irradiados com HL foi 6.5 ± 1.5 °C para HP, e 7.5 ± 1.1 °C usando HPM; enquanto para os grupos usando LED, foram 2.8 ± 0.7 °C usando HP, e 3 ± 0.8 °C para HPM. Não houve diferença estatística entre o aumento da temperatura pulpar e superficial entre os grupos usando os diferentes géis ($p < 0,05$). As médias de temperatura foram significativamente maiores para os grupos usando HL quando comparados com aqueles irradiados com LED ($p < 0,05$). Ainda foi investigada a variação de temperatura superficial e assim determinado o tempo de demora para ser atingida a variação crítica de temperatura (5,5 °C para câmara pulpar e 10 °C para o periodonto). Quarenta e cinco incisivos inferiores humanos foram divididos em 3 grupos e submetidos ao clareamento dental com HP ativado por fontes HP, LED e DL respectivamente. As médias de temperatura e desvios padrão do aumento da temperatura intrapulpar foram para o HL 6.47 ± 2.78 °C, para o DL foram $15.34 \pm$

8.81 °C e 1.90 ± 0.97 °C para LED. A temperatura superficial para a HL foi 9.13 ± 2.19 °C, para o DL foi 25.66 ± 18.89 °C e 2.58 ± 1.40 °C para LED. A média de temperatura foi significativamente (teste de *Tukey* $p > 0,05$) maior para o grupo irradiado com DL se comparado como irradiado com HL e LED. Aplicando-se os limites inferiores do intervalo de confiança a 95% foi encontrado um tempo de aplicação de 38,68 segundos para a HL e 4,38 segundos para DL. A fonte de luz LED não atingiu as temperaturas críticas para a polpa nem para o periodonto, mesmo quando irradia da por 360 segundos. Investigou-se também a microdureza do esmalte durante o clareamento dental com peróxido de hidrogênio a 35%, sob irradiação de fontes HL, LED e DL. Cento e cinco blocos obtidos de terceiros molares inclusos tiveram a superfície de esmalte planificada e foram feitas medidas iniciais de microdureza *Knoop* com a carga de 25g por 5s. Procedeu-se o tratamento clareador com HP: Grupo A, B e C ativados por HL, LED e DL respectivamente; grupo D, E e F tratados apenas com HP pelos mesmos tempos dos grupos A, B e C, respectivamente, e o grupo G (controle) foi mantido apenas em saliva artificial pelo mesmo período dos outros grupos experimentais. As médias percentuais de dureza e desvio padrão obtidos foram: A: $97,8 \pm 13,1$; B: $95,5 \pm 12,7$; C: $84,2 \pm 13,6$; D: $128,6 \pm 20,5$; E: $133,9 \pm 14,2$; F: $123,9 \pm 14,2$; G: $129,8 \pm 18,8$. A análise estatística (*Tukey* $p < 0,05$) mostrou que as médias percentuais de microdureza dos grupos irradiados pelas fontes de luz são significativamente menores que dos grupos não irradiados. Além disso, os grupos não irradiados mostraram que a saliva artificial foi capaz de aumentar a microdureza durante o tempo de armazenagem. Segundo a análise térmica os resultados sugerem que o LED pode ser usado com segurança para os tecidos pulpar e periodontal quando usados os parâmetros desse trabalho, já a HL e o DL necessitam de maiores cuidados. Considerando as limitações desse estudo, a microdureza do esmalte foi diminuída quando as fontes de luz foram utilizadas durante o processo de clareamento e a saliva artificial foi capaz de aumentar a microdureza quando nenhuma luz foi irradiada.

Palavras chave: Clareamento de dente; periodonto; polpa dentária; calor; dureza; fonte de luz.

Kabbach W. Bleaching with 35% hydrogen peroxide active for several light sources, thermal and enamel microhardness analyses. [Dissertação de Mestrado]. Araraquara: Faculdade de Odontologia da UNESP; 2008.

ABSTRACT

The objective of this in vitro study was to evaluate the variation of temperature and Knoop microhardness on enamel surface during the bleaching process with 35% hydrogen peroxide under irradiation by halogen light with the wavelength of 400 to 500nm (HL), LED with the wavelength of 460 to 480nm and high intensity diode laser LED with the wavelength of 810nm (LD) on extracted human teeth. Initially were investigated the variation of surface temperature (infrared thermography) and the temperature of the interior of the pulp chamber (thermocouples) in human mandibular incisors when subjected to dental bleaching with 35% hydrogen peroxide in the colors red (HP) and green(HPM) irradiated by: HL and LED. Four groups (n = 10) were divided according to the bleaching gel and light source. The results were submitted to the analysis of variance and Tukey test ($p < 0.05$). The mean values and standard deviations of the temperature increase inside the chamber pulp with HL were 4.4 ± 2.1 °C using HP and 4.5 ± 1.2 °C using HPM; while in LED groups, were 1.4 ± 0.3 °C to HP, and 1.5 ± 0.2 °C using HPM. For all surface temperatures, the maximum variation of the groups irradiated with HL was 6.5 ± 1.5 °C to HP and 7.5 ± 1.1 °C using HPM; while for the groups using LED, were 2.8 ± 0.7 °C using HP, and 3 ± 0.8 °C for HPM. There were no statistical difference between the increase of pulp and superficial temperature between groups using the various gels ($p < 0.05$). Mean temperatures were significantly higher for groups using HL compared with those irradiated with LED ($p < 0.05$). Was investigated the variation of surface temperature and thus given the time-delay to hit the critical variation of temperature (5.5 °C for the pulp chamber and 10 °C for periodontal). Forty-five human inferior incisors were divided into 3 groups and submitted to dental bleaching using HP and activated by HL, LED and DL sources respectively. The mean and standard deviation of the increase of pulpal temperature were 6.47 ± 2.78 °C for HL; 15.34 ± 8.81 °C for DL and 1.90 ± 0.97 °C for LED. The surface temperature for HL was

9.13 ± 2.19 °C, for DL was 25.66 ± 18.89 °C and for LED was 2.58 ± 1.40 °C. The mean temperature was significantly (Tukey test, $p > 0.05$) higher for the group irradiated by DL as compared as groups irradiated by HL and LED. Applying the lower limits of the confidence interval to 95% was found a time of 38.68 seconds for HL and 4.38 seconds for DL. The LED light source not reached the critical temperature for pulp or the periodontal, even when irradiated by 360 seconds. It was also investigated the dental enamel microhardness during the bleaching with hydrogen peroxide to 35%, under irradiation of HL, LED and DL sources. One hundred and five blocks were obtained from third molars, the enamel surface were planned and were made initial measures of Knoop microhardness with the burden of 25g per 5s. Then, the bleaching treatment with HP was made: A, B and C groups activated by HL, LED and DL respectively; D, E and F groups treated only with HP for the same time used on A, B and C groups, respectively, and G Group (control) was maintained only in artificial saliva for the same period of the other experimental groups. The average percentage of hardness and standard deviations obtained were: A: 97,8 ± 13,1; B: 95,5 ± 12,7; C: 84,2 ± 13,6; D: 128,6 ± 20,5; E: 133,9 ± 14,2; F: 123,9 ± 14,2; G: 129,8 ± 18,8. The statistical analysis (Tukey $p < 0.05$) showed that the mean percentage of microhardness for the groups irradiated by the light sources are significantly lower than the non-irradiated groups. Moreover, the non-irradiated groups showed that the artificial saliva was able to increase the microhardness during the time of storage. According to the thermal analysis, results suggest that the LED can be used safely for the pulp and periodontal tissue when used the parameters of this work. But the HL and DL require greater care. Considering the limitations of this study, the microhardness of the enamel was reduced when the light sources were used during the bleaching process and artificial saliva was able to increase the microhardness when no light was irradiated.

Keywords: Tooth bleaching; periodontal; dental pulp; heat; hardness; light sources.

1 Introdução

1 Introdução

A sociedade contemporânea exige cada vez mais a estética do sorriso, as quais se definem por dentes claros, alinhados e bem contornados. Este padrão denota além de dentes atraentes, saúde². Todavia muitos procedimentos clínicos realizados para alcançar tais padrões de beleza são invasivos. Já o clareamento dental é tido como tratamento mais conservador¹⁹, além de ser a forma mais comumente utilizada para satisfazer o desejo dos pacientes em clarear seus dentes.

Existem numerosas formas descritas na literatura para o clareamento dental, as quais utilizam diferentes agentes clareadores, concentrações, tempos de aplicação, formato de produto, modo de aplicação e o uso de luz²⁴. O uso de agentes clareadores pode ser realizado pela forma caseira e/ou de consultório. No uso caseiro o profissional fornece uma moldeira individual para o paciente, o qual irá carregá-la com um agente clareador de baixa concentração. Usualmente, o produto utilizado é o peróxido de carbamida em concentrações de 10% a 22%^{5, 6, 17, 20, 22, 23, 27} ou peróxido de hidrogênio, em baixas concentrações (2% a 7%)¹⁵, mantidos em contato com os dentes de 2 a 8 horas^{3, 16}. Existem também os vendidos sem prescrição do profissional que

podem ser encontrados na forma de moldeira e gel, de tira, de pincel, ou associados a dentifrícios.

Para o clareamento de consultório, o profissional aplica peróxido de hidrogênio em concentrações elevadas, que variam de 15% a 38%^{5, 14} ou, menos freqüentemente, o peróxido de carbamida de 30 a 37%¹². Os produtos podem ou não ser indicados em associação com uma fonte de luz e/ou calor. A energia fornecida por essas fontes tem por finalidade acelerar a degradação do peróxido de hidrogênio e proporcionar um melhor efeito em um tempo clínico mais curto⁷.

Entre as vantagens do clareamento de consultório em relação ao caseiro destacam-se a proteção dos tecidos moles e a não ingestão de material, além de produzir resultados imediatos, trazendo grande satisfação e motivação aos pacientes²¹. Entre as desvantagens destaca-se o custo superior, devido a necessidade de requerer múltiplas visitas ao consultório^{24, 27}. Porém, na tentativa de diminuir o tempo para o clareamento dos dentes fontes de luz e/ou calor podem ser utilizadas¹⁹.

O emprego da luz proveniente de fontes halógenas, LED's, lasers, entre outras, têm o propósito de acelerar a reação de quebra do peróxido de hidrogênio para otimizar o clareamento. Porém, a inevitável transmissão de calor para os tecidos dentais pode

danificá-los. Segundo a literatura, a elevação de 5,5°C da polpa²⁷ e 10°C do periodonto¹¹ já são suficientes para causar inflamações nestes tecidos.

Ainda, como os protocolos de clareamento incluem o contato direto do agente clareador com a superfície dos dentes¹³ em uma reação de oxi-redução que ocorre nos tecidos duros¹⁴, alterações na dureza do esmalte podem ocorrer, podendo ser agravada se somado a energia fornecida durante a irradiação de fontes de luz.

A manutenção da integridade dos tecidos dentais e periodontais são fatores de grande importância e que devem ser respeitados nos tratamentos estéticos, como no clareamento dental. Sendo assim, cresce a necessidade de investigações dos efeitos das fontes de luz indicadas para o clareamento dental sobre os tecidos dentais.

2 Proposição

2 Proposição

O objetivo do presente estudo foi avaliar in vitro:

- 1) A variação de temperatura superficial e intrapulpar de incisivos inferiores humanos quando submetidos ao clareamento dental com dois géis de peróxido de hidrogênio a 35% de diferentes cores, (vermelho e verde) ativado por HL e LED.
- 2) A variação de temperatura superficial e intrapulpar de incisivos inferiores humanos quando submetidos ao clareamento dental com peróxido de hidrogênio a 35% ativado por HL, LED e DL de alta intensidade, bem como determinar o tempo para se atingir as temperaturas limites da polpa (5,5 °C) e do periodonto (10 °C).
- 3) A influência das fontes de luz HL, LED e DL, utilizadas durante o clareamento com peróxido de hidrogênio a 35% na microdureza superficial do esmalte dental.

3 Capítulos

3.1 *Capítulo 1* - A THERMAL INVESTIGATION DURING DENTAL BLEACHING IN VITRO STUDY. ¹

¹ Artigo aceito para publicação dia 21 de Janeiro de 2008 no periódico Photomedicine and Laser Surgery. (Anexo 2)

ABSTRACT

Objective: Investigate the surface temperature variation of cervical region (infrared thermography) as well as the temperature within pulpal chamber (thermocouples) of human mandibular incisors when submitted to dental bleaching using two different 35% hydrogen peroxide gels, red (HP) and green (HPM) activated by Halogen light (HL) and LED. **Background data:** temperatures above 5.5°C can be considered potentially threatening to pulp vitality while higher than 10°C can result in periodontal injury. **Methods:** The samples were randomly divided into 4 groups (n=10), according to the bleaching agent and catalyst light source. **Results:** Mean values and standard deviations of the temperature increase inside the pulpal chamber in the HL groups was 4.4 ± 2.1 °C using HP, and 4.5 ± 1.2 °C using HPM; whereas in the groups using LED, it was 1.4 ± 0.3 °C for HP, and 1.5 ± 0.2 °C using HPM. For the root surface, the maximum temperature variations in the groups irradiated with HL were 6.5 ± 1.5 °C for HP, and 7.5 ± 1.1 °C using HPM; whereas in the groups irradiated with LED, it was 2.8 ± 0.7 °C using HP, and 3 ± 0.8 °C for HPM. There were no statistically significant differences in pulpal and surface temperature increases between the groups using different gels although the mean temperature increase values were significantly higher for the groups irradiated with HL when compared with those irradiated with LED (Tukey test, $p < 0.05$). **Results:** LED could be safe for periodontal and pulpal tissue when using this method, but HL should be used with care.

INTRODUCTION

The bleaching procedure has been exceptionally enhanced by the development of new technologies, and also because of patients increasing desire to obtain immediate outcomes. This technique requires the use of high concentration bleaching agents, such as hydrogen peroxide at concentrations ranging from 30 to 50%(1, 2). Products may be indicated in association with a light and/or heat source(3, 4). The aim of the energy supplied by these sources is to accelerate the degradation of hydrogen peroxide, as well as to provide more effective results in a shorter clinical treatment(5, 6).

In order to choose an irradiation parameter for a clinical application, the fluences used must be safe for the vitality of pulp and periodontal tissues(7, 8). Dental pulp is highly vascularized and its viability may be compromised by thermal injury transmitted through the enamel and dentin(9). Previous studies have indicated that temperature increments above 5.5°C can be considered potentially threatening to pulp vitality, and increments in excess of 16°C can result in complete pulpal necrosis(10). Furthermore, the heat generated by the different light sources used in dental bleaching technique may also increase temperature around the periodontal ligament. During the bleaching procedure, the crown irradiation provides heat transmission towards the cervical region, which consequently heats periodontal tissues. If the transmitted temperature leads to an increase exceeding 10°C, there may be periodontal injury(11, 12). To minimize thermal increase, dentists rely on the application of a pigmented bleaching agent in order to restrict the heat generated by the light absorption to the superficial gel layer(13, 14). Therefore, investigating heat generation during

light activated bleaching is the first step when choosing a light parameter for any clinical application(7, 15).

The objective of the present study was to evaluate the surface temperature variation of the root, as well as the temperature within the pulpal chamber of human mandibular incisors when submitted to dental bleaching with 35% hydrogen peroxide, associated with two energy sources: halogen light (HL) and LEDs, to find a protocol for light activated bleaching of vital teeth, which results in a temperature variation that prevents injuries to both periodontal and pulpal tissue.

MATERIALS AND METHODS

After approval by the Ethics Commission, 40 human mandibular permanent incisors were selected, cleaned and stored in 0.5% chloramine for seven days for disinfection. As the aim of the present study was to evaluate temperature increase generated during the light activated dental bleaching technique, the teeth were submitted to a process of accelerated darkening, using a staining mixture that consisted of equal parts of black tea, dry red wine, cola soft drink, pipe tobacco and artificial saliva (16) modified by (17). The teeth and substances were mixed to stain dental structures and taken for thermocycling of 1,000 cycles in alternate baths of 5°C and 55°C. Afterwards, the teeth were kept immersed in the stain mixture, in an oven at 37°C, for seven more days. Next, the already stained teeth were submitted to coronal polishing so as to remove superficial enamel stains. This was performed with a Robinson brush in a low-speed handpiece, using a pumice and water paste.

The teeth were darkened from 3 ± 2 to 15 ± 1 , according to the Vita Lumin Shade Guide (Vita Zahnfabrik H. Rauter GmbH & Co., Bäd Sackingen, Germany) ranking according to value. These proceedings were performed, because the highest temperature is reached during light activated dental bleaching in dark teeth with low mass and thin walls.

For the bleaching process, a 35% hydrogen peroxide (HP) *Whiteness HP* (FGM, Joinvile, Brazil) and 35% hydrogen peroxide (HPM) *Whiteness HP Maxx* (FGM, Joinvile, Brazil) gels that differed in color were used. Initially, the HP is red and gradually becomes colorless, whereas the HPM is green and becomes light green when activated by different energy sources: Halogen light (HL) with the intensity of $400\text{mW}/\text{cm}^2$ and wavelength of 400 to 500nm, (Optilight Plus – Gnatus, Ribeirão Preto, Brazil) and LED with $350\text{mW}/\text{cm}^2$ intensity and wavelength of 460 to 480 nm (Ultra Blue IV – DMC, São Carlos, Brazil).

The 40 human mandibular permanent incisors, artificially darkened as previously described, were randomly divided into 4 groups (n=10), according to the bleaching agent and the activating light source:

Group 1: teeth bleached with HP activated by HL;

Group 2: teeth bleached with HPM activated by HL;

Group 3: teeth bleached with HP activated by LED;

Group 4: teeth bleached with HPM activated by LED.

Optical Absorption of Bleaching Agents

The absorption spectrum of the bleaching gels was determined with the use of a temporal double beam absorption spectrometer (USB650-Red Tide - Ocean Optics, Dunedin, USA), calibrated in the spectral range from 400nm to 950nm, with resolution of 2nm. An integration time of 60 milliseconds was used, with repetition of 8 readings for each curve of the sample, obtaining each absorption curve at time intervals of 60 seconds, the result of the spectrum being the mean of these readings. The spectra were normalized for optic density, having an empty cubette with an optic path of 0.1 mm as reference; then the next sample was put in place for analysis.

Temperature Monitoring

The temperature was recorded in real-time using two different methods. Infrared thermography imaging allowed surface temperature distribution monitoring and thin thermocouples allowed the temperature inside the pulpal chamber to be monitored.

In order to measure the temperature in the pulpal region during the bleaching procedure, a 125- μ m thick type-K thermocouple (*chromel-alumel* - Omega, Stamford, EUA), with a response time of 0.1 seconds and sensitivity of 0.1°C was introduced into each specimen via the apical foramen, so that the thermocouple edge would coincide with the highest part of the pulpal chamber. This procedure required the pulpal chambers and root canals of all specimens to be chemically-mechanically adapted. A rotary instrument, Largo number 2, 0.9

mm in diameter, (Maillefer, Ballaigues, Switzerland) was used in a low-speed handpiece under constant irrigation with 1% sodium hypochlorite (Halex Istar, Goiânia, Brazil). After this step, a radiograph was taken of each specimen, with proximal incidence, to verify whether Largo rotary instrument had reached the pulpal chamber(18, 19). The canals were filled, using a hypodermal needle (BD, Juiz de Fora, Brazil), with black stain diluted in a ratio of 0.1ml per 30ml of distilled and deionized water(20). This is a standard procedure for the contact between the thermocouple tip and the area to be evaluated; in this case the mean thermal conductivity of the highest part of the pulpal chamber is maintained at a value equal or higher than that of the pulp (Table 1). After the thermocouple was introduced, root apices were sealed with composite resin (Filtek Z250, 3M, Minnesota, USA). Samples were radiographed once more in order to ensure that the junction was in contact with the buccal wall.

	Diffusivity (m ² /s) (21)	Specific heat (J/g °C) (22)	Thermal conductivity (W/cm °C) (22)
Enamel	2.27 x 10 ⁷	0.71	9.34 x 10 ⁻³
Dentin	1.92 x 10 ⁷	1.59	5.69 x 10 ⁻³
Water	1.3 x 10 ⁷	4.18	5.61 x 10 ⁻³

Table 1- Thermal property of the tooth

The temperature changes were monitored with an infrared thermographic camera using a quantum well infrared photodetector (SC 3000, Boston, MA) with a sensitivity of 0.01 °C and response time of 0,01 seconds. The camera was calibrated considering the dentin emissivity to be 0.91 within the temperature range of 20 to 100 °C with data acquisition of 60 Hz, with the ambient temperature stabilized at 20 °C, for two hours before and during the experiment, at the distance of 0.1 meter between the sample and the camera, and with the ambient humidity (46%).

The data were processed using commercial software (ThermaCam Research 2001, Boston, MA) to determine the maximum temperature rise at the root. The temperature variation of each irradiation was calculated using computer software (Origin 7.0, Northampton, USA).

Exposure to the bleaching agent

The teeth were placed in a thermal bath (Precision Scientific Co., Chicago, USA) with water at $37 \pm 1^\circ\text{C}$, to reproduce the temperature conditions found in the oral cavity during the irradiation, as well as to improve the transmission of the heat generated during the test.

The bleaching agent was handled until a gel consistency was reached, in a ratio of 3 drops (0.3 ml) of hydrogen peroxide per 1 drop (0.1 ml) of thickening, in accordance with the instructions given by the manufacturer. The gel was applied over the crown buccal surface of each specimen, in a layer of approximately 1.0 mm. The gel was left on the enamel surface for 1 minute after application. This period was suggested by the manufacturer to allow the bleaching agent to penetrate into dental structure.

In order to standardize and maintain the distance of 5 mm between the activating sources and the teeth, which would directly influence the intensity of irradiation, the appliances were fixed on an optical mount (OPTRON, Campinas, Brazil) and the distance was measured with a pachymeter (Mitutoyo, Suzano, Brazil). The measurements taken with the thermocouple were made with an acrylic device, so as to establish the vertical position of the tooth in relation to the

water surface, maintaining the whole root submerged and exposing the buccal aspect towards the activating source. Whereas, for the measurements with the thermographic camera, the tooth was fixed horizontally in a thermal bath with the root buccal aspect turned upwards, out of the water, allowing the images to be captured by the camera.

The light exposure time followed the manufacturer's recommendations, with 40 seconds being used for HL and 3 minutes for the LED.

RESULTS

The absorption spectra can be seen in Figures 1 and 2. For the HP the presence of one peak is centered on 522 nm with 95 nm of Full Weight of Half Maximum (FWHM) and one band between 750 and 950nm (complete decay not seen due to the diffraction grade used). For the WHM the first peak centered on 525 nm, with a FWHM of 100 nm, and the longer the time, the longer the wavelength this peak displaces towards, reaching 600 nm, but at an optic density well below the initial one. A very broad band is also observed, as in HP, starting at 750 nm and being maintained up to 950 nm.

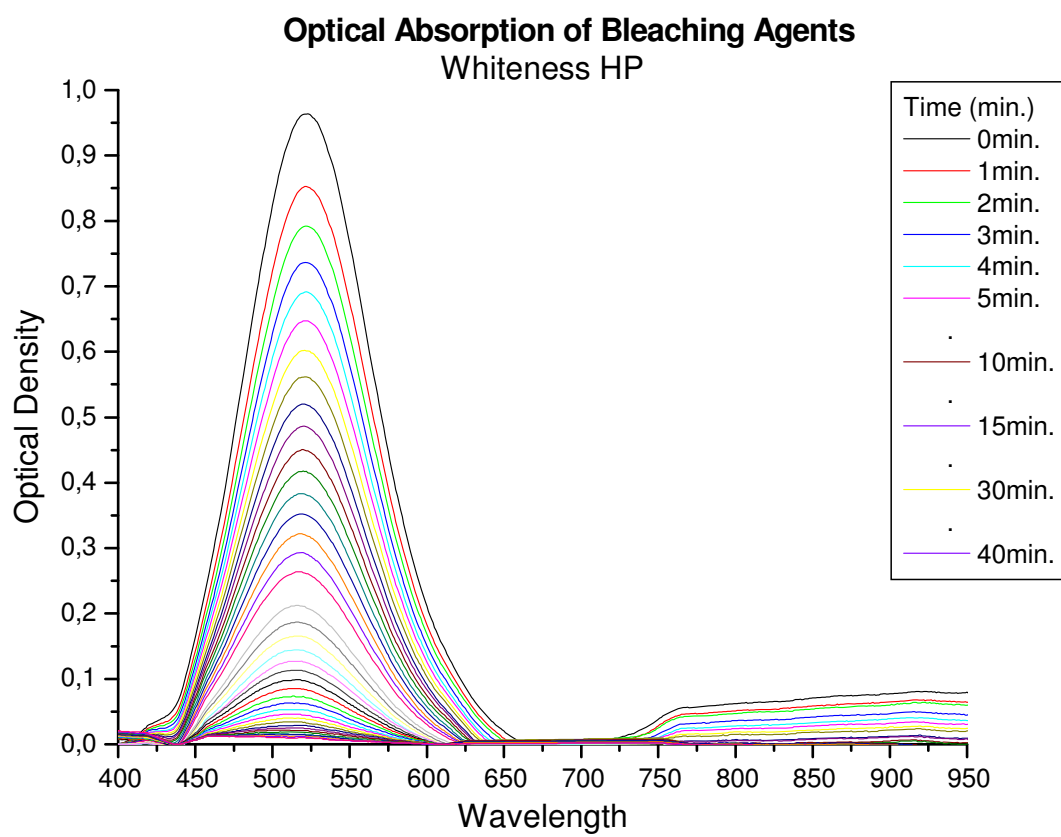


Figure 1 – Absorption spectrum of Whiteness HP gel.

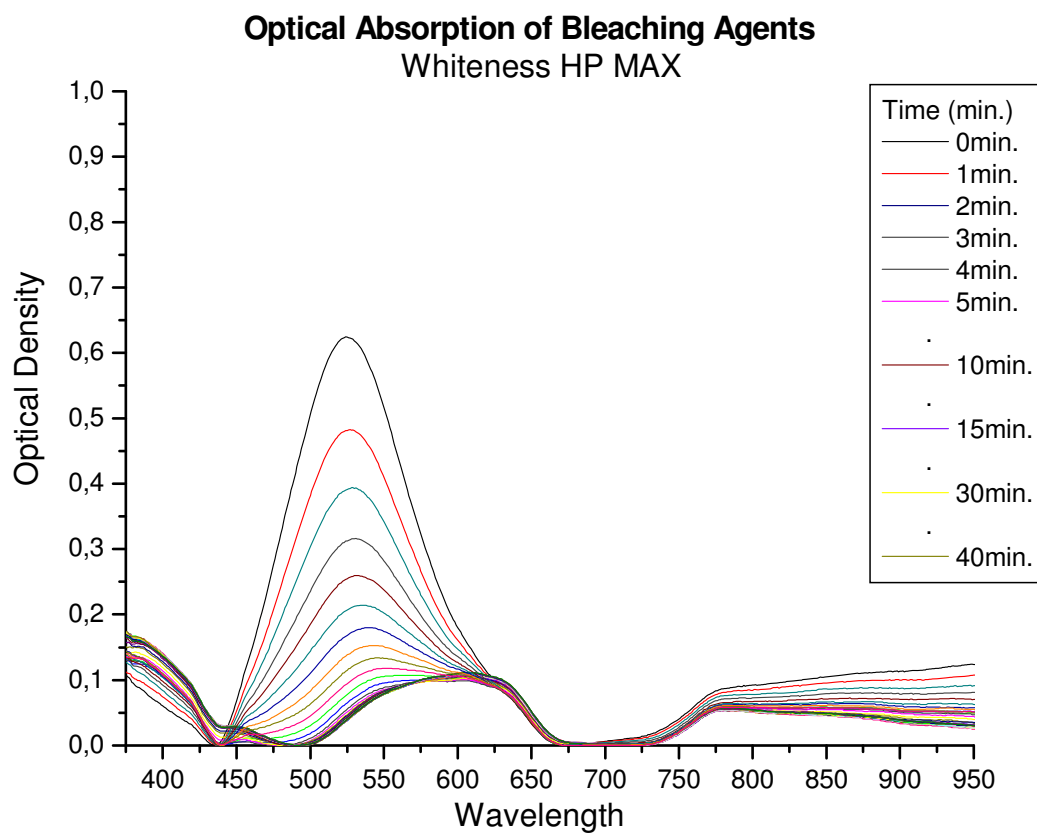


Figure 2 – Absorption spectrum of WPM.

The temperature was monitored by both the thermocouple and the thermographic camera in real-time, during the bleaching procedure and for 20 more seconds. Next, the data collected were submitted to both Analysis of Variance and the Tukey test.

The means and standard deviations of the temperature increases within pulpal chamber in the HL groups (Groups 1 and 2) were $4.4 \pm 2.1^{\circ}\text{C}$ using HP, and $4.5 \pm 1.2^{\circ}\text{C}$ using HPM; whereas in the groups that used LED (Groups 3 and 4), the temperature variation was $1.4 \pm 0.3^{\circ}\text{C}$ for HP, and $1.5 \pm 0.2^{\circ}\text{C}$ using HPM.

At the root surface, the temperature variation in the groups irradiated with HL (Groups 1 and 2) was $6.5 \pm 1.5^{\circ}\text{C}$ with HP, and $7.5 \pm 1.1^{\circ}\text{C}$ using HPM; while in the groups irradiated with LED (Groups 3 and 4), the temperature variation was $2.8 \pm 0.7^{\circ}\text{C}$ using HP, and $3 \pm 0.8^{\circ}\text{C}$ for HPM (Figure 3).

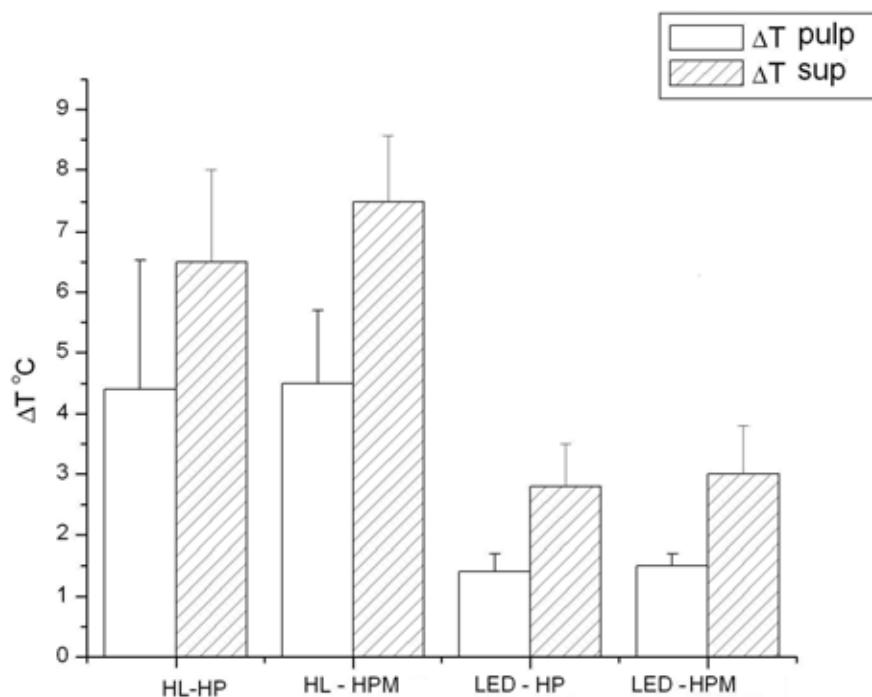


Figure 3 – Maximum temperature increase on root surface and within pulpal chamber.

The mean temperatures both on the root surface and within the pulpal chamber showed no statistically significant increases between the groups using different gels ($p < 0.05$). The mean temperature increase values were significantly higher for the groups irradiated with HL (Group 1 and Group 2) when compared with those irradiated with LED (Group 3 and Group 4) ($p < 0.05$).

DISCUSSION

Although light interacts with the gel during bleaching, there is a possibility that remaining heat will propagate and can reach the pulpal and periodontal tissue. The mechanism of heat conversion depends directly on the tissue constituents and the irradiation wavelength. The tooth absorption coefficient is low for the wavelength used in this work ($400 < \lambda < 500$ nm), thus scattering predominates over absorption(23). This leads to photons being absorbed far away from the irradiation surface. According to the modified Beer-Lambert law and diffusion theory, the light intensity exponentially decreases in deeper layers of the tissue; thus, the resulting temperature at the surface is higher than internal temperatures(19). Nevertheless, the absorption of the scattered photons in deep layers can result in a temperature rise that is harmful to the pulpal and periodontal tissue. For all these reasons, it is extremely necessary to know the estimated temperature rise for a certain irradiation condition, before undertaking any clinical application.

The low thermal diffusivity value of dental tissues (Table 1), which is defined as the thermal conductivity divided by the product of the density and heat capacity(22), is the parameter which characterizes the transient temperature change within a material when the material is exposed to an environmental temperature stimulus. Thus, it is important for protecting dental tissue against thermal shock(24). Some studies(3, 10, 14) that used different teeth for thermal investigation mention that small teeth are more susceptible to heat, therefore the choice of teeth for this study was decisive, since mandibular incisors are the smallest teeth in human dentition. The results obtained in this study could be

safety extrapolated to other dental groups, if the operating conditions are respected.

Coloring matter is added to bleaching gels, not only for the purpose of increasing light absorption, but also as an indicator of reaction, because soon after being manipulated, the gel presents an intense red color for HP and green for HPM(15, 25), which over the course of time weakens until the product becomes completely colorless. When comparing the activity of the gels under the presence of noncoherent light: HL at a wavelength between 400 and 500nm and LED with a wavelength from around 460 to 480 nm, it was observed that these sources were well absorbed by the two bleaching gels in the first minutes of manipulation, and that their absorption diminished with the passage of time, as the color became less intense.

Any in vitro study does not replicate the clinical environment completely. In vivo, the surrounding tissues, which have much lower thermal conductivity than air, together with blood flow, constitute potential heat sinks. Thus, the thermal energy also dissipates more rapidly in vivo than in vitro because of the vital circulation of blood in adjacent structures(26). The present study showed that the medians of temperature variation did not exceed 10°C in either of the experimental groups. The maximum mean temperature variation between the irradiated samples was $7.5 \pm 1.1^\circ\text{C}$. But with the use of HL, the medians of temperature variation were close to 5.5°C with the maximum mean of $4.4 \pm 2.1^\circ\text{C}$; for clinical use care must be taken, perhaps by reducing the light irradiation time.

It was evidenced that the LED light source showed the lowest temperature increase values for both pulp and root surface when the two types of gel were evaluated, when comparing them with the HL source values. This result is in agreement with the results obtained by Eldeniz et al., 2005(14), who evaluated intrapulpal temperature during the bleaching procedure activated by high-intensity HL (850 mW/cm^2), Conventional HL (450 mW/cm^2), LED (380 mW/cm^2), and diode laser. Other results shows by Sulieman, Addy and Ress, 2005(3), gotten the lowest value of temperature for light source with low intensity (plasma arc lamp 650 mW/cm^2) when compared with high-intensity HL (1000 mW/cm^2). The low intensity provided by LED has probably generated little heat, which resulted in lower temperature values when compared with the other activating light source evaluated.

According to Baik et al., 2001(27), the light emitted by the LED source induces photochemical effects and just minimal thermal effects when interacting with dark pigments in dentin, enhancing the bleaching process. Since the bleaching agent, and not dental structure, is heated by the light source, it allows the peroxide to break down.

The presence of bleaching gel between the activating light source and the tooth strongly reduces pulpal temperature increase(3, 14). Moreover, in order to prevent higher temperature increases of the dental element, either stains or photosensitive agents have been added to the bleaching gel. Thus, the light energy and heat are concentrated in the

bleaching gel, and therefore better used to accelerate hydrogen peroxide breakdown and hydrogen penetration(15).

The temperature increase associated with light activated bleaching can be minimized by reducing the incidence time as well as the use of certain substances in the gel that raise energy absorption, decreasing its transmission to the tooth(28). The green laser energy can be absorbed in Rhodamine B red dye and nowadays there is this light source available and the manufacturer claims that is the best light source for red gel(25). However, there were no statistically significant differences between the bleaching gels. The HP is initially red and gradually becomes colorless, therefore blue light was well absorbed; and for the HPM, which is green and becomes light green, red light would be more absorbed than blue. In this study, since the two light sources were in the blue region of spectra, the HP was able to present the least temperature rise.

The ideal bleaching treatment must provide a bleaching result, as good as possible, without causing injuries to dental tissues(29). Therefore, it is fundamentally relevant to perform a thorough analysis of the outcomes of current studies, with regard to the adverse effects of bleaching therapy.

CONCLUSION

The results of this study showed that the use of different bleaching gels did not statistically alter the mean temperature increase within the pulpal chamber and at the root surface. These results suggest that LED could be safe

for periodontal and pulpal tissue when using this method, but the use of HL needs more care.

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**3.2 *Capítulo 2* - A THERMAL ANALYSIS DURING THE
BLEACHING PROCESS ACTIVATED FOR SEVERAL LIGHT
SOURCES – IN VITRO STUDY.***

* Artigo submetido à publicação no periódico Laser Physics Letters.

ABSTRACT

Objective: This study investigated the surface temperature variation in the cervical region (infrared thermography) and temperature within the pulpal chamber (thermocouples), and also determined the time lag for reaching the critical temperature variation of 5.5 °C for the pulp chamber and 10 °C for the periodontium) of 45 human mandibular incisors when submitted to dental bleaching using 35% hydrogen peroxide gel activated by: Halogen light (HL), LED and high intensity diode laser (DL). The samples were randomly divided into 3 treatments (n=15), according to the catalyst light source. The results of temperature variation were submitted to the analysis of variance and Tukey test with $p < 0.05$. The mean values and standard deviations of the temperature increase inside the pulpal chamber in the HL groups was 6.47 ± 2.78 °C; in the DL groups it was 15.34 ± 8.81 °C; and 1.90 ± 0.97 °C for LED. For the tooth surface, the temperature variations in the groups irradiated with HL were 9.13 ± 2.19 °C; with DL were 25.66 ± 18.89 °C; and with LED were 2.58 ± 1.40 °C. The mean temperature increase values were significantly higher for the groups irradiated with DL when compared with those irradiated with HL and LED ($p < 0.05$). When applying the bottom limits of the interval of confidence of 95%, an application time of 38.68 seconds was found for HL, and 4.38 seconds for DL. The LED light did not attain the critical temperatures for pulp or the periodontium, even when irradiated for 360 seconds. The HL and DL light sources may be used for dental bleaching, provided this is done for a short period of time, whereas the LED sources did not heat the tissues excessively when used within the parameters used in this study.

INTRODUCTION

The desire to obtain white teeth in increasingly short times grows together with the demand for new dental bleaching products and techniques.

The use of hydrogen peroxide as the active principle for dental bleaching has shown interesting effectiveness without the use of light sources[1]. Nevertheless, many clinics have used different light sources for the dental bleaching technique associated with 30 to 35% hydrogen peroxide[2, 3] in an endeavour to accelerate dental bleaching[4, 5].

Previous investigations have demonstrated that the use of light sources leads to an increase in temperature at the tooth surface and inside the pulp chamber[6, 7]. Dental pulp is highly vascularized and its viability may be compromised by thermal injury transmitted through the enamel and dentin[8]. Previous studies have indicated that temperature increments above 5.5°C can be considered potentially threatening to pulp vitality[9]. Furthermore, studies have demonstrated that the heat generated by the different light sources used in dental bleaching techniques may also increase temperature around the periodontal ligament. During the bleaching procedure, crown irradiation provides heat transmission towards the cervical region, which consequently heats periodontal tissues. If the temperature transmitted leads to an increase exceeding 10°C, it may cause periodontal injury[10, 11].

Although scientific literature has shown that intense light sources generate tissue heating, and that this may cause damage, little is known with

respect to the time these sources take to attain these temperatures. Therefore, investigating heat generation during light activated bleaching is the first step in choosing a light parameter for any clinical application[12, 13]. Therefore, the aim of this study was to determine the irradiation times for dental bleaching, with the limit being the surface temperature of the cervical region (10 °C) and the temperature inside the pulp chamber (5.5 °C), of human mandibular incisors, when submitted to dental bleaching with 35% hydrogen peroxide, associated with three different energy sources: diode laser ($\lambda = 810 \text{ nm}$); LEDs (460nm $<\lambda < 480 \text{ nm}$) or Halogen light (400 nm $<\lambda < 500\text{nm}$).

MATERIALS AND METHODS

To prepare the teeth, 45 human freshly extracted mandibular permanent incisors were selected, cleaned and stored in 0.5% chloramine for seven days for disinfection. As the present study evaluated the temperature increase generated during the light activated dental bleaching technique, the teeth were submitted to a process of accelerated darkening, using a staining mixture that consisted of equal parts of black tea, dry red wine, cola soft drink, pipe tobacco and artificial saliva[14] modified by[7, 15]. The teeth and darkening substances were mixed to stain dental structures and subjected to thermal cycling (Ética - Equipamento Científicos S. A., São Paulo, Brazil) of 1,000 cycles in alternate baths of 5°C and 55°C . Afterwards, the teeth were kept immersed in the stain mixture, in an oven at 37°C, for another 7 days. Next, the already stained teeth were submitted to coronal polishing to remove superficial enamel stains. This was performed with a Robinson brush in a low-speed handpiece, using a pumice and water paste.

The teeth were darkened from 4 ± 2 to 15 ± 1 , according to the Vita Lumin Shade Guide (Vita Zahnfabrik H. Rauter GmbH & Co., Bäd Sackingen, Germany) ranking according to value. These procedures were performed, because the highest temperature is reached during light activated dental bleaching in dark teeth with low mass and thin walls, such as the incisors.

For the bleaching process, a 35% hydrogen peroxide (HP) Whiteness HP (FGM, Joinvile, Brazil) was used. Initially, this gel is red and gradually becomes colourless when activated by different energy sources: Halogen light (HL) with the intensity of 400 mW/cm^2 and wavelength of 400 to 500 nm, (Optilight Plus – Gnatus, Ribeirão Preto, Brazil), a high intensity diode laser with the intensity of $800 \pm 40 \text{ mW/cm}^2$ and wavelength of 810 nm (OPUS 10 – Opusdent, Jerusalem, Israel) and LED with 350 mW/cm^2 intensity and wavelength of 460 to 480 nm (Ultra Blue IV – DMC, São Carlos, Brazil). The light exposure time were 90 seconds being used for HL, 60 seconds for DL and 360 seconds for the LED.

The human mandibular permanent incisors, artificially darkened as previously described, were randomly divided into 3 treatments (n=15), according to the activating light source, Table 1.

Table 1: Division of Groups.

Group	n	Light Source
HL	15	bleached with HP activated by HL
DL	15	bleached with HP activated by DL
LED	15	bleached with HP activated by LED

Conducting the Experiment

Thermographic monitoring and recording of the thermal curves arising from the temperature increase generated inside the pulp chamber and at the vestibular cervical surface during irradiation of the teeth was performed by means of two methods simultaneously: an internal measuring system with thermocouples to monitor the temperature in the pulp chamber, and to monitor the surface temperature of the cervical region. With the data coming from the thermographic camera, the intention was to measure the temperature increase in the cervical surface region, in order to analyze any eventual occurrence of damage to the periodontal ligament.

In order to measure the temperature in the pulpal region during the bleaching procedure, a 125- μ m thick type-K thermocouple (*chromel-alumel* - Omega, Stamford, USA), with a response time of 0.1 seconds and sensitivity of 0.1°C was introduced into each specimen via the apical foramen, so that the thermocouple edge would coincide with the highest part of the pulpal chamber. This procedure required the pulpal chambers and root canals of all specimens to be chemically-mechanically adapted. A rotary instrument, Largo number 2, 0.9 mm in diameter, (Maillefer, Ballaigues, Switzerland) was used in a low-speed handpiece under constant irrigation with 1% sodium hypochlorite (Halex Istar, Goiânia, Brazil). After this step, a radiograph was taken of each specimen, with proximal incidence, to verify whether Largo rotary instrument had reached the pulpal chamber[16, 17]. The canals were filled, using a hypodermal needle (BD, Juiz de Fora, Brazil), with black stain diluted in a ratio of 0.1ml per 30ml of distilled and deionized water[18]. This is a standard procedure for the contact between the thermocouple tip and the area to be evaluated; in this case the

mean thermal conductivity of the highest part of the pulpal chamber is maintained at a value equal to or higher than that of the pulp (Table 1). After the thermocouple was introduced, root apices were sealed with composite resin (Filtek Z250, 3M, Minnesota, USA). Samples were radiographed once more in order to ensure that the thermocouple junction was in contact with the buccal wall.

Table 1- Thermal property of the tooth

	Diffusivity (m ² /s)[19]	Specific heat (J/g °C)[20]	Thermal conductivity (W/cm °C)[20]
Enamel	2.27×10^{-7}	0.71	9.34×10^{-3}
Dentin	1.92×10^{-7}	1.59	5.69×10^{-3}
Water	1.3×10^{-7}	4.18	5.61×10^{-3}

The superficial cervical temperature changes were monitored with an infrared thermographic camera using a quantum well infrared photo detector (SC 3000 FLIR , Boston, MA, USA) with a sensitivity of 0.01 °C and response time of 0.01 seconds. The camera was calibrated considering the dentin emissivity to be 0.91 within the temperature range of 20 to 100 °C, with data acquisition taken at 60Hz, using a lens with a focal distance of 0.1 meter between the sample and the camera. The ambient humidity was 46% and the room temperature was stabilized at 20 °C for two hours before and during the experiment.

The bleaching agent was manipulated until gel consistency was reached, in a ratio of 3 drops (~0.3ml) of hydrogen peroxide per 1 drop (~0.1ml) of

thickening, in accordance with the instructions given by the manufacturer. The gel was applied over the crown buccal surface of each specimen, in a layer approximately 1.0mm thick. The gel was left on the enamel surface for 1 minute after application. This period was suggested by the manufacturer to allow the bleaching agent to penetrate into dental structure.

The teeth were placed in a thermal bath (Precision Scientific Co., Chicago, USA) with water at $37 \pm 1^\circ\text{C}$, to reproduce the temperature conditions found in the oral cavity during irradiation, as well as to improve the transmission of the heat generated during the test.

In order to standardize and maintain the distance of 5 mm between the activating sources and the tooth, which would directly influence the intensity of irradiation, the appliances were fixed on an optical mount (OPTRON - Campinas-SP) and the distance was measured with a digital pachymeter (Mitutoyo, Suzano, Brazil). A device was made from an acrylic base to maintain the tooth position fixed at a fixed angle of ($\sim 60^\circ$) to the surface of the water, maintaining a large part of the tooth submerged and exposing the vestibular cervical region so that it faced the thermographic camera.

Statistical treatment of data

Evaluation of the bleaching treatments as regards Knoop microhardness of the treated tooth surfaces was performed by the Analysis of Variance, considering repeated measures in different periods of time. This analysis was

complemented by multiple comparisons of measures by the Tukey test. A level of significance of 5% was adopted for taking a decision.

The temperature was monitored by both the thermocouple and the thermographic camera in real-time, during the bleaching procedure and the sets of data collected were both submitted to Analysis of Variance. This analysis was complemented by multiple comparisons of measures by the Tukey test. A level of significance of 5% was adopted for taking a decision. The interval of confidence of 95% of the irradiation time until the temperature considered safe was reached [(5.5°C intrapulpal[9]) monitored by the thermocouple and (10°C periodontium[10]) monitored by the thermographic camera] of the different light sources was also calculated.

RESULTS

The means and standard deviations of the temperature increases within pulpal chamber in the HL groups was 6.47 ± 2.78 °C; in the groups using DL it was 15.34 ± 8.81 °C; and 1.90 ± 0.97 °C for LED. At the tooth surface, the temperature variation in the groups irradiated with HL was 9.13 ± 2.19 °C; for groups irradiated with DL it was 25.66 ± 18.89 °C; and for LED it was 2.58 ± 1.40 °C. The mean temperature increase values were significantly higher for the groups irradiated with DL when compared with those irradiated with HL and LED ($p < 0.05$).

Once the temperature curves were obtained, the mean and interval of confidence of 95% of the irradiation times for each treatment were obtained, Figure 1.

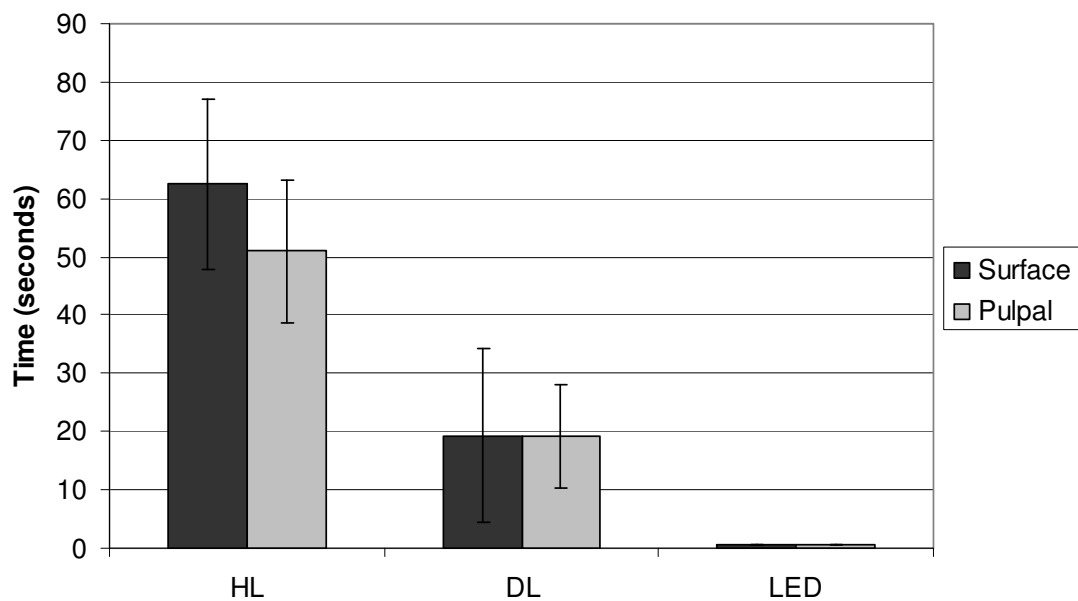


Figure 1- Sampling Means (columns) and intervals of confidence of 95% for the population means (vertical bars) of limit heating times, having the surface and intrapulpal temperatures.

Observing the temperature increase curves of each light source, it is possible to note the increase in temperature at each instant of irradiation, for the treatment with LED both the intrapulpal and surface limit temperatures were not attained.

As the aim was to find a safe irradiation time for each activation source under the conditions used in the study, the lowest bottom limit (IL) of the Interval of confidence (IC) of 95% between the thermocouple and thermographic camera was considered the safe time, since one understood that the longer the irradiation time, the more heat would be generated. Therefore, one arrived at the following time values in seconds:

Table 5 – Irradiation times for the activation sources.

<i>Light</i>	<i>IC 95%</i>
	IL
HL	38.68
DL	4.38
LED	None

As the temperature limits were not attained in the treatment with LED, even after irradiating the teeth for 360 seconds, and the graph of temperature increase as a function of time indicated a tendency for temperatures to stabilize around 4 °C for the intrapulpal region and 7 °C at the cervical surface, the time to be used for this light source in the second stage of the project will be 360 seconds.

DISCUSSION

Hydrogen peroxide has been used clinically at concentrations levels of 30% to 38% for bleaching teeth for many years, but the process has required various stages. Heat and light are used empirically in attempts to catalyze hydrogen peroxide decomposition and speed tooth bleaching[21]. When choosing a light parameter for any clinical application the first step is to investigate heat generated during light activated bleaching[12, 13] in order to assume the confidence parameters for future clinical application. The irradiation time is a simpler parameter for the dentist to control, therefore, in this in vitro study, the times that HL, DL and LED sources take to reach a limit temperature for pulp (5,5 °C)[9] and periodontal tissue (10 °C)[10] during 35% HP bleaching were increased?. Nevertheless, the confidence interval of 95% was calculated to suggest a safe application time for the use of these light sources.

In order to choose a parameter for safe use, the worst clinical situation needs to be reproduced: low mass, thin walls. Some studies[2, 6, 9] that used different teeth for thermal investigation mention that small teeth are more susceptible to heat, therefore the choice of teeth for this study was decisive, since mandibular incisors are the smallest teeth in human dentition. Moreover, natural teeth have blood circulation that acts to decrease the heat, but in a limit situation this circulation could be reduced or nonexistent[22]. The results obtained in this study could be safely extrapolated to other dental groups, if the operating conditions are respected.

In this study it can be observed (Figures 2, 3, 4) that the temperature recorded by the thermographic camera, for the greater part of the time, was

higher than that recorded by the thermocouples. This occurs due to the low thermal diffusivity value of dental tissues (Table 1), which is defined as the thermal conductivity divided by the product of the density and heat capacity[20], which is the parameter that characterizes the transient temperature change within a material when the material is exposed to an environmental temperature stimulus. Thus, it is important for protecting dental tissue against thermal shock[22]. This phenomenon is not even more evident, because the presence of bleaching gel between the activating light source and the tooth strongly reduces pulpal temperature increase[2, 6].

The laser light source attained a surface temperature of 10 °C and pulp temperature of 5.5 °C in times close to the shortest time. The mechanism of heat conversion depends directly on the material irradiated and the light source wavelength. The tooth absorption coefficient is low for the wavelength used in this study ($400 < \lambda < 500$ and 810 nm), thus scattering predominates over absorption[23]. However, the laser wavelength used in this study was not well absorbed by the red colour of the bleaching gel, allowing a large part of the energy provided to reach the tooth[7]. In the literature, when different light sources were compared[2, 6, 16, 24] the authors reported high temperatures for high light intensities, certainly, in this study, the high intensity of laser light contributed to a fast rise in temperature, when compared with the other light sources used.

Whereas, the HL and LED light sources not only have relatively lower intensity values, thus making available a lower quantity of energy per area irradiated, but they also have a wavelength that is well absorbed by the bleaching gel, and therefore presented lower temperature values[7].

Even without scientifically proved efficiency, light has been used in the bleaching treatment, but it is important to know whether it is the effect of the light or heat on the chemical bleaching product (gel) rather than on the tooth substance itself, and whether it is the chromophores it contains that may lead to an increased bleaching effect[12]. At the time of establishing a protocol for the use of light for dental bleaching, no rise in temperature above the biologic tolerance must occur. Therefore, the LED light source was the one that best met this requirement, as it did not attain the limit temperatures when used for 360 seconds.

CONCLUSIONS

The results of this study showed that the use of different light sources alters the mean temperature increase within the pulpal chamber and at the tooth surface. These results suggest that LED could be safe for periodontal and pulpal tissue when using this method, HL may be used for 38 seconds and DL for only 4 seconds, the latter two requiring greater care.

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**3.3 *Capítulo 3* - THE EFFECT OF POWER BLEACHING
ACTIVED BY SEVERAL LIGHTS SOURCES IN ENAMEL
MICROHARDNESS***

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ABSTRACT

The purpose of this study was to evaluate the influence of different light sources for in-office bleaching on surface microhardness of human enamel. One hundred and five blocks of third molars were distributed among seven groups. The facial enamel surface of each block was polished and baseline *Knoop* microhardness of enamel was assessed with a load of 25g for 5 s. Subsequently, the enamel was treated with 35% hydrogen peroxide bleaching agent: groups A, B and C activated by halogen light, LED and high intensity diode laser respectively, group D, E, and F were only treated with the bleaching agent for the same time as specimens in groups A, B and C. The control, group G was kept in saliva for the same period as the other groups. Microhardness was reassessed after 1 day of the bleaching treatment, and after 7 and 21 days storage in artificial saliva. The mean percentage and standard deviation of microhardness in Knoop Hardness Number were: A 97.8 ± 13.1 KHN; B 95.5 ± 12.7 KHN; C 84.2 ± 13.6 KHN; D 128.6 ± 20.5 KHN; E 133.9 ± 14.2 KHN; F 123.9 ± 14.2 KHN; G 129.8 ± 18.8 KHN. Statistical analysis ($p < 0.05$; Tukey test) showed that microhardness percentage values were significantly lower in the groups irradiated with light when compared with the non-irradiated groups. Furthermore, for the non-irradiated groups it showed that saliva was able to enhance the microhardness during the measurement times. Within the limitations of this *in vitro* study the enamel microhardness was decreased when light sources were used during the bleaching process and

the artificial saliva was able to increase microhardness when no light was used.

INTRODUCTION

Tooth whitening has become one of the most rapidly growing oral care treatments, fuelled by patient demands for both healthy teeth and cosmetically attractive smiles. A number of methods and approaches have been described in the literature for bleaching vital teeth, for example, methods using different bleaching agents, concentrations, application times, product format, application mode and light activation[1]. The great majority of bleaching agents have hydrogen peroxide as an active ingredient. Hydrogen peroxide is capable of oxidizing a wide range of coloured organic and inorganic compounds, causing decolourisation and hence bleaching of the substrate[2].

In-office-bleaching or power bleaching procedures seem to be an appropriate alternative, especially in the case of very severe discolorations, of single teeth, lack of patient compliance or if a rapid treatment is desired[1]. It differs from at-home methods because it generally uses relatively high levels of whitening agents; it is applied to the teeth after the soft tissues have been protected, and the peroxide may be further activated by light source.

The idea of using light sources, such as quartz-tungsten-halogen lamp, LED and lasers is to reduce the total in-office bleaching time; in theory this occurs through the ability to heat the hydrogen peroxide, thereby increasing the rate of decomposition of oxygen to form the oxygen free radicals necessary for bleaching[3]. It is the effect of the heat generated by light absorption on the chemical bleaching product (gel), rather than on the tooth substance itself, or its chromophores that may lead to an increased bleaching effect[1], but the combination of a high concentration of hydrogen peroxide and light may bring about an undesired effect on the teeth.

The microhardness study is widely used in the literature[4-6], and as it concerns a mechanical property that evaluates surface alterations quantitatively [7], it must serve as one of the parameters in the choice of the best treatment. The aim of this study was to investigate the effect of 35% hydrogen peroxide gel activated by quartz-tungsten-halogen lamp, LED and high intensity diode laser on the superficial microhardness of enamel.

MATERIALS AND METHODS

Specimen preparation

After approval by the Ethics Commission, 40 freshly extracted human third molars were selected, cleaned and stored in 0.5% chloramine for seven days for disinfection[8].

The crowns of the teeth were separated from the roots by a cut made with a diamond disk, approximately 3 mm beyond the amelo-cement limit. The vestibular and lingual surfaces of each tooth were used to make 4 human enamel and dentin blocks, measuring 3 x 3 x 3 mm, by cuts using a diamond disk adapted to a low speed motor under cooling. Thus 160 blocks were obtained, from which 105 were selected for this study after examination under optic microscope at 30x magnification, excluding those that presented fractures or cracks. The blocks were embedded in self-polymerizable acrylic resin with the enamel face (3 x 3 mm) facing outward, to facilitate handling and flattening. The samples were flattened and polished with a polishing machine (Panambra DP-10, Struers - São Paulo, Brazil) with 330, 400, 600, 1000, 1400 grit silicon carbide abrasive papers under water cooling, and felt disk with diamond paste. This procedure is of fundamental importance to obtain a flat, parallel and smooth surface, to enable the Knoop hardness test to be performed.

A fragment area of 4 mm² (2 x 2mm) was determined for performing the measurements. Afterwards the fragments were divided into the experimental groups and stored in artificial saliva, at pH = 7.0, at 37°C.

The Knoop hardness measurements of the enamel were made at the following time intervals: Before treatment (baseline); 1, 7 and 21 days after bleaching treatment, for each of the 105 blocks.

Division of the Experimental Groups

The bleaching agent used was 35% hydrogen peroxide (Whiteness HP – FGM, Joinville, Santa Catarina - Brazil). During all time the blocks were immersed in artificial saliva, the group G did not receive any type of bleaching treatment; they were only kept in saliva as control. Table 1 shows the division of the groups and treatments performed.

Table 1 – Groups and Treatments Performed

Group	n	Light source	Bleaching	Time
A	15	Halogen light (Optilight Plus – Gnatus, Ribeirão Preto, São Paulo - Brazil)	Applied	38 sec
B	15	LED (Ultra Blue IV – DMC, São Carlos, São Paulo - Brazil)	Applied	360 sec
C	15	Diode laser $\lambda = 810$ nm (OPUS 10 – Opusdent, Jerusalém, Israel)	Applied	4 sec
D	15	Without light	Applied	38 sec
E	15	Without light	Applied	360 sec
F	15	Without light	Applied	4 sec
G	15	Without light	Not Applied	Zero

Conducting the Experiment

The blocks were removed from the artificial saliva, washed in running water and dried with absorbent paper. The bleaching gel was manipulated and a layer of 1 mm applied to the fragments, followed by waiting for the time of 1 minute, in accordance with the manufacturer's instructions. Activation with the energy sources was standardized at 5 mm from the irradiated surface. The application times were determined in a previous study, in which the increase in temperature in the pulp and pulpal region were also analyzed. After irradiation, the gel was removed with gauze and abundantly washed with water. The blocks were again placed in artificial saliva until the next hardness measurements were taken.

Obtainment of the enamel hardness measurements

The blocks were submitted to the Knoop hardness test, by using a microhardness meter Micromet 2100 (Buehler, Lake Bluff, Illinois, USA). For each situation, three marks were made on the specimen surfaces, using a Knoop diamond with a 25 g load for five seconds. [9]

Statistical Methodology

Evaluation of the bleaching treatments as regards Knoop microhardness of the treated tooth surfaces was performed by the Analysis of Variance, considering repeated measures in different periods of time.

This analysis was complemented by multiple comparisons of measures by the Tukey test. A level of significance of 5% was adopted for taking a decision. The conditions of analysis, i.e. the homogeneity of variance, normality of the residues and sphericity used were proved by the Levene, Shapiro-Wilk and Mauchley tests, respectively.

RESULTS

For all groups baseline microhardness measurements were in the range of 361.8 ± 69.8 KHN. The microhardness measurements after bleaching treatment and with one day storage in artificial saliva were in the range of 378.4 ± 23.4 KHN, and revealed a range of 397.9 ± 71.1 KHN after 7 days storage in artificial saliva, and after 15 days storage the range was 405 ± 62 KHN.

To improve the comparison among the groups, the percentages of variation in hardness in comparison with the baseline were used for analysis. The means and standard deviations in percentages of variation in hardness of each group in the different periods of measurement are shown in Table 2.

The analysis of variance identified significant effects on hardness of group, time and the interaction between these two factors ($p < 0.001$). As the interaction was significant, there was evidence that the effect of the different treatments on hardness depended on the times at which the

measurements were taken. To clarify this dependence, multiple comparisons were made by the Tukey test at the level of significance of 5%. The result is resumed in Table 2, in which means on the same line with different lower case letters indicate statistically significant difference (comparisons among means within one and the same group). Analogously, means accompanied by different capital letters in a column indicate statistical significance (compared among groups within one and the same time).

Table 2 - Means and standard deviations (SD) of percentage variation in hardness in each period in comparison with baseline (means followed by the same lower case letters in the horizontal or the same capital letters in the vertical, do not differ significantly)

Group	Statistics	Time (days)				
		1	7		21	
A	Mean	94.3	^{AB} _a 100.4	^{ABC} _a	98.6	^A _a
	SD	15.3	12.2		11.9	
B	Mean	100.1	^{ABC} _b 88.4	^{AB} _a	98.1	^A _{ab}
	SD	10.7	12.6		14.8	
C	Mean	82.8	^A _a 83.0	^A _a	86.8	^A _a
	SD	15.1	11.9		13.9	
D	Mean	121.4	^{BC} _a 130.6	^{CD} _{ab}	133.7	^{BC} _b

	SD	17.6	22.8	21.0
E	Mean	119.2 ^{AB} _a	127.6 ^C _a	154.9 ^D _b
	SD	14.1	11.4	17.2
F	Mean	112.6 ^{ABC} _a	119.3 ^{BC} _a	139.7 ^{AB} _b
	SD	16.1	12.6	13.8
G	Mean	114.9 ^{ABC} _a	121.6 ^C _a	152.9 ^C _b
	SD	18.7	19.2	18.6

The graph in Figure 1 contains the representation of the sample means of percentages of variations in hardness and intervals of confidence of 95% for the population means. The intervals of confidence that include the proportion 100% suggest that there was no significant alteration in hardness in comparison with the baseline value.

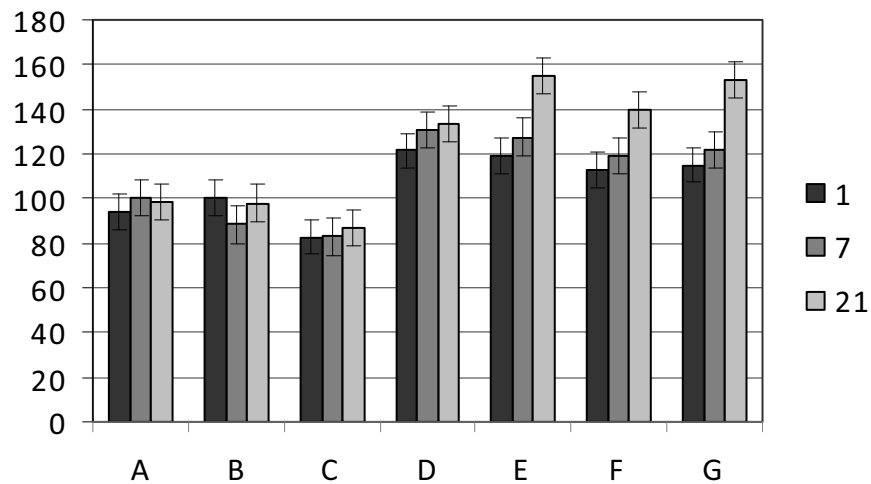


Figure 1 – Sample Means (columns) of percentage variation in hardness at each evaluation time (1, 7 and 21 days) in comparison with baseline and intervals of confidence of 95% for the population means (vertical bars)

The results obtained evidence that the means of percentage variation in hardness for the groups with light (A, B and C) are reasonably equivalent in the different periods. However, the mean relative to Group B at the time of 7 days was significantly lower than the mean of the previous period. For Group C the intervals of confidence of 95% in the three measurement times did not contain the percentage 100%, being below this level. This indicated that there was a significant but equivalent reduction in hardness in the three periods in Group C.

For the other groups, by the intervals of confidence, an increase in the mean hardness of at least 20% is noted. Groups D, E, F and G presented a significantly higher mean increase in hardness at 21 days than the variation obtained on day 1, but not significant in relation to day 7. The means related to these groups do not differ significantly among them throughout the periods of evaluation.

Finally, the Control Group G had a significant initial increase in hardness, which was maintained in the following period and increased even more in the final period. These increases were obviously higher than all the variations in Groups A, B and C. With regard to the other groups, at 7 days no significantly different percentage variation occurred in comparison with the first day of measurement, but at 21 days, the mean percentage was significantly higher.

DISCUSSION

Power bleaching is achieved by using 35% hydrogen peroxide directly on the enamel surface, enhanced by a light source to accelerate the process, but the use of high concentration of hydrogen peroxide in association with light causes concern with regard to enamel demineralisation. When power bleaching is compared with the at-home bleaching process, some aspects must be observed with regard to

microhardness evaluation. It has been reported that a higher peroxide concentration caused higher diffusion of the peroxide towards the pulp [16]. Another difference is that in at-home bleaching, small amounts of saliva might diffuse into the applied gel. It is conceivable that this would lead to some dilution and to a buffering of the bleaching agent, thus reducing the decrease in microhardness[10]. These are basic differences that harm the microhardness of enamel when the in-office technique is applied.

The results found cannot be directly extrapolated to *in vivo* situations; in the present study, sample surfaces were initially polished to allow proper microhardness measurements to be made. By removing the outer layers of enamel, the hypermineralised layer (often containing fluoroapatite and thus being more resistant to mineralloss) was discarded. This is not comparable to the clinical situation; however, since the control samples were affected during the remineralization period of the study, this could address such issues. Furthermore, in order to simulate the clinical situation and to standardize the experimental conditions at the same time, the samples were stored in artificial saliva for 21 days after bleaching treatments. Saliva is known to be an important factor in enamel remineralisation[11]. Thus, in the present study artificial saliva, with a neutral pH was used as a remineralising solution, since it has been proved that it acts as an effective agent for rehardening softened enamel in several *in vitro* studies[12, 13]. Concentrations of calcium, phosphate and fluor in the artificial saliva were 1.5 and 0.9mM, respectively. These values are as high or even higher when compared with the amounts of calcium and (inorganic) phosphates

described for natural human saliva.[14] Therefore, it was concluded that the artificial saliva used in the present study was saturated with the above-mentioned minerals. Moreover, artificial saliva was chosen in order to minimize the influence presumed to be exerted by the variation in the composition of human saliva collected from different subjects.

The use of light sources as coadjuvant to the dental bleaching process has been indicated even without scientifically proved efficiency. In the present study, a notable reduction in the microhardness values was observed for the groups in which light was used (A, B, C). Irradiation of the samples by light sources inevitably generates heat, and this heat can alter the surface composition of enamel [15], in addition to act by increasing the hydrogen peroxide penetration [16] and can accelerate its breakdown [17] into free radicals responsible for the bleaching process [18]. Another interesting effect occurred with the groups in which light was used; there was apparent resistance to remineralisation, when compared with the control group, even when stored in saliva for 21 days. This may suggest that permanent modifications occurred in the enamel structure, however more specific research must be conducted to verify this finding. A percentage drop of approximately 20% in comparison with the microhardness measured initially was found in Group C, in which the laser light source was used; the high intensity ($800 \pm 40 \text{ mW/cm}^2$) of this light source could probably have been responsible for the initial reduction in hardness and also for not allowing the initial hardness to be regained.

Demineralisation may increase susceptibility to tooth wear caused by abrasive factors like tooth brushing[19].

The time hydrogen peroxide remains on the tooth would seem to have a strong influence on the microhardness of enamel and dentin. Pécora et al.[20] applied various bleaching agents directly onto dentine slices for 72 h and observed that 30% hydrogen peroxide solution caused the greatest reduction in dentine microhardness, followed by 10% carbamide peroxide. Suleiman et al.[21] reported that there were no significant changes in hardness values for enamel and dentin after bleaching with 35% hydrogen peroxide after a 30-min application. The results of the present study showed that without the use of light a significant increase in microhardness occurred on 1 day after immersion in artificial saliva, as also observed in the control group. This could have occurred due to the short period the gel remained on the samples (from 4 to 360 seconds), which was not observed in the groups in which light was used.

Whether this demineralisation can be remineralised in the longer term by saliva and/fluorides should be further investigated. In addition, future *in vivo* studies with treatment controls are needed to verify these results.

Under the conditions of this *in vitro* study, the application of 35% hydrogen peroxide gel on enamel reduces its microhardness when light sources are used, and artificial saliva is capable of increasing the microhardness of enamel. It is recommended that the use of light sources

on bleaching agents should be carefully considered in patients susceptible to tooth wear.

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4 Discussão

4 Discussão

A interação da luz com o gel clareador inevitavelmente gera calor, que durante o processo de clareamento dental se propaga e pode resultar em danos pulpares e periodontais. Corantes são adicionados aos géis clareadores não apenas para tentar minimizar a geração de calor nos tecidos dentais, mas também como indicador da reação^{7, 28}, pois quando manipulado, o HP e o HPM apresenta uma coloração intensa que com o passar do tempo vai se tornando cada vez mais incolor.

Observando os espectros de absorção dos géis sobre as HL (comprimento de onda de 400 a 500nm) e LED (comprimento de onda de 460 de 480nm), nota-se que a absorção desses comprimentos de onda foi alta apenas nos primeiros minutos e com o passar do tempo os géis vão perdendo a cor e menos luz é absorvida. Portanto, mais luz é transmitida e atinge diretamente o dente e conseqüentemente mais calor é gerado. Esse aspecto deve ser observado quando se pretende utilizar fontes de luz por vários minutos.

Quando se deseja escolher um parâmetro para o uso de luz em qualquer aplicação clínica, o primeiro passo é investigar a geração de calor nos tecidos^{7, 18}. Este trabalho utilizou duas formas de mensurar a elevação de temperatura uma para o interior da

câmara pulpar (termopares) e outra para a superfície do dente (termografia de infravermelho), metodologia muito utilizada na literatura^{1, 8, 10, 26} e que pôde assim, fornecer informações precisas sobre as alterações de temperatura durante o clareamento dental assistido por luz.

Com base nas curvas de aquecimento, ficou evidente que a fonte LED, quando comparado às fontes HL e DL (comprimento de onda de 810nm), mostrou os menores valores de aumento de temperatura, tanto para o interior da polpa quanto superficial. Esses resultados estão de acordo com o trabalho de Eldeniz et al.¹⁰ (2005) e Sulieman et al.²⁶ (2005).

A baixa intensidade fornecida pelo LED gera, provavelmente, pouco calor, o que resulta baixas temperaturas. De acordo com Baik et al.⁴, 2001, a luz emitida pelo LED induz efeitos fotoquímicos e mínimo efeito térmico quando interage com os pigmentos escuros do interior do dente, melhorando o efeito do clareamento, enfatizando, assim, ser o aquecimento do agente clareador e não da estrutura dental que produz o efeito aumentado do processo.

O aumento de temperatura associado ao uso de luzes pode ser minimizado pelo controle do tempo de aplicação da mesma. Dos dados obtidos nesse estudo pode-se chegar ao tempo de irradiação de 38 segundos para HL e 4 segundos para DL, dessa forma as

temperaturas suficientes para comprometer os tecidos (elevação de 5,5 °C da polpa²⁷ e 10 °C do periodonto¹¹) não são atingidas por essas fontes. Já a fonte LED, mesmo aplicada por 360 segundos não gerou elevação de temperatura no dente acima dos níveis críticos, o que sugere que fontes LED's podem ser mais bem indicadas para o clareamento dental quando considerado o aquecimento dos tecidos.

No clareamento de consultório o peróxido de hidrogênio a 35% entra em contato direto com a superfície de esmalte, juntamente irradiação com fontes de luz para acelerar o processo. Assim o uso de altas concentrações do peróxido de hidrogênio somado com a energia fornecida pelas fontes de luz traz preocupações relacionadas à desmineralização do esmalte. Já no clareamento caseiro, são utilizadas menores concentrações de peróxido de hidrogênio, que portanto se difundem menos através do dente²⁵. Além disso, a saliva se difunde para dentro da placa e dissolve o gel clareador, atuando de forma a não causar grandes diminuições da microdureza do esmalte²⁸.

A irradiação das amostras gera calor e esse calor pode alterar a composição superficial do esmalte⁹, além de atuar aumentando a penetração do peróxido de hidrogênio²⁵ e acelerando a quebra¹⁴ em radicais livres responsáveis pelo processo de clareamento². Em

nosso trabalho foi observado uma notável redução nos valores de microdureza para os grupos em que se empregou luz. Outro efeito interessante que ocorreu com os grupos que utilizaram luz foi uma aparente resistência à remineralização, se comparado ao grupo controle, mesmo quando armazenados em saliva por 21 dias. Isso pode sugerir que ocorreram modificações permanentes na estrutura do esmalte, entretanto o trabalho em questão poderá servir de suporte para pesquisas que venham investigar o efeito da luz sobre esse fato.

5 Conclusão

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De acordo com a metodologia aplicada e os resultados obtidos julgamos lícito concluir que:

- 1) O uso de géis com diferentes cores não alterou significativamente o aumento médio de temperatura para a polpa e superfície dental;
- 2) O laser de diodo foi a que apresentou maior aquecimento dos tecidos quando comparada com as outras fontes estudadas;
- 3) O LED pode ser indicado para o clareamento dental quando se considera o aquecimento dos tecidos;
- 4) O uso dos comprimentos de onda empregados em associação com o gel clareador utilizado nesse estudo reduziu a dureza superficial do esmalte;
- 5) A saliva artificial foi capaz de remineralizar o esmalte clareado.

6 Referências

6 Referências*

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7 Anexos

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