

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE ODONTOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA
ÁREA DE CONCENTRAÇÃO CLÍNICA ODONTOLÓGICA – MATERIAIS DENTÁRIOS

Linha de pesquisa: Biomateriais e Técnicas Terapêuticas em Odontologia

AVALIAÇÃO DO EFEITO DO DIÓXIDO DE TITÂNIO ATIVADO POR LUZ
VISÍVEL NO CLAREAMENTO DENTAL

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Porto Alegre, junho de 2015

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Dissertação apresentada como requisito obrigatório
para obtenção do título de Mestre em Odontologia
na área de concentração em Clínica Odontológica

Porto Alegre, 2015

CIP - Catalogação na Publicação

Oliveira de Souza, Marcela
AVALIAÇÃO DO EFEITO DO DIÓXIDO DE TITÂNIO ATIVADO
POR LUZ VISÍVEL NO CLAREAMENTO DENTAL / Marcela
Oliveira de Souza. -- 2015.
30 f.

Orientador: Fabrício Mezzomo Collares.

Dissertação (Mestrado) -- Universidade Federal do
Rio Grande do Sul, Faculdade de Odontologia,
Programa de Pós-Graduação em Odontologia, Porto
Alegre, BR-RS, 2015.

1. Clareamento Dental. 2. Peróxido de hidrogênio.
3. Dióxido de titânio. I. Mezzomo Collares, Fabrício ,
orient. II. Título.

"A verdadeira viagem de descobrimento não
consiste em procurar novas paisagens,
e sim em ter novos olhos."

Marcel Proust

DEDICATÓRIA

Dedico este trabalho aos meus pais, *Cezar e Eloá*, que além do amor incondicional, transmitiram-me os preciosos valores que levo para minha vida.

À minha irmã, *Laila*, acima de tudo, minha amiga e companheira.

Ao meu namorado *Thiago*, pelo apoio, incentivo e paciência.

AGRADECIMENTOS

Ao Professor ***Fabício Mezzomo Collares***, orientador, pela dedicação e tempo dispensados, pelo crescimento pessoal e oportunidades a mim proporcionadas.

À Professora ***Susana Maria Werner Samuel*** responsável pela oportunidade de fazer parte do laboratório e por apresentar-me à área da pesquisa científica.

Ao professor ***Vicente Castelo Branco Leitune*** por todo apoio e incentivo prestados.

À Professora ***Carmen Beatriz Borges Fortes*** pelo acolhimento e colaboração.

À Professora ***Annelise Koop Alves*** pela acolhida no LACER e pela disponibilidade.

A todos os colegas do ***Laboratório de Materiais Dentários (LAMAD)*** pela convivência e colaboração durante todos os meus anos de laboratório.

À técnica e amiga ***Letícia Moreira***, por todo o auxílio prestado na pesquisa e os momentos de conversa.

Às minhas amigas e colegas ***Priscila Bohn, Natália Bertella, Aline Caume, Cecília Meller, Camilla Nascimento, Vivian Wagner e Gabriela Goldenfum*** por todas as horas de carinho e companheirismo.

À ***Faculdade de Odontologia da UFRGS***, ao ***PPGODO***, à ***UFRGS*** e à ***CAPES*** pela oportunidade de estudo, conhecimento e crescimento pessoal.

RESUMO

Clareamento dentário é atualmente um dos tratamentos estéticos mais requisitados na odontologia devido ao crescimento da exigência estética por parte dos pacientes. Assim, pesquisas sobre o tema tem avançado buscando aprimorar materiais e protocolos. Este estudo tem como objetivo avaliar o efeito da ação fotocatalizadora do dióxido de titânio (TiO_2) ativado por luz visível adicionado à baixa e alta concentração de peróxido de hidrogênio (H_2O_2) e sua eficácia no processo clareador dentário diante da redução do período de aplicação. A ação fotocatalizadora do TiO_2 foi avaliada utilizando uma solução de H_2O_2 3,5 e 35% e azul de metileno (AM). A análise de cor (ΔE) foi realizada em dentes bovinos empregando H_2O_2 gel 6% e 35%. As amostras foram fotoativadas durante 5min utilizando um aparelho de LED. O tempo total de de ação foi de 15min; apenas para o grupo controle aplicado em dente o total foi de 45min (H_2O_2 35% sem TiO_2). Os grupos contendo H_2O_2 3,5 ou 35%, AM e TiO_2 seguido de fotoativação apresentaram diferença estatisticamente significativa quando comparado com os demais grupos testados. Entretanto, quando as duas concentrações foram comparadas também houve diferença significativa, apresentando com 35% maior diminuição da concentração de AM. Nos testes realizados em dentes bovinos, 35% H_2O_2 gel e TiO_2 não apresentaram diferença para o grupo controle. Quando a concentração (6%) e tempo de aplicação foram conjuntamente reduzidos houve diferença em relação ao controle. Porém, todos os grupos avaliados apresentaram ΔE acima de 3,3. H_2O_2 em alta e baixa concentração com adição de TiO_2 , luz e redução do tempo de ação foram considerados efetivos para aplicação em clareamento dentário.

Palavras-chave: Clareamento Dental. Peróxido de hidrogênio. Dióxido de titânio.

ABSTRACT

Tooth whitening is currently one of the most requested aesthetic treatments in dentistry. Thus, research studies have attempted to improve the materials and protocols used in this treatment. This study evaluated the photocatalyst effects of visible light activated titanium dioxide (TiO_2) employed in low and high hydrogen peroxide (H_2O_2) concentrations with a reduced application period. TiO_2 effect was evaluated using seven H_2O_2 solutions (3.5 and 35%) and methylene blue (MB). Color analysis (ΔE) was performed on bovine teeth using H_2O_2 gel 6 and 35% ($\text{GH}_2\text{O}_2 35\%$, $\text{GH}_2\text{O}_2 35\% + \text{TiO}_2$ and $\text{GH}_2\text{O}_2 6\% + \text{TiO}_2$). The samples were light activated (LA) for 5 minutes using an LED device. Total action time was 15min. For the control group ($\text{GH}_2\text{O}_2 35\%$), the action time was 45min. Solutions containing MB, H_2O_2 at 3.5 or 35%, and TiO_2 , followed by LA, showed a statistically significant difference when compared with the other groups tested. However, when the two concentrations were compared, significant difference was also found, showing greater MB reduction for 35%. In tests employed on bovine teeth, $\text{GH}_2\text{O}_2 35\% + \text{TiO}_2$ gel showed no difference in comparison to $\text{GH}_2\text{O}_2 35\%$ (the control group). When the concentration and application time were reduced ($\text{GH}_2\text{O}_2 6\% + \text{TiO}_2$), a statistical difference was found ($p < 0.05$). Nevertheless, all groups showed ΔE higher than 3.3. Titanium dioxide and hydrogen peroxide association was affected by light activation. Thus, high and low H_2O_2 concentrations showed color difference in comparison to the baseline color and did so after a shorter application period when combined with TiO_2 , suggesting the effectiveness of this material and being promising of use to tooth bleaching.

Keywords: Tooth bleaching. Hydrogen peroxide. Titanium dioxide

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1 INTRODUÇÃO

Clareamento dental vem sendo cada vez mais requisitado em consultórios dentários devido à grande exigência estética por parte dos pacientes (1). Como alteração de cor nos dentes é um aspecto que prejudica significativamente o sorriso e há cada vez mais valorização de procedimentos menos invasivos, a técnica de clareamento dental representa uma importante opção de tratamento estético (2, 3). Atualmente, três técnicas de clareamento são utilizadas: clareamento de dentes desvitalizados, técnica de clareamento caseiro com utilização de moldeiras e técnica de clareamento em consultório. O clareamento de dentes vitais é um procedimento relativamente simples, conservador e eficaz, proporcionando resultados satisfatórios (4).

A técnica de clareamento de consultório utiliza peróxido de hidrogênio (H_2O_2) em concentrações entre 20 e 38% como agente clareador. O procedimento é realizado no consultório e, portanto, com acompanhamento direto do cirurgião dentista, exigindo mais tempo de atendimento clínico e conseqüentemente maior custo para o paciente. É indicado quando o paciente não adere ao tratamento caseiro ou deseja reduzir o tempo de clareamento. Apresenta como vantagem maior controle da técnica, não dependendo da colaboração do paciente, controle dos locais de aplicação como a possibilidade de clarear dentes isolados e de ser um acelerador inicial do clareamento quando associado à técnica caseira (5).

Os agentes clareadores atuam por oxidação devido a sua habilidade de formar radicais livres: moléculas de oxigênio reativo e ânions de peróxido de hidrogênio. A substância clareadora penetra na estrutura dental pelo baixo peso molecular e pela desnaturação proteica que aumenta a passagem e o trânsito do agente clareador pelo esmalte e dentina até atingir o pigmento formado por compostos chamados de cromóforos (5-7). Estes compostos apresentam cadeias longas com peso molecular relativamente alto, muitas ligações insaturadas e anéis aromáticos. Quanto maior a insaturação de uma molécula, mais escura a cor do pigmento e maior o índice de absorção de luz do mesmo. A quebra das cadeias cromóforas pode ocorrer pelo rompimento de uma ou mais ligações existentes em sua composição (4, 5).

Atualmente, as fontes de luz utilizadas em Odontologia tem sido utilizadas como potencializador na técnica de clareamento de consultório, mas relatos na literatura

confrontam o uso de fontes auxiliares de luz, como LED, halógena ou laser, mostrando que estes não potencializam os resultados do procedimento clareador (8, 9). Assim, o crescente interesse por uma melhor aparência estética do sorriso, tem incentivado o desenvolvimento significativo de novos materiais e técnicas propiciando uma importante evolução na odontologia estética.

Nesse contexto, um semicondutor, o dióxido de titânio tem sido estudado devido à sua ação em processos de oxidação e redução mediados pela luz (10). Ele absorve fótons com energia em um comprimento de onda menor que 380nm (Ultravioleta - UV) o que induz vibração molecular, havendo transferência de elétrons para um nível superior de energia e degradando o peróxido de hidrogênio (11). Devido a este mecanismo, alguns estudos tem introduzido a utilização de dióxido de titânio (TiO_2) como agente fotocatalisador com o objetivo de aumentar a eficácia do gel clareador quando exposto à uma fonte luz, através da formação de um radical hidroxila por meio da sua ação fotocatalítica (12-14).

Entretanto, a exposição prolongada à radiação UV é uma das principais causas exógenas que conduz a dano celular e imunossupressão, podendo levar a câncer de pele, tornando-a inapropriada para clareamento dental (15). Além disso, os aparelhos utilizados em odontologia são de luz visível e apresentam comprimento de onda dentro do espectro azul, como por exemplo, os aparelhos que utilizam LED e emite na faixa de comprimento de onda de 440 a 500nm (16-18), tornando necessário que um agente clareador à base de H_2O_2 contendo como fotocatalisador dióxido de titânio, mesmo exibindo maior absorbância na região UV, apresente também forte absorção da luz visível (13, 14).

Diante do exposto, TiO_2 pode ser uma promissora alternativa para acelerar o processo de clareamento. O seu emprego poderá viabilizar tanto a diminuição do tempo de consulta e número de aplicações quanto o custo, bem como minimizar possíveis danos à estrutura dentária e tecido pulpar (19) pela possibilidade de utilizar uma baixa concentração de H_2O_2 .

2 OBJETIVO

Este estudo tem como objetivo avaliar o efeito da ação fotocatalizadora do dióxido de titânio ativado por luz visível adicionado à baixa e alta concentração de peróxido de hidrogênio e sua eficácia no processo clareador dentário diante da redução do período de aplicação .

3 MANUSCRITO

Esta dissertação de mestrado é composta pelo seguinte manuscrito:

Effect of visible light-activated titanium dioxide photocatalysis on hydrogen peroxide

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3.1 ABSTRACT

Aim This study evaluated the photocatalyst effects of visible light activated titanium dioxide (TiO_2) employed in low and high hydrogen peroxide (H_2O_2) concentrations with a reduced application period.

Methods TiO_2 effect was evaluated using seven H_2O_2 solutions (3.5 and 35%) and methylene blue (MB). Color analysis (ΔE) was performed on bovine teeth using H_2O_2 gel 6 and 35% ($\text{GH}_2\text{O}_2 35\%$, $\text{GH}_2\text{O}_2 35\% + \text{TiO}_2$ and $\text{GH}_2\text{O}_2 6\% + \text{TiO}_2$). The samples were light activated (LA) for 5 minutes using an LED device. Total action time was 15min. For the control group ($\text{GH}_2\text{O}_2 35\%$), the action time was 45min.

Results Solutions containing MB, H_2O_2 at 3.5 or 35%, and TiO_2 , followed by LA, showed a statistically significant difference when compared with the other groups tested. However, when the two concentrations were compared, significant difference was also found, showing greater MB reduction for 35%. In tests employed on bovine teeth, $\text{GH}_2\text{O}_2 35\% + \text{TiO}_2$ gel showed no difference in comparison to $\text{GH}_2\text{O}_2 35\%$ (the control group). When the concentration and application time were reduced ($\text{GH}_2\text{O}_2 6\% + \text{TiO}_2$), a statistical difference was found ($p < 0.05$). Nevertheless, all groups showed ΔE higher than 3.3.

Conclusion Titanium dioxide and hydrogen peroxide association was affected by light activation. Thus, high and low H_2O_2 concentrations showed color difference in comparison to the baseline color and did so after a shorter application period when combined with TiO_2 , suggesting the effectiveness of this material and being promising of use to tooth bleaching.

Keywords: Tooth bleaching. Hydrogen peroxide. Titanium dioxide

3.2 INTRODUCTION

Tooth bleaching treatment is reported for produce changes in oral health and quality of life and is considered one of the most important dentistry treatments for patient satisfaction (1). White teeth cause direct impact on smiling, laughing and have been linked to social competence, intellectual ability, successful interpersonal relationships, and even psychological stability (2, 3). Esthetic dentistry has focused its attention to this matter and a series of techniques and materials are constantly developed for this purpose.

Tooth whitening success depends mainly on the combination of peroxide concentration and application period (4, 5). In-office bleaching products generally contain high levels of the hydrogen peroxide (20-38%) and are applied for shorter time periods when compared to the home bleaching agents. Furthermore, has advantages in terms of professional control, avoidance of material ingestion, possibility of immediate results and treatment time reduction (6, 7). Energy sources have been used in association with in-office bleaching agents to produce faster and more effective treatment. Existing literature reports activation of bleaching agents by light using halogen, light emitting diode-LED or laser (8, 9). Light activated bleaching procedures, leads to increased release of hydroxyl radicals through a rise in temperature. However, this heat may cause irreversible damage to pulpal tissues (9, 10). Although the large debate, the effectiveness of light source for in-office bleaching has been controversial and it appears not to influence by speed, extent or durability of the bleaching effect (11-13).

Titanium dioxide has been known as an important semiconductor photocatalyst for a wide range of environmental applications and is a great option due to its high oxidizing properties, absence of toxicity, photo and chemistry stability in a wide pH band (14-16). The energy of its band gap is +3.2 eV, and therefore, titanium dioxide absorbs ultraviolet light (< 387 nm), producing vital oxygen and hydroxyl radicals (17). However, titanium dioxide shows to be able to react at visible light exposure of 400 nm. Thus, it appears that the light source might be an important determinant for bleaching effect when hydrogen peroxide and titanium dioxide are associated (18, 19).

Bleaching may be enhanced by generation of oxidative hydroxyl radicals though titanium dioxide photocatalyst and depends strongly of absorption properties in the range of visible light in the vicinity of 400nm (20-23). In this way, low concentration

hydrogen peroxide solution containing a titanium dioxide have recently been introduced for in-office tooth bleaching, with the claim of increased safety and efficacy over conventional high concentration formulations, but H_2O_2 concentrations, TiO_2 proportion and time application protocols not are defined (18-20).

Therefore, this study aims 1- to evaluate the photocatalyst effects of visible light activated titanium oxide, 2- the employ in low and high hydrogen peroxide concentrations with reduced application period.

3.3 MATERIALS AND METHODS

This study evaluated the effects of titanium dioxide using Methylene Blue dye for photocatalysis analysis and extracted bovine incisors for color measurements.

Photocatalysis analysis:

Preparation of experimental solutions

Seven experimental solutions were prepared by mixing hydrogen peroxide (H_2O_2) and/or titanium dioxide (TiO_2)—AEROXIDE[®]-P25 (30nm size particle)—with Methylene Blue $\text{C}_{16}\text{H}_{18}\text{ClN}_3\text{S}$ - 20 ppm solution (MB). In the solutions containing hydrogen peroxide, the latter was used in two different concentrations: 35% and 3.5%, obtaining as a result, two groups to compare these two different concentrations. For each group, the seven solutions were developed separately, and 4.0 mL of each experimental solution was poured into a quartz cell. In the solutions containing TiO_2 , 10 mg were added, according to the following formulations:

Sol_{MB}: MB (control group)

Sol_{MB+H₂O₂}: MB + H_2O_2 without light activation (15 minutes of action)

Sol_{MB+H₂O₂+LA}: MB + H_2O_2 + light activation for 5 minutes + 10 minutes waiting period

Sol_{MB+TiO₂}: MB + TiO_2 without light activation (15 minutes of action)

Sol_{MB+TiO₂+LA}: MB + TiO_2 + light activation for 5 minutes + 10 minutes waiting period

Sol_{MB+H₂O₂+TiO₂}: MB + H_2O_2 + TiO_2 without light activation (15 minutes of action)

Sol_{MB+H₂O₂+TiO₂+LA}: MB + H_2O_2 + TiO_2 + light activation for 5 minutes + 10 minutes waiting period

Light activation

The visible light curing unit employed in this study was Emitter - LED unit, 700mW/cm², (Schustter – Santa Maria, RS, Brazil). Each specimen (quartz cell) was masked by an aluminum foil and the top of cell solutions were light activated (LA) with continuous irradiance for 5 minutes. During light irradiation, the solution was stirred using a magnetic stirrer. After the light activation, the aluminum foil was removed and the cell was left for 10 minutes, totaling 15 minutes of action. Each solution was repeated three times (n=3). LED unit intensity was verified before each utilization (ECEL Radiometer – Ribeirão Preto, SP, Brazil).

Table 1. Formulations of the experimental solutions for H₂O₂ at 35% and 3.5%.

Solutions	MB	H₂O₂	TiO₂	Light activation
Sol_{MB}	+	—	—	—
Sol_{MB+H₂O₂}	+	+	—	—
Sol_{MB+H₂O₂+LA}	+	+	—	+
Sol_{MB+TiO₂}	+	—	+	—
Sol_{MB+TiO₂+LA}	+	—	+	+
Sol_{MB+H₂O₂+TiO₂}	+	+	+	—
Sol_{MB+H₂O₂+TiO₂+LA}	+	+	+	+

Absorbance analysis

Before the analysis of the photocatalysis, TiO₂ was removed using a filter (0.22 µm) coupled with a syringe and the solutions were placed into another cell. Absorbance at 660 nm was measured using a spectrophotometer (Agilent, Santa Clara, CA, USA) to determine concentration as an indicator of the bleaching effect. In the experimental solutions, the MB remaining concentrations were determined through the absorbance of a sample solution (*A_s*) compared with a pure MB solution (*A_c*) and were calculated according to the following equation, based on the previous study (18):

Concentration of MB after irradiation [ppm]:

$$C = \frac{A_s}{A_c} \times 10$$

A_s = absorbance of experimental solution after irradiation.

A_c = absorbance of pure solution MB 20ppm (control group) before irradiation.

After tests using an organic dye, this study evaluated the behavior of TiO₂ when added to bleaching products currently marketed.

Teeth color change analysis:

Teeth preparation

Thirty-six extracted bovine incisors were used in this study. The crowns were cleaned by removing soft tissue remnants with a scalpel, the roots were cut using a diamond disc, and pulpal tissue was removed.

A stain solution was prepared by coffee immersion (100g – Mellita, Brazil) in 1L of boiled water. Specimens were immersed into the solution and stored in a refrigerator for 7 days at 4°C.

After being removed from the solution, the specimens were rinsed, dried, and stored in distilled water at 4°C. Labial surfaces were covered with a masking tape containing a 6 mm-diameter hole purposed to fit the spectrophotometer's scanner. This procedure enabled the measuring area to be at the same spot in each step.

Bleaching treatment

The specimens were randomly assigned into three groups ($n=12$).

G_{H₂O₂35%}: office bleaching agent H₂O₂ at 35% (Whiteness HP, FGM, Joenville, SC, Brazil) without TiO₂ addition (control group). Each tooth was light activated with continuous irradiance for 5 minutes and the bleaching agent was left on the specimens for a further 40 minutes, totaling 45 minutes of gel action, according to the manufacturer's instructions.

G_{H₂O₂ 35%+TiO₂}: office bleaching agent H₂O₂ at 35% (Whiteness HP, FGM, Joenville, SC, Brazil) with the addition of TiO₂. Each tooth was light activated with continuous irradiance for 5 minutes and the bleaching agent was left on the specimens for a further 10 minutes, 15 minutes of gel action.

G_{H₂O₂6%+TiO₂}: home bleaching agent H₂O₂ at 6% (White Class, FGM, Joenville, SC, Brazil) with the addition of TiO₂. Each tooth was light activated with continuous irradiance for 5 minutes and the bleaching agent was left on the specimens for a further 10 minutes, totaling 15 minutes of gel action.

Bleaching gel was applied to the experimental surface to be whitened using a disposable brush. The specimens in each group were photo-cured for five minutes using the LED unit. After treatment time, the bleaching gel was removed using a damp gauze. The specimen was washed thoroughly under tap water and was dried gently.

Color measurement

Labial surfaces were measured using a spectrophotometer (CM2600 Konica Minolta, Osaka – Japão). The CIE *L*a*b** values were then assessed using the CIELab (Commission Internationale L'Eclairage) color coordinate system. When a color is expressed in CIELAB, *L** defines lightness, *a** denotes the red/green value, and *b** denotes the yellow/blue value. ΔL values indicate that the sample is lighter than the standard (baseline readout), with negative values indicating that the sample is darker than the standard. Given ΔL , Δa , and Δb , the total difference or distance on the CIELAB diagram can be stated as a single value, known as ΔE .

As baseline data, the tooth surfaces were recorded prior to bleaching for the first time. Bleaching treatments and measurements were subsequently repeated 3 times on the specimen with one week intervals; each color measurement was carried out 24h after the procedure.

The color difference (ΔE) was calculated according to the following equation:

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$$

Statistical analysis

For each experimental solution, the mean and standard deviation were calculated. To evaluate titanium dioxide photocatalysis using methylene blue, a two-way ANOVA test was applied. Holm-Sidak test was used as a post hoc test for multiple comparisons.

ΔE data of the bovine teeth groups was subjected to statistical analysis with the one-way ANOVA test. Holm-Sidak test was used as a post hoc test for multiple comparisons. The statistical significance was defined as $p < 0.05$.

3.4 RESULTS

Table 2. Changes in MB concentration [ppm] at each H_2O_2 solution

Solutions	35% Mean (SD)	3.5% Mean (SD)
Sol_{MB}	10 (0) Aa	10 (0) Aa
Sol_{MB+H₂O₂}	3.46 (0.18) Cb	8.95 (0.34) Ba
Sol_{MB+H₂O₂+LA}	2.67 (0.17) Db	8.72 (0.38) Ba
Sol_{MB+TiO₂}	9.25 (0.23) Ba	9.04 (0.13) Ba
Sol_{MB+TiO₂+LA}	9.06 (0.17) Ba	9 (0.25) Da
Sol_{MB+H₂O₂+TiO₂}	0.95 (0.16) Eb	1.85 (0.38) Ca
Sol_{MB+H₂O₂+TiO₂+LA}	0.02 (0.01) Fb	1.28 (0.23) Da

Different capital letters indicate statistical difference in the same columns.

Different small letters indicate statistical difference in the same line $P < 0.05$

The two-way ANOVA results are shown in Table 2. H_2O_2 at 35%, non-activated and without TiO_2 addition, showed a statistically significant difference when compared to light activation (Sol_{MB+H₂O₂} and Sol_{MB+H₂O₂+LA}). For the same groups, using H_2O_2 at 3.5% showed no difference in terms of light application. The solutions Sol_{MB+TiO₂} and Sol_{MB+TiO₂+LA}, which used only MB and TiO_2 , showed little decrease in MB concentration despite light activation. In contrast, the concentration of MB was significantly influenced by the experimental solution when H_2O_2 , TiO_2 , and light activation were associated (Sol_{MB+TiO₂+LA}), with the highest value of MB concentration decrease shown in H_2O_2 at 35% and 3.5%. The association between H_2O_2 and TiO_2 also showed an MB decrease even without light activation (Sol_{MB+H₂O₂+TiO₂}). Among the two different concentrations of H_2O_2 , 35% resulted in the most significant reduction in MB; however 3.5% with the addition of TiO_2 also decreased MB concentration.

Table 3. ΔE mean of each experimental group

Groups H ₂ O ₂ 6% and 35%	Delta E Mean (SD)
G_{H₂O₂ 35%} (45 min)	5.39 (1.11) A
G_{H₂O₂ 35%+TiO₂} (15 min)	4.17 (1.62) AB
G_{H₂O₂ 6%+TiO₂} (15 min)	3.38 (2.01) B

Different capital letters indicate statistical difference. $p < 0.05$

ΔE mean and standard deviation of experimental groups are shown in Table 3. G_{H₂O₂35%}, without TiO₂ and with a major application time did not show significant difference in comparison with G_{H₂O₂ 35%+TiO₂. However, when H₂O₂ concentration was reduced (G_{H₂O₂ 6%+TiO₂), a statistically significant difference was found. The groups containing TiO₂ did not show a statistically significant difference despite the two different H₂O₂ concentrations. ΔE , ΔL , Δa , and Δb values of each application time in each group are shown in figures 1–3. After bleaching, ΔL and ΔE values gradually increased in all groups, while Δa and Δb showed a gradual decrease. Figure 4 summarizes the ΔL change for each group.}}

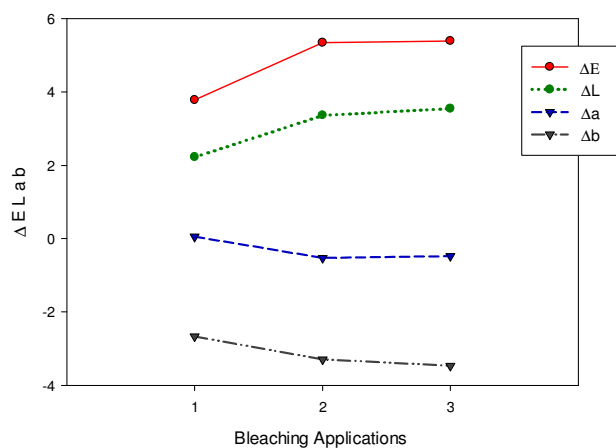
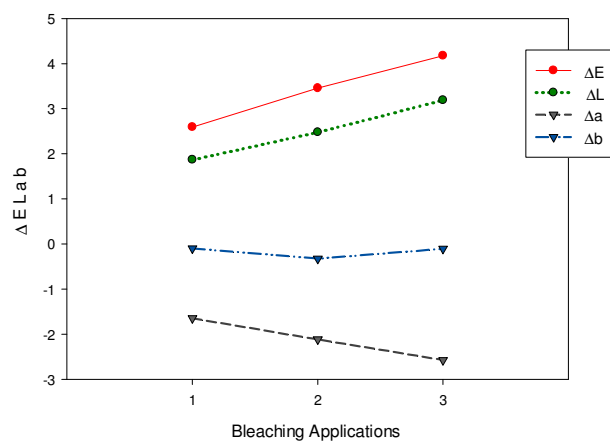


Figure1: The mean ΔE , ΔL , Δa , and Δb , values of GH_2O_2 35% group



The mean ΔE , ΔL , Δa , and Δb , values of GH_2O_2 35%+TiO₂ group

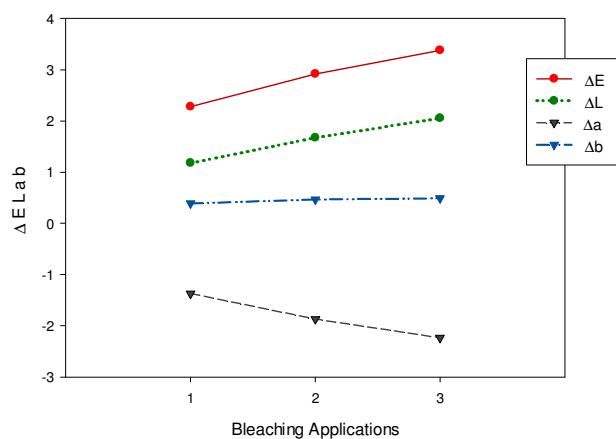


Figure 3:The mean ΔE , ΔL , Δa , and Δb , values of GH_2O_2 6%+TiO₂ group

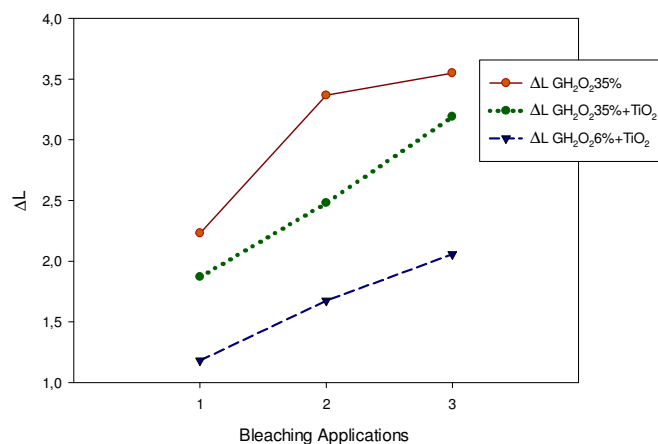


Figure 4:ΔL of each experimental group

Ascending order of ΔE is shown in Figure 5. Among the three procedures to GH_2O_2 35%, two applications were enough to produce a bleaching effect. GH_2O_2 6%+TiO₂ also did not show a statistically significant difference despite the lower ΔE value. However, a third gel application appears to be an important factor in terms of the reduction in the time application (GH_2O_2 35%+TiO₂).

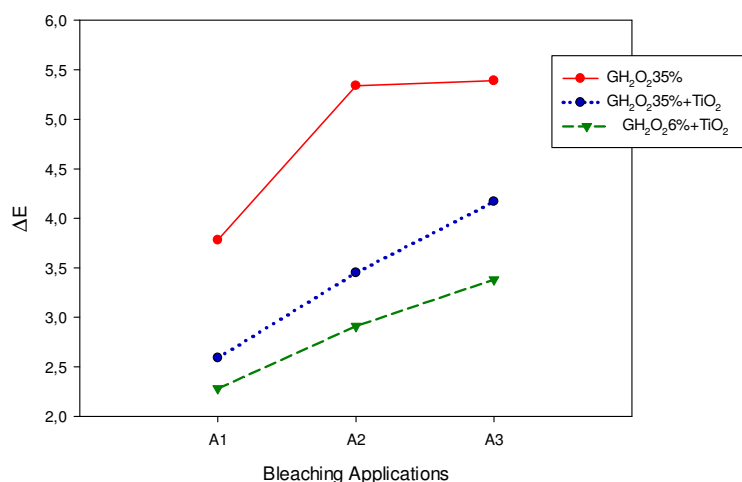


Figure 5: The ΔE value of each experimental group

3.5 DISCUSSION

This *in vitro* study evaluated the effects of visible light activated titanium dioxide photocatalyst on bleaching using different methodologies. This design intended to be useful as a screening test for bleaching with hydrogen peroxide to establish their application methods before teeth application. Hydrogen peroxide acts as a strong oxidizing agent through the formation of free radicals, reactive oxygen molecules and hydrogen peroxide anions. Bleaching process occur by reduce or cleave of the double bonds pigments in the conjugated chain, in more diffusible molecules able to out of the tooth (4, 7). Low H₂O₂ concentrations gels catalyzed by titanium dioxide have been studied, due concern of cellular damage to pulp cells caused by typical concentrations of bleaching gel (35%), hypersensitivity and the caustic nature of the high concentrations hydrogen peroxide. Currently, *in vitro* models have been used to evaluate the efficacy of TiO₂ in tooth bleaching products, but the majority of these models concentrated in testing the effects of various light sources on the bleaching action (18-20). This study utilized only LED curing unit (440 to 500 nm) to analyze if TiO₂ absorption in the

visible region may speed up the oxidation reaction of H_2O_2 , and consequently enhance effectiveness and speed of bleaching.

On the first method employed, no teeth were used so that suitable evaluations could be realized with minimal variation and better reproducibility. The hydrogen peroxide was tested in high concentration (35%), normally used in-office bleaching, and in a low concentration (3.5%). To identify the bleaching effects, methylene blue dye (an organic material) was used and its decomposition was determined through the absorbance of a sample solution.(18, 24)

Hydrogen peroxide solution 35% and light irradiation, showed statically significant difference when compared to no light exposure ($\text{Sol}_{\text{MB}+\text{H}_2\text{O}_2}$ and $\text{Sol}_{\text{MB}+\text{H}_2\text{O}_2+\text{LA}}$). This did not occur with lower concentrations of H_2O_2 (3.5%). In consequence of light activation, a release of hydroxyl-radicals from high concentrations of H_2O_2 may be possible through direct excitation by light (photolysis), which then induces the molecular vibration and degradation of peroxide, subsequently leading to rise in temperature (9). The heat induced might cause damage to adjacent soft tissues and pulp. Moreover, the temperature increase of 5.5 °C is regarded as the critical value, which should not be exceeded to avoid irreversible pulp damage (25, 26). Although temperature increase was not measured, previous studies relate that heating of the irradiated objects by LED lights is expected to be minimal, producing the least thermal injury when compared to halogen or plasma arc units (27-29); for this reason, this light source was employed in the present investigation.

In experimental solutions containing only TiO_2 photocatalyst, there were little changes in the bleaching outcome. In other hand, association between H_2O_2 and TiO_2 showed MB decreased even no light exposition ($\text{Sol}_{\text{MB}+\text{H}_2\text{O}_2+\text{TiO}_2}$). It was likely that either TiO_2 or H_2O_2 produced less free radical than a combination of both the components. Additionally, when these components were combined with light irradiation MB concentration decreased effectively. Among the two different concentrations of H_2O_2 , 35% resulted in the most significant reduction in MB; however 3.5% with TiO_2 addition decreased MB concentration (Table 2).

According to tests using an organic dye, this study evaluated the behavior of TiO_2 added to bleaching products currently marketed. Bovine teeth were used as the substrate, it has been the most widely used substitute for human teeth and reported similar properties (20, 30). A solution of coffee was used for develop an intrinsic stain,

to simulate tooth discoloration observed clinically. Bleaching gel containing H_2O_2 35% was utilized as the total time indicated by the manufacturer, besides, in the same product TiO_2 was added and time action was reduced. Low concentration H_2O_2 bleaching gel (6%) employed only as home bleaching also was tested with TiO_2 addition like in-office regime; bleaching procedures were repeated 3 times, simulating 3 visits to dentist office.

Color difference has been used extensively in dental research and applications, including perceptibility and/or acceptability descriptions, despite this, several methods of color measurement after in-office bleaching described in the existing literature (CIELAB system, colorimeters, spectrophotometers and shade guides) become complicated this comparison. Different CIELAB ΔE units to an acceptable clinical comparison are cited ($\Delta E=1$, $\Delta E=2$, $\Delta E=3.3$), the variability of materials and methods employed, probably results in this wide range of color change. (31-34).

In this study, color readout was performed by a spectrophotometry following the CIE $L^*a^*b^*$ system and $3.3\Delta E$ values are considered acceptable (33) and color shifts occurred higher this value for all groups. H_2O_2 35% bleaching gel without TiO_2 addition and with major application time (GH_2O_2 35%), did not show significant difference in comparison to GH_2O_2 35%+ TiO_2 . Each tooth was light activated for 5 minutes in two groups. Bleaching agent was left on the teeth for more 40 minutes in GH_2O_2 35 and for more 10 minutes in GH_2O_2 35%+ TiO_2 , totalizing 45 and 15 minutes of gel action respectively. Thereby, formulation that comprised both H_2O_2 and a TiO_2 photocatalyst showed the same efficacy and had reduction of 30 minutes in tooth, indicating that TiO_2 containing gel can accelerate the bleaching process and may be an alternative to decreasing the clinical time required, considered as disadvantage, because makes the procedure more expensive for the patient.

Hydrogen peroxide concentration and contact time are two factors discussed, that determine tooth bleaching outcomes. The literature suggests that low concentration of bleaching agent with a longer contact time (home bleaching) is effective as the in-office bleaching with a highly concentrated bleaching agent with a shorter contact period (4, 5). When H_2O_2 concentration and time contact were reduced (GH_2O_2 6%+ TiO_2) a statically significant difference between control group was found. This way, a drastic reduction of two factors influenced tooth bleaching. In the other hand, low and high peroxide concentrations, in groups containing TiO_2 , applied during 15 minutes did not show statically significant difference in ΔE values. Bleaching effectiveness was defined

as a change in ΔE units, which represents an increase in luminosity and previous clinical studies have often used ΔL to assesses changes in the lightness of a tooth (5, 35, 36). Sustaining ΔE values found, ΔL values gradually increased in all groups, while Δa and Δb showed a gradual decrease. (Figure 1-4)

Among the three procedures, to $\text{G}_{\text{H}_2\text{O}_2\ 35\%}$ two applications were enough to bleaching effect. $\text{G}_{\text{H}_2\text{O}_2\ 6\%+\text{TiO}_2}$ also did not show statically significant difference between second and third sessions, despite the lower ΔE value (Figure 1). Third gel application appears to be an important factor to high H_2O_2 concentration and time application reduction ($\text{G}_{\text{H}_2\text{O}_2\ 35\%+\text{TiO}_2}$). Conforming to finds, all treatments showed a change between baseline color; nevertheless, over peroxide concentration and duration of gel on the tooth, number of gel applications also have to be considered as a factor determining to efficacy in TiO_2 containing bleaching and achieve better performance of this compound.

3.6 CONCLUSION

Titanium dioxide and hydrogen peroxide association was affected by light activation. Thus, high and low H_2O_2 concentrations showed color difference in comparison to the baseline color and did so after a shorter application period when combined with TiO_2 , suggesting the effectiveness of this material and being promising of use to tooth bleaching.

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4 CONSIDERAÇÕES FINAIS

Apesar de amplamente estudado e discutido, o clareamento dentário ainda é um tema que gera constantes esforços, visando o desenvolvimento de materiais e protocolos que aprimorem seu desempenho e diminuam possíveis reações adversas. Neste estudo foi proposto a adição de um agente fotocatalisador - dióxido de titânio – ao peróxido de hidrogênio em alta e baixa concentração, assim como sua ativação por luz visível e redução do tempo de contato entre peróxido e dente. Frequentes investigações têm sido realizadas empregando dióxido de titânio em procedimentos clareadores, pois espera-se que esta associação potencialize a geração de radicais livres, aumentando segurança e eficácia do tratamento.

Os resultados apresentados no artigo, mostram que TiO_2 foi capaz de absorver a luz gerada pelo aparelho LED e apresentar significativas reduções na concentração de azul de metileno, assim como diferença nos valores de ΔE após ser aplicado em dentes bovinos previamente pigmentados. Além disso, conseguiu-se reduzir o tempo de exposição do dente ao gel a um terço do recomendado pelo fabricante. Desta forma, sua ação fotocatalizadora apresenta-se como uma promissora alternativa para acelerar o processo de clareamento, diminuindo tempo de tratamento e custo, além de minimizar possíveis danos ao tecido pulpar pela possibilidade de utilizar uma baixa concentração de H_2O_2 . Os achados obtidos justificam novos investimentos em avaliações e ajustes, como tempo de fotoativação, concentração de peróxido, duração, número de sessões e reações adversas para que um gel de clareamento dentário contendo TiO_2 possa ser de fato considerado eficaz e seguro.

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