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EFEITO DO LASER DE ER:YAG NA RESISTÊNCIA DE UNIÃO AO ESMALTE E À DENTINA SUBMETIDOS AO CLAREAMENTO DENTAL

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Co-Orientador : Prof. Dr. José Augusto Rodrigues

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Guarulhos, 22 de Fevereiro de 2010.

Dedico esta dissertação à minha
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esteve muito perto de mim.

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“É muito melhor arriscar coisas grandiosas para alcançar o triunfo e glórias, mesmo expondo-se à derrota, do que formar fila com os pobres de espírito que nem gozam muito, nem sofrem muito, porque vivem em uma penumbra cinzenta, que não conhecem vitória nem derrota”

T. Roosevelt

RESUMO

Este trabalho teve como objetivo avaliar a influência de diferentes parâmetros de densidade de energia de laser de Er:YAG (2,94μm) na resistência de união por microtração (RU) e na morfologia de dentes bovinos clareados em esmalte (artigo 1) e dentina (artigo 2) à resina composta. Para a avaliação em esmalte, dentes bovinos foram clareados (n=30) ou não (n=30) com peróxido de carbamida 16%, em seguida foram lixados e divididos em dois subgrupos de acordo com a irradiação de Er:YAG: 25,56 J/cm^2 (ablativo, modo contato, LA) ou 4,42 J/cm^2 (sub-ablativo, modo não-contato, LB). Os fragmentos foram restaurados com adesivo Adper Single Bond 2 e resina Filtek Z250 (3M/ESPE), seccionados em forma de ampulhetas e submetidos ao teste de RU. Para a avaliação da dentina 40 fragmentos foram divididos em 2 grupos, clareados (n=20) ou não (n=20), que foram desgastados até dentina, sendo um grupo irradiado com laser de Er:YAG (25,52 J/cm^2, modo focado) e preparados para ensaio de RU. Os valores de RU foram analisados por two-way ANOVA e teste Tukey (α=0.05). O modo da fratura foi determinado em aumento de 100X usando lupa estereomicroscópica e um fragmento tratado foi avaliado em microscopia eletrônica de varredura (MEV). Alta porcentagem de falha adesiva foi encontrada para superfície de esmalte clareado e irradiado. Com base nos resultados concluiu-se que a superfície de esmalte clareado não foi estatisticamente diferente da superfície de esmalte não clareado para os valores de RU. A irradiação com Er:YAG (LA) antes do procedimento adesivo na superfície de esmalte não teve influência na RU e promoveu ablação com base nas análises de MEV. Por outro lado, a irradiação com laser de Er:YAG (LB) antes do procedimento adesivo não alterou os valores de RU e não promoveu ablação observada em MEV. O procedimento clareador não afetou os valores de RU para adesão em dentina. A irradiação com laser de Er:YAG com densidade de energia de 25,52 J/cm^2 antes do procedimento adesivo não afetou a RU à dentina de dentes clareados mas diminuiu a RU de dentes não clareados e promoveu ablação na superfície observada sob MEV.

PALAVRAS-CHAVES

Ablação com laser, Laser Er:YAG, Microscopia eletrônica de varredura, Microtração, Resistência de união, clareamento dental

ABSTRACT

The aim of this study was to evaluate the influence of different energy parameters of Er:YAG laser (λ= 2.94μm) on microtensile bond strength (μTBS) and morphology of bleached bovine teeth in enamel (manuscript 1) and dentin (manuscript 2) to resin composite. For enamel evaluation bovine teeth were bleached (n=30) or not (n=30) with 16% carbamide peroxide, afterwards flattened and divided in two subgroups according Er:YAG irradiation: 25.56 J/cm^2 (ablative, contact mode, LA) or 4.42 J/cm^2 (sub-ablative, non-contact mode, LB). The slabs were restored with Adper Single Bond 2 and Filtek Z250 (3MESPE), sectioned in hour-glass shape and were subjected to μTBS test. For dentin evaluation, 40 slabs were divided and 2 groups bleached (n=20) or not (n=20) that were flattened to expose dentin, being one group Er:YAG laser irradiated (25.52 J/cm^2, focused mode), prepared for μTBS test, failure type. μTBS data were analyzed by two-way ANOVA and Tukey test (α=0.05). Failure mode was determined at a magnification of 100X using a stereomicroscope and one treated slab of each group was selected for scanning electron microscope (SEM) analysis. A high amount of adhesive failure was recorded for bleached and lased enamel surface tested. Based on the results, one can conclude that bleached enamel surface did not show statistical difference from the non-bleached enamel surface for μTBS values. Er:YAG laser irradiation (LA) prior to adhesive procedure on bleached enamel surface had no influence on μTBS and promoted ablation as observed using SEM. On the other hand, Er:YAG laser irradiation (LB) prior to adhesive procedure did not affect the μTBS and did not promote ablation observed under SEM. Bleaching procedure did not affect μTBS values on dentin. Er:YAG laser irradiation with 25.52 J/cm^2 prior to bonding did not affect μTBS on dentin of bleached teeth but impaired μTBS of non-bleached teeth and promoted ablation observed under SEM.

KEY WORDS

Laser ablation, Er:YAG Laser, Scanning electron microscope, Microtensile bond strength, Dental Bleaching

SUMÁRIO

1. INTRODUÇÃO E JUSTIFICATIVA

Desde o início da vida humana, a boca é o órgão de ligação do bebê com o mundo externo, na vida adulta ela é o órgão da expressão e comunicação social. Desta forma, as pessoas procuram dentes brancos por diversas razões e essas podem ser: sociais, profissionais e psicológicas (Baratieri et al., 2004).

Dentes claros contribuem para um sorriso agradável, com uma aparência jovem mantendo-os dentro dos padrões de beleza, já o escurecimento dental pr problemas estéticos para a grande maioria dos pacientes (Goldstain & Garber, 1996).

As preocupações com dentes escurecidos são de longa data. Nos tempos modernos, pesquisadores vêm usando substâncias para clarear os dentes desde a década de 1860. Várias substâncias têm sido empregadas para clarear os dentes como Ácido Oxálico, Cloreto de Cálcio, Solução Clorada, Cloreto de Alumínio, Dióxido de Enxofre, Hipoclorito de Sódio, entre outras (Fasanaro, 1992).

Somente em 1989, Haywood & Heymann introduziram o sistema de aplicação de Peróxido de Carbamida a 10% com a utilização de moldeiras individuais para clareamento de dentes vitais, técnica denominada “caseira”. Nesta técnica utiliza-se moldeiras individuais para a aplicação de agentes clareadores a base de peróxido de hidrogênio ou peróxido de carbamida sobre os dentes escurecidos, promovendo-se o clareamento dos mesmos (Potocnik et al., 2000; Rodrigues et al., 2007).

Haywood & Heymann desenvolveram este estudo com base nos estudos de um Ortodontista de Arkansas, o Dr. Bill Klusmier no final da década de 1960, através da observação de seus pacientes que tinham quadro de gengivite. Sendo assim, o mesmo prescrevia um anti-séptico bucal, de nome Gly-Oxide, para ser utilizado durante a noite em uma moldeira semelhante a um protetor bucal esportivo de silicone.

Com o passar dos anos a técnica evoluiu, moldeiras individuais confeccionadas em plastificadoras a vácuo com silicone de fina espessura são utilizadas, a concentração de Peróxido de Carbamida aumentou para 16% e 22%, como forma de acelerar o processo clareador, diminuindo o tempo de tratamento para o paciente (Gurgan et al., 2009).

Assim, como alternativa eficaz para o tratamento do escurecimento dental e considerado um tratamento conservador estético, simples e de custo relativamente baixo, tem-se o clareamento dental, sendo que o clareamento caseiro de dentes vitais é a mais utilizada dentre as técnicas existentes, (Baratieri et al., 1993; Ritter et al., 2002; Rodrigues et al., 2005).

Os mecanismos com que os peróxidos atuam no processo de clareamento não são completamente compreendidos, mas especula-se que envolvem uma reação de oxi-redução, na qual as moléculas de peróxido de hidrogênio se quebram formando radicais livres de oxigênio, hidrogênio e peridroxil, que provocarão a quebra sucessiva das macromoléculas de pigmentos, transformando-as em moléculas menores, que são eliminadas da estrutura dental por um processo de difusão, promovendo o clareamento dental (Goldstain & Garber,1996).

O peróxido tem baixo peso molecular, característica que facilita sua penetração nas estruturas dentais, que associado à permeabilidade dental, permite a difusão do oxigênio pelo esmalte e dentina, age sobre as estruturas orgânicas do dente e desta forma efetuam o clareamento (Haywood & Robinson, 1997).

Clinicamente, o processo não apresenta efeitos colaterais que contra-indiquem este tratamento (Goldstain & Garber,1992; Potocnik et al., 2000; Rodrigues et al., 2005, Rodrigues et al., 2007), porém, diversos trabalhos demonstram que microscopicamente pode-se observar o desenvolvimento de porosidades, erosões, perda de microdureza e aumento da rugosidade superficial (Toko et al., 1993; Potocnik et al., 2000; de Oliveira et al,. 2003; Worschech et al., 2003; Rodrigues et al., 2005, Rodrigues et al., 2007).

Além disso, os radicais livres presentes no esmalte e dentina logo após o tratamento clareador podem ser responsáveis por prejudicar a adesão de materiais resinosos, nos casos que exigem a substituição de restaurações estéticas (Titley et al., 1991; Haywood 1992; Stokes et al., 1992; Potocnik et al., 2000; Shinohara et al., 2001; de Oliveira et al., 2003; Shinohara et al., 2004).

Os agentes à base de peróxido de carbamida ou peróxido de hidrogênio não têm poder clareador sobre a cor dos materiais restauradores. Freqüentemente após o tratamento há necessidade de troca das restaurações pré-existentes, com a utilização de procedimentos restauradores estéticos adesivos (da Silva Machado et al., 2007).

Testes de cisalhamento de resinas compostas com dentes humanos tratados previamente com agentes clareadores e não tratados, revelaram uma queda significativa na resistência de união (Titley et al., 1988; Torneck et al.,1990; Titley et al., 1991; Toko et al., 1993; Potocnik et al., 2000), devido a alterações no mecanismo de adesão da resina-dente o qual foi atribuído a uma inibição no processo de polimerização das resinas pela presença de oxigênio (Gurgan et al., 2009), entretanto, a resistência adesiva retorna ao normal após 15 a 21 dias (Titley et al., 1988; Crim et al., 1992; Basting et al., 2004; Shinohara et al., 2004).

Shinohara et al., em 2001, demonstraram em seu estudo que há um aumento da microinfiltração marginal em dentina, em restaurações classe V, após o clareamento com peróxido de carbamida 37% e perborato de sódio, por 21 dias, porém não houve diferença quando analisadas as margens em esmalte.

Lai et al., em 2002, demonstraram que dentes clareados com peróxido de carbamida 10%, por 8 horas e restaurados após 10 minutos, sofrem uma diminuição da adesão ao esmalte, quando submetidos ao teste de microtração. Em 2004, Shinohara et al., mostraram que há uma diminuição da força de adesão em dentina, nos dentes clareados com perborato de sódio e submetidos ao teste de cisalhamento, e não houve diferença com o controle, quando analisada a adesão em esmalte. Também em 2004, Türkün & Türkün clarearam dentes com peróxido de carbamida 10%, por 8h diárias por 7 dias, realizaram teste de microinfiltração e constataram um aumento da microinfiltração nos dentes clareados em relação ao grupo controle. Em 2006, Cadenaro et al., utilizaram peróxido de hidrogênio a 38% por 30 minutos e analisaram imediatamente o grau de conversão de adesivos em dentina, constataram uma queda no grau de conversão nos dentes clareados. Porém, Loretto et al., em 2004, não obtiveram resultados diferentes, com relação ao grupo controle, quanto a adesão em esmalte, em dentes clareados com peróxido de carbamida a 10%, por 4 horas, por 14 dias e submetidos ao teste de cisalhamento. Do mesmo modo Basting et al., em 2004, não obtiveram resultados diferentes de adesão em dentina, quando submeteram dentes clareados com peróxido de carbamida a 10%, por 42 dias, ao teste de cisalhamento.

Chuang et al., em 2009, utilizaram peróxido de carbamida 10% por 8 horas diárias, por 14 dias. Após este período os espécimes foram restaurados imediatamente e foi realizado teste de microtração. Os dentes clareados tiveram uma diminuição da força de adesão com relação ao grupo não clareado. Eldin et al. (2006) obtiveram resultados que mostraram que há queda nos valores de adesão em esmalte, em dentes clareados com peróxido de carbamida a 10% e submetidos ao teste de cisalhamento, obtiveram também 100% de falhas adesivas na análise dos padrões de fratura. Bulut et al. (2006) clarearam dentes com peróxido de carbamida a 10%, por 8 horas, por 7 dias e realizaram as restaurações imediatamente, estes dentes demonstraram queda em MPa, quando submetidos ao teste de cisalhamento.

Estudos realizados com dentes bovinos, considerados substitutos eficazes dos dentes humanos na avaliação *in vitro* da adesão dos compósitos (Haywood,1992; Saleh & Taymour, 2003, Reis et al., 2004), mostraram resultados semelhantes aos de dentes humanos. Os dentes clareados restaurados com resinas compostas apresentaram uma queda na força de adesão (Nakamichi et al., 1983; Kantorowitz et al., 1998; Meister et al., 2006), devido a presença de resíduos de peróxido próximos à superfície do esmalte, alterando a adesão da resina (Titley et al., 1988; Torneck et al.,1990; Stokes et al., 1992; Toko et al., 1993).

A perda da resistência adesiva logo após o tratamento clareador está relacionada ao tempo do tratamento clareador, da concentração aplicada e do período da realização dos procedimentos adesivos após o clareamento dental. (Leonard et al., 2002; Marson et al., 2008). A maioria dos estudos aponta para a necessidade de se aguardar de 2 a 3 semanas para a troca de restaurações, período necessário para que os radicais livres sejam eliminados (Perdigão et al., 1988; Titley et al., 1991; Crim et al., 1992; Barkhodar et al., 1997; Shinohara et al., 2001; de Oliveira et al., 2003; Basting et al., 2004; Shinohara et al., 2004; Shinohara et al., 2005).

Com base na premissa de que os radicais livres atrapalham o processo de polimerização e consequentemente a adesão, e a remoção destes pode promover uma adesão similar ao dente não clareado e que estes radicais são altamente instáveis e reativos, o aumento de energia térmica liberada pela irradiação de laser poderia levar a redução e total eliminação dos mesmos (Dishman et al., 1994; Cassoni & Rodrigues, 2007).

Laser é o acrônimo de "*Light Amplification by Stimulated Emission of Radiation*", que significa "Ampliação de Luz por Meio da Emissão Estimulada de Radiação", ou seja, o laser é uma luz que quando emitida promove fenômenos físicos e interage com os tecidos, a diferença é que é uma luz com comprimento de onda específico emitida em um feixe monocromático (uma única cor), coerente (os fótons se movem em sincronia) e colimado (feixes paralelos de luz) que pode ser facilmente focado para aplicação no tecido desejado obtendo interação ou efeito terapêutico (Perito et al., 2009).

O laser quando incide sobre um material pode sofrer, em combinação ou não, quatro fenômenos físicos: reflexão, quando a luz é refletida em outra direção; transmissão, quando a luz atravessa diretamente o material e não causa nenhum efeito; difusão, quando a luz penetra no material, mas se difunde no mesmo e absorção, quando a luz é absorvida. Desses, a absorção é o fenômeno mais desejado sobre os tecidos dentais, pois é através deste que a energia do laser se transforma em calor e promove alterações nos tecidos dentais (Aoki et al., 2004).

Um dos primeiros relatos encontrados na literatura com relação aos lasers utilizados na Odontologia foi com laser de rubi em alta densidade de energia (9.000 J/cm^2) que carbonizavam a estrutura dental (Goldman et al., 1964). Em 1989, Hibst & Keller foram os pioneiros na utilização do laser de Er:YAG para realização de preparos cavitários sem efeitos térmicos indesejáveis (Hibst & Keller, 1989). Desde então, lasers que possuem boa interação com o esmalte e dentina e pouca difusão de calor, como os de Er,Cr:YSGG e Er:YAG, podem ser utilizados para preparos cavitários (Hadley et al., 2000; Apel et al., 2003), para o condicionamento do esmalte e dentina (Botta et al., 2007; Bevilácqua et al., 2008) e para prevenção de cárie (Perito et al., 2009a; Perito et al., 2009b).

O laser de Er:YAG apresenta como meio ativo sólido, o cristal de ítrio-alumínio-granada dopado com érbio, com comprimento de onda de 2,94μm, infra-vermelho e invisível que coincide com o espectro de absorção da água com completa transformação em energia térmica (Lee at al., 2007). Os lasers de érbium promovem ablação de estruturas dentais efetivas devido a sua alta absorção pela água e baixa penetração térmica (Delme et al., 2007). A evaporação da água ocorre quando atinge a temperatura entre 100 e 650^{o}C junto com o início da perda de carbonato (Bevilácqua et al., 2008).

Os lasers de Érbio possuem a característica de serem altamente absorvidos pela superfície dental e muito pouco difundidos para as superfícies adjacentes. Quando utilizado para preparo cavitário, portanto utilizando uma potência ablativa, gera uma temperatura intrapulpar semelhante a proporcionada por uma ponta diamantada montada em alta rotação refrigerada a *spray* de água (Mollica et al., 2008).

Geraldo-Martins et al., em 2005, irradiaram dentina bovina com laser de Er:YAG refrigerado a *spray* de água e constataram uma variação de temperatura intrapulpar entre 0,03 e 2,5 ºC, concluindo que este procedimento é aceitável para a estrutura pulpar, estando abaixo do valor crítico de 5,5 ºC (Zach & Cohen, 1965).

Er:YAG em baixa densidade de energia atua na dentina criando uma superfície livre de esfregaço dentinário e com os túbulos dentinários abertos (Soares et al., 2006; Chousterman et al., 2009), pela afinidade do laser Er:YAG com a água a dentina intertubular é mais marcantemente ablasionada do que a dentina peritubular, mais mineralizada, e um padrão rugoso pode ser observado por meio de microscopia (Soares et al., 2006; Raucci-Netto et al., 2009).

Os lasers de alta potência como os de CO_2 e Argônio são utilizados durante o clareamento em consultório como fonte de energia, pela liberação de calor, para favorecer a decomposição dos peróxidos e acelerar os resultados clínicos (Cassoni & Rodrigues, 2007). Sabe-se que os lasers indicados para preparo cavitário e alteração morfológica dos tecidos dentais são os mais absorvidos pela água e pela hidroxiapatita (Eversole & Rizoiu, 1995; Kantorowitz et al., 1998; Hossain et al., 2002; Hossain et al., 2003; Hossain et al., 2003; Liu et al., 2006; Meister et al., 2006; Metz et al., 2007; Soares et al., 2007) e quando a energia do laser é absorvida pelos tecidos dentais, há um grande aumento de temperatura, o que pode favorecer a eliminação dos radicais livres.

Alguns autores têm afirmado que, mesmo quando o laser de Érbio é utilizado com energia abaixo do limiar de ablação, é observada uma redução na desmineralização de dentes submetidos a desafio cariogênico (Fowl & Kuroda, 1986; Apel et al., 2000; Young et al., 2000; Perito et al., 2009a; Perito et al., 2009b). Hossain et al., em 2003, demonstraram que após a irradiação com o laser de Er:YAG observa-se um aumento na proporção de cálcio e fósforo no tecido dental, sem modificar a razão entre estes minerais e está de acordo com o estudo de Liu & Hsu, em 2007, que relatam que a quantidade de minerais após a irradiação com este laser é a mesma, o que ocorre é a diminuição do conteúdo orgânico e consequentemente um aumento na proporção do conteúdo mineral em relação ao orgânico. Teoria esta denominada “organic blocking theory” (Ying et al., 2003).

Não se encontram trabalhos que descrevam o efeito do laser de Er:YAG na superfície de tecidos dentais clareados, com o objetivo de melhorar a adesão em esmalte e dentina.

Desta forma, se os radicais livres absorverem a energia do laser de Er:YAG e forem completamente eliminados do esmalte e dentina, poderia ser possível obter-se valores de adesão em dentes clareados semelhantes aos dentes não clareados, visto que com o grande emprego do clareamento dental e da necessidade de se realizar restaurações estéticas definitivas, é importante determinar se o uso do laser de Er:YAG, pode reverter a perda de adesão temporária, causada pelos agentes clareadores sobre a estrutura dental, aos materiais restauradores.

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2. PROPOSIÇÃO

O objetivo deste estudo *in vitro* foi investigar a influência do laser de Er:YAG em dois diferentes parâmetros, na resistência de união em esmalte e um parâmetro na dentina de dentes bovinos, clareados com peróxido de carbamida 16% por meio de 2 artigos:

1- Effect of Er:YAG laser on the microtensile bond strength of resin composite to bleached enamel.

2- Microtensile bond strength of resin composite to dentin treated with Er:YAG laser of bleached teeth.

METODOLOGIA E RESULTADOS
3. 1 CAPITULO 1

EFFECT OF Er:YAG LASER ON THE MICROTENSILE BOND STRENGTH OF RESIN COMPOSITE TO BLEACHED ENAMEL

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ABSTRACT

The objective of this study was to evaluate the influence of different energy parameters of Er:YAG laser (λ= 2.94μm) on microtensile bond strength (μTBS) and morphology of bovine enamel bleached with 16% carbamide peroxide. Sixty enamel blocks (7x3x3mm^3) were randomly assigned to six groups (n=10): G1- bleached and irradiated with Er:YAG laser (Kavo Key II Laser II – KaVo, Biberach, German) with 25.56 J/cm^2(LA); G2- bleached and irradiated with Er:YAG laser with 4.42 J/cm^2(LB); G3- bleached and restorated; G4- non-bleached and irradiated with Er:YAG laser (LA); G5- non-bleached and irradiated with Er:YAG laser (LB); G6- control, non-treated. G1 to G3 were bleached for 6h during 21 days. Afterwards all blocks were abraded with 600grit abrasive papers and G1, G2, G4 and G5 were irradiated according to their groups. Blocks were immediately restored with 4-mm thick composite resin (Adper Single Bond 2; Z-250-3M/ESPE). After 24hs of storage at 37^{o}C in relative humidity, the restored blocks (n=9) were sectioned and trimmed to an hour-glass shape of approximately 1 mm^2 area at the bonded interface tested in tension with a cross-head speed of 1mm/min. μTBS data was analyzed by Two-way ANOVA (α=0.05) and no statistical differences were found. Failure mode was determined at a magnification of X100 using a stereomicroscope. One treated block of each group was selected for scanning by electron microscope (SEM) analysis. Results: Mean bond stregths (SD) in MPa were: G1- 30.4 (6.2); G2- 27.9 (8.5); G3- 32.3 (3.9); G4- 23.7 (5.8); G5- 29.3 (6.0); G6- 29.1 (6.1) (P>0.05). A high amount of adhesive failures was recorded for bleached and lased enamel surface tested. Conclusions: The bleached enamel surface did not present significant difference from the non-bleached enamel surface for μTBS values. Er:YAG laser irradiation with LA prior to adhesive procedure of bleached and non-bleaching enamel surface had no influence on μTBS. In the same way, Er:YAG laser irradiation with LB prior to adhesive procedure of bleached and unbleached enamel surface had no influence on the microtensile bond strength. The LA promoted ablation of enamel surface observed under SEM and LB did not promote ablation.

KEY WORDS

Er:YAG Laser, Scanning electron microscope, Microtensile bond strength, bleached enamel

INTRODUCTION

Dental bleaching is an esthetic procedure that changes tooth color as it becomes lighter. The oxygenating agents, like hydrogen peroxide, diffuses through enamel and dentin.[1] After bleaching procedures is very frequent the replacement of old restoration. However, it has been reported that dental bleaching treatment reduces immediate bond strength of composite resin to enamel[2,3] and dentin due to the presence of residual bleaching agent.[4,5] The residual oxygen in dental tissue may inhibit the polymerization and reduces the bond strength of composite resin restorations.[6]

Bond strength of restoration on enamel can be reestablished to standard values up to 3 weeks after bleaching an elapsed time is needed for oxygen release,[5,7,8] several authors inquire about a technique to revert this side effect and to enable the replacement of esthetic restoration as soon as possible.

On the other hand, due to a wavelength of 2.94µm the use of Er:YAG laser with low parameter may promote a chemical and/or morphological alteration of enamel surface,[9,10,11] which coincides with the water absorption spectrum results in thermal energy and surface temperature rise due to absorption by water molecules.[12] Therefore, hydrogen peroxide and free oxygen are highly reactive and an increase in temperature may release them. Er:YAG used in low energy density may increase the surface temperature in the replacement of a restoration. The Er:YAG laser irradiation could accelerate the removal of bleaching residual agents and free oxygen by treating the target surface to improve adhesion resulting in standard bond strength values.

Since there is no report in literature of a study that evaluated the treatment with Er:YAG laser in low energy density after enamel bleached with 16% carbamide peroxide prior to adhesive restoration of dental resin composite, the objective of this study was to evaluate the effect of two parameters of Er:YAG irradiation on the adhesion to bleached enamel.

2- MATERIAL AND METHODS

Sixty extracted bovine incisors stored in 0.1% thymol solution at 4°C were used in this study. Blocks with 7x3x3 mm^3 were sectioned from the buccal surface of bovine teeth with double-faced diamond disks (n° 7020; KG Sorensen, Barueri, SP, Brazil) used at a low speed (Kavo, Joinville, SC, Brazil). The samples were randomly assigned to six experimental groups according to the bleaching and laser treatment performed (Table 1).

Bovine Dental Fragments Bleaching

Dental bleaching has been performed during 21 days in G1 to G3. An individual tray for each block was manufactured using 0.4mm thick ethyl vinyl acetate (EVA, Bio Art Equipaments Odontológicos Ltda., São Carlos, Brazil) polymer in a vacuum forming machine. The vital bleaching agent, 16% carbamide peroxide (Whiteness Perfect 16%, FGM, Denstcare Ltda, Joinville, Brazil) was applied for 6 hours a day by covering the dental block with 0.04ml of bleaching material and the individual custom tray. The blocks were stored in 14 ml of artificial saliva at 37^oC that was changed daily. The artificial saliva contained calcium and phosphate in a known degree of saturation to mimic the remineralizing properties of natural saliva (50 mmol/l KCl, 1.5 mmol/l Ca, 0.9mmol/l PO^4, 20 mmol/l tri-hydroxymethil-aminomethan, pH 7.0).[13,14]

To simulate the cavity preparation and obtain a flat standardized surfaces for microtensile bond strength test, the blocks were abraded with 600-grit silicon carbide sandpaper disks (Carburundum Abrasivos Ltda, Vinhedo, Brasil) using a polishing machine Teclago (PL02 RB LAB Com. Técnica Ltda, São Paulo, Brasil).

Laser Irradiation

The Er:YAG laser (KaVo KEY Laser II – KaVo, Biberach, German), which presents a wavelenght of 2.94 µm, and pulsed duration of 250-400 µs, was used to irradiate the blocks with the handpiece no. 2055, fiber 50/10 with beam diameter of 0.47 mm, intput power of 100mJ, and repetition rate fixed at 2 Hz with a transmitting factor of 0.54 without refrigeration in all specimens. The output power was 82mJ. It was measured with a power meter (Coherent, Newport, United States).

Blocks of G1 and G4 were irradiated in “contact mode” with 25.52 J/cm^2. All area of enamel was irradiated uniformly by hand in a grid pattern for 40s by a single trainned operator.

In G2 and G5 the handpiece was positioned 3mm away from target surface to decrease the energy density at the surface, and they were irradiated in “non-contact” mode resulting in irradiation with 4.42 J/cm^2 of energy density. The standardization of the distance was achieved with an endodontic K-file fixed to the laser hand-piece.

Table 1- Experimental groups according to bleaching procedure and laser treatment.

Experimental Groups	Bleaching Treatment	Laser treatment (n=10)
G1	Yes	Er:YAG laser – contact
G2	Yes	Er:YAG laser - non-contact
G3	Yes	-
G4	No	Er:YAG laser – contact
G5	No	Er:YAG laser - non-contact
G6	No	-

Microtensile Bond Strength Test

After surface treatment, an etch-and-rinse adhesive system Adper Single Bond 2 (3M/ESPE) was used according to the manufacturers’ instructions. Table 2 lists composition, lot number, and application instructions of the selected materials. The adhesive system was applied in all groups with microbrush disposable tips to avoid excess and pooling of adhesive at teeth surface and air dried gently for 5s. After bonding procedure, restorations were made at 4-mm height with micro-hybrid resin composite Filtek Z250 – A2 (3M/ESPE). The activation of adhesive and resin composite was performed with quartz-tungsten halogen lamp with a 700mW/cm^2 output (Optilux, Demetron/Kerr, Danbury, CT, USA) according to manufacturers’ instructions. The bonded specimens were placed in relative humidity at 37^{o}C for 24h.

Table 2. Composition, lot number and application mode of the selected materials

Product	Composition	Directions for use
Adper Single Bond 2 (3M-ESPE, Irvine, CA, USA) Lot: 9WBBR	HEMA, bis-GMA, DMAs, methacrylate functional, copolymer of polyacrylic and polyitaconic acids, water, ethanol, nanofilller, photoinitiator	Two coats were consecutively applied, gently air-dried and light-cured for 10 seconds
Z250 – A2 (3M-ESPE, Irvine, CA, USA) Lot: 9EP	Bis-GMA, Bis-EMA, UDMA e camphorquinone. Fillers: Zirconia-sílica	Light activate for 20 seconds each increment

Abbreviations: bis-GMA=bisphenol glycidyl methacrylate; Bis-EMA= bisphenol A polyethylene glycol diether dimethacrylate; UDMA=urethane dimethacrylate DMA=dimethacrylates; HEMA=2-hydroxyethyl methacrylate

The specimens were sectioned mesiodistally to serial slabs using a diamond saw (IsoMet 1000; Buehler Ltd, Lake Bluff, IL, USA) under water cooling into 1.0mm-thick sections. The adhesive interface was trimmed at the interface to an hour-glass shape (‘trimmed’micro-specimens) with approximately 1 mm^2 of bonded area which were subjected to µTBS test using an universal testing machine (EZ Test, Shimadzu, Kyoto, Japan) at a cross-head speed of 1mm/min.[15] The specimens were attached to a microtensile apparatus, using a cyanoacrylate adhesive (Loctite Super Bond Gel; Henkel, Düsseldorf, Germany). Dimensions of each side of the debonded specimen were measured using a digital micrometer (Mitutoyo Co., Tokyo, Japan) and the values of µTBS in MPa were calculated for each specimen by the ratio of the fractured load and measured surface area. The mean of the five µTBS values of each specimen was used as the specimen microtensile value. A two-way ANOVA method was used to evaluate the statistical significance of mean µTBS (MPa) data and was compared at the 95% level of confidence.

The failure mode was observed at a magnification of 100X using a stereomicroscope (PanTec, Panambra Ind. e Técnica SA, São Paulo, Brazil) and scored according to standardized criteria into 1 of 3 types: cohesive failure in enamel (CE), adhesive failure between enamel and resin (AD) and cohesive failure in resin (CR). Instead of classifying failure as mixed, the area percentage of each type of failure in each hour glass was recorded. The failure modes were given as percentage. A statistical descriptive method treatment was given to debonded sample analysis.

Scanning electron microscopy analysis of the enamel surface

The samples were cleaned on ultrasonic bath for 10 minutes and fixed in Karnovsky solution for 1 hour. The samples were rinsed with distilled water and then dehydrated in a graded series of alcohol solutions [70, 90, and (2x) 100%] for 10 minutes at each concentration. These samples were sputtered with 15 µm thick platinum.

One treated block of each group was kept for SEM evaluations and one representative failure type was selected during failure mode analysis. Photographs were taken at 200, 500 and 2,000X magnifications (Figures 2 to 8) and representative failure modes selected among fractured specimens and enamel surface were examined with a scanning electron microscope (FEI; Quanta 600F, Nederland, NE).

RESULTS

Microtensile Bond Strength Test

As data presented normal distribution they were submitted to two-way ANOVA ($\alpha=0.05$) considering the factors of bleaching and laser treatment and their interaction. Table 3 presents the mean µTBS values of each experimental group and studied factors, the standard deviation and pre-test failures.

No statistically significant differences were observed for bleaching ($p=0.08$), laser treatment ($p=0.20$) or for the interaction bleaching and laser treatment ($p=0.13$).

Table 3. Mean Microtensile bond strength values (MPa) of each experimental group and studied factors, standard deviation [SD] and pre-test failures.

Groups	Bleaching Treatment	Laser treatment (n=10)	µTBS (MPa) (n=9)	Pre-test Failures
G1	Yes	Er:YAG laser – contact	30.4 [±6.2]	5%
G2	Yes	Er:YAG laser - non-contact	27.9 [±8.5]	8%
G3	Yes	-	32.3 [±3.9]	11%
G4	No	Er:YAG laser - contact	23.7 [±5.8]	36%
G5	No	Er:YAG laser - non-contact	29.3 [±6.0]	8%
G6	No	-	29.1 [±6.1]	0%

The distribution of failure modes among adhesive interface is shown in Figure 1.

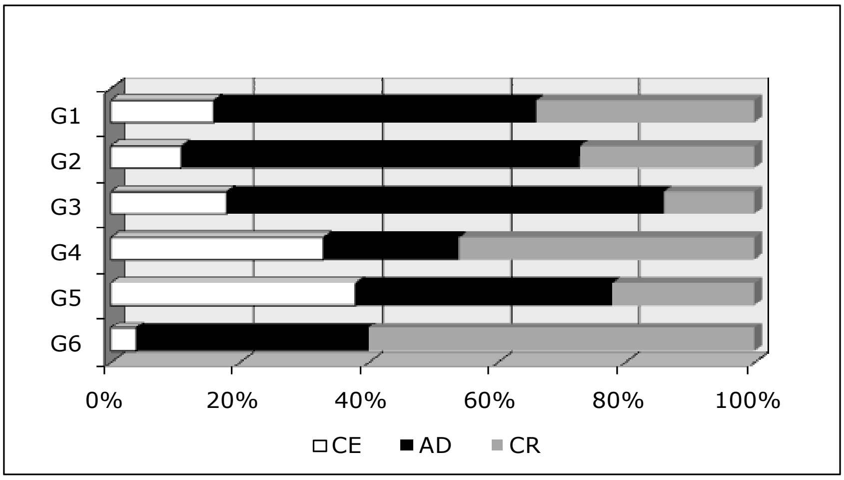

Figure 1. Distribution of failure modes within groups. CE, cohesive failure in enamel; AD, adhesive failure between enamel and adhesive; CR, cohesive failure in resin composite.

The predominant fracture in control group G6 (60%) and G4 (46%) was CR, but G6 showed 4% of CE and G4 (35%). In the other groups, the predominant fracture was AD (G1- 50%; G2- 62%; G3- 68%; and G5- 40%), however the least predominant type of fracture was different and to G1 (16%) and G2 (11%) was CE and CR to G3 (14%).

The pre-test failure recorded was G1- 5%, G2- 8%, G3- 11%, G4- 36%, G5- 8% and G6- 0%.

Scanning electron microscopy evaluation

A representative SEM image of each failure mode is shown in Figure 2. Figure 2A is a SEM image of cohesive failure in enamel (CE); Figure 2B is the SEM image of adhesive failure between enamel and resin (AD) and Figure 2C is the SEM image of 50% cohesive failure in resin (CR) and 50% adhesive failure.

The SEM appearances of each group of surface treatment are present in figures 3 to 7. Contact Er:YAG laser irradiation promoted ablation of enamel surface observed under SEM for bleached (Figure 3) and unbleached teeth (Figure 6). Non-contact Er:YAG laser irradiation prior to adhesive procedure promote no ablation of bleached enamel surface (Figure 4). There were no morphological alterations on the enamel surface of bleached and flattened teeth (Figure 5) compared with control that was covered by smear layer (Figure 7).

DISCUSSION

Vital dental bleaching is a conservative and safe procedure to solve tooth discoloration.[16,17] The clinical use of 10% carbamide peroxide has been described by Haywood and Heymann (1989) and it is widely used in modern dentistry.[18] This agent is effective for bleaching procedure,[17] and the use of peroxide with higher concentration has grown.[19,20] The residual oxygen of bleaching procedure may reduce the bond strength of composite resin restorations.[6] Carbamide peroxide agent demineralizes the enamel[21-23] and even with the strength on bleached teeth reversed, the bonding quality is decreased.[1] It has been reported reduced penetration of adhesive system in enamel after dental bleaching.[8] However; our study showed no statistical differences among the studied groups.

The absence of differences in the µTBS may be due the selected study design model, which simulated 21 days of bleaching treatment. Also in the study model the bleaching treatment was performed on the enamel surface and the bond strength test in the inner enamel. Due to the low molecular weight of peroxides, it was expected a penetration of the bleaching in the inner enamel that was exposed after 21 days by wearing the enamel to simulate a cavity preparation with smear layer (Figure 5). This way, the accumulation of residual peroxide and free oxygen in the area prepared for the adhesion test could be smaller than the superficial surface directed exposed to bleaching agents and it did not impair the µTBS values.

However, the failure mode clearly demonstrated that the bleaching procedure damaged adhesive procedure, even with no differences in µTBS. Bleached group (G3) recorded a high percentage of adhesive failure (68%) compared with control group (G6- 36%). It is also important to note that group 4 recorded the highest pre-test failure (36%).

The group bleached and treated with Er:YAG laser in contact mode (G1) showed no difference of µTBS compared with the group treated with non-contact mode (G2) and both groups presented a predominant adhesive failure mode pattern with more percentage in the G2 but less cohesive failure in enamel and adhesive failures than the group bleached and non-irradiated (G3). Even though the µTBS was not statistically different among the bleached and control groups; a high percentage of cohesive failure in enamel in irradiated groups was observed.

By the SEMs microphotographs it can be observed that the contact mode irradiation with 25.52 J/cm^2 lead to total removal of the smear layer creating a non-homogeneous surface with some evidence of ablation with cracks and few melted enamel (Figure 3). In the non-contact irradiation mode, it was observed a partial removal of the smear layer with a flat surface without ablation or melting (Figure 4). In both groups, the laser energy must have been absorbed by enamel and changed into thermal energy. This thermal energy may change enamel chemical composition even in subablative or by temperature diffusion in ablative energy densities.[24]

In contrast to the groups irradiated in contact mode (G1 and G4) which had no statistical difference in the µTBS value they presented different failure pattern. The bleached group showed less cohesive enamel failure (16%) than unbleached group which showed 33% of cohesive enamel failure. This is probably due to the interaction of Er:YAG laser with the enamel under the treated surface which caused detrimental effects. Structural defects on enamel can be responsible for this fact because Figure 3 and 6 showed morphological alteration with evidence of ablation.

Also, Chuang et al. (2009) related low microtensile bond strengh values after bleaching treatment with 10% carbamide peroxide 8h/day during 14 days.[1] However, in Chuang et al. (2009) study, adhesive procedure was performed without flattening enamel surface and this fact can explain the decreased µTBS values. In the same way, Gurgan et al. (2009) related decreased shear bond strength after bleaching procedure with 16% carbamide peroxide to a self-etching adhesive system compared with conventional adhesive technique and Er,Cr:YSGG laser treatment.[20]

In the present study the enamel flattening step intention was to create a clinical condition of restoration replacement and the removal of superficial enamel could be favorable to adhesive condition.

There are no comparable studies analysing the influence of Er:YAG laser irradiation in subablasive energy density on the µTBS in enamel after dental bleaching when the tooth will be restored with an adhesive procedure. In addition, the hypothesis that Er:YAG laser treatment with two different energy densities, by contact or non-contact mode was evaluated.

In summary, since no statistical significant damage in µTBS of enamel after bleaching treatment was observed, the surface treatment with Er:YAG laser irradiation with contact and non-contact mode also had no improvement on µTBS of bleached and unbleached enamel. Contact mode laser irradiation promoted ablation of enamel surface observed under SEM.

DISCLOSURE

The authors have no interest in any of the companies or products mentioned in this article.

CONCLUSIONS

Adhesive procedures may be performed in margins of cavities prepared soon after vital dental bleaching without damaging on microtensile bond strength. Er:YAG laser irradiation with 25.52 J/cm^2 prior to adhesive procedure had no influence on µTBS and promoted ablation of enamel surface observed under SEM. On the other hand, Er:YAG laser irradiation with 4.42 J/cm^2 prior to adhesive procedure did not promote ablation.

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FIGURES

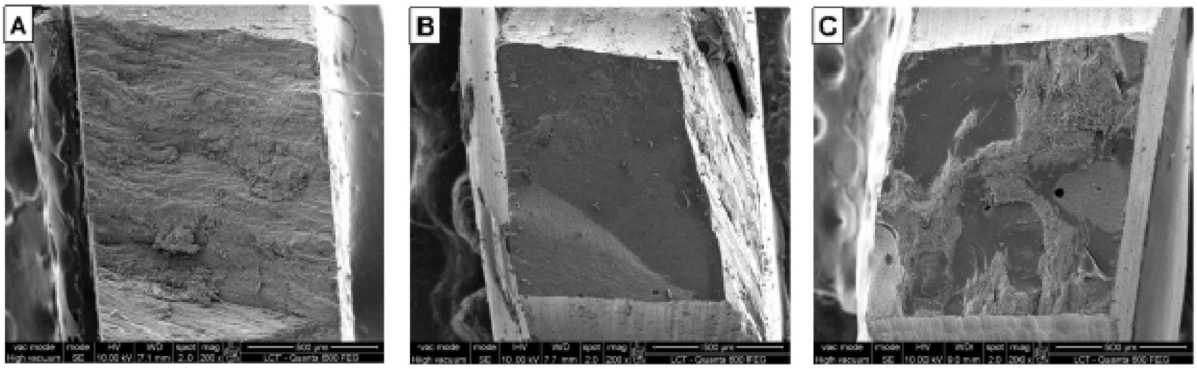

Figure 2. Scanning electron microphotographs (SEMs) of the three representative failure types (**A**) cohesive failure in enamel; (**B**) adhesive failure between enamel and resin (**C**) 50% cohesive failure in resin and 50% adhesive failure (original magnification 200X, bar represents 500 µm).

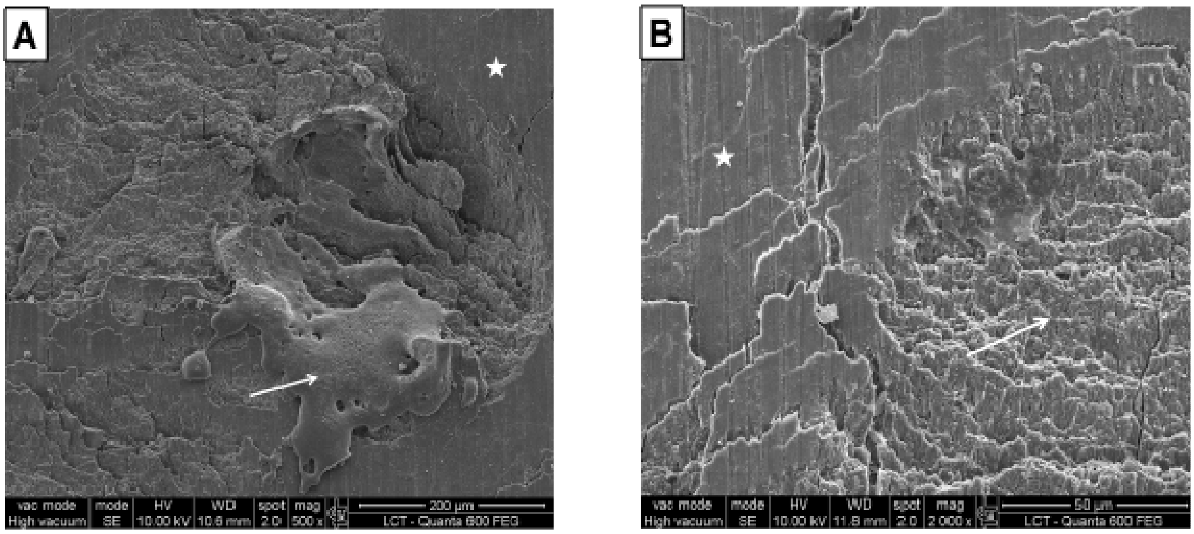

Figure 3. Scanning electron microphotographs (SEM) of the enamel surface of bleached teeth irradiated in contact mode with 25.52 J/cm^2 (Group1). (**A**) The irradiation pattern is not homogeneous; some areas were not irradiated (star) and irradiated surface presents evidence of ablation. There are cracks and craters on the irradiated surface with melted area, pointed with an arrow. (original magnification 500X, bar represents 200 µm); (**B**) areas

were not irradiated (star) and irradiated surface presents evidence of ablation with some superficial irregularity, pointed with an arrow (original magnification 2,000X, bar represents 50 μm).

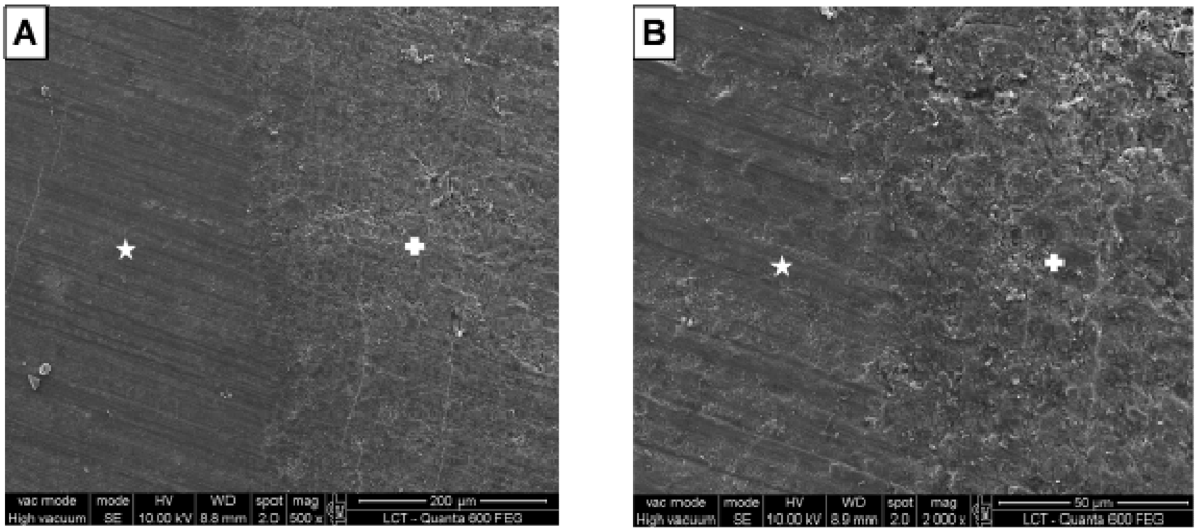

Figure 4. Scanning electron microphotographs (SEM) of the bleached enamel irradiated in non-contact mode with 4.42 J/cm^2 (Group 2). (**A**) The irradiation pattern is not homogeneous; some areas present smear layer (star) in others a irradiated surface (cross), all flatten with no evidence of ablation (original magnification 500X, bar represents 200 μm); (**B**) scarce morphological alteration of irradiated surface (cross) compared with no irradiated enamel (star) (original magnification 2,000X, bar represents 50 μm).

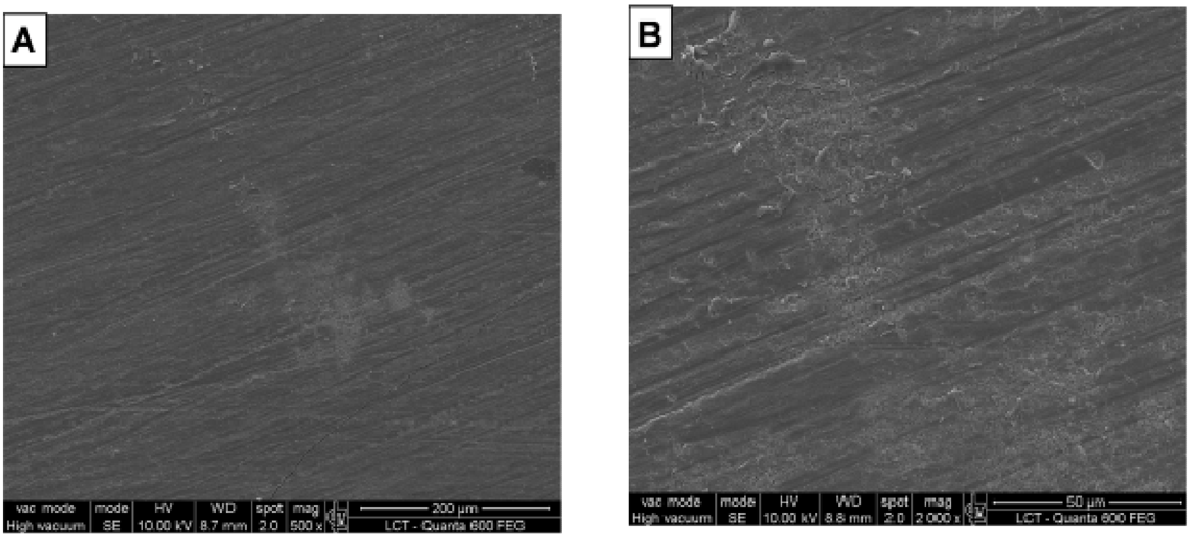

Figure 5. Scanning electron microphotographs (SEM) of the surface of bleached and prepared enamel (Group 3) demonstrate (**A**) flatten surface covered with a smear layer

(original magnification 500X, bar represents 200 μm); (**B**) no morphological alterations on surface of the bleached enamel (original magnification 2,000X, bar represents 50 μm).

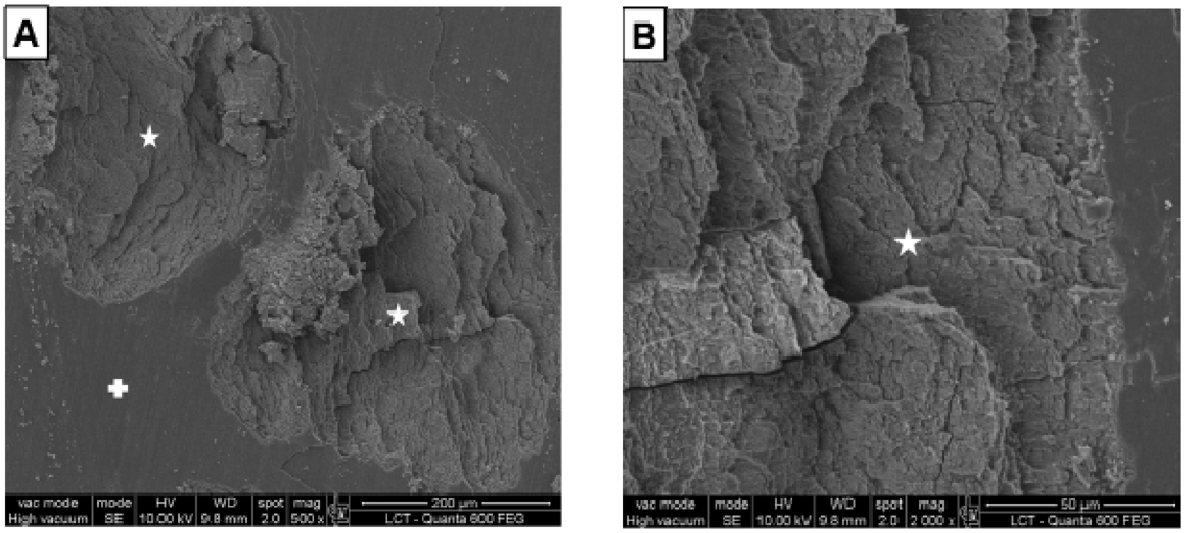

Figure 6. Scanning electron microphotographs (SEM) of the bleached enamel irradiated with 25.52 J/cm^2 (Group 4). (**A**) There are cracks and craters on the irradiated enamel surface (star) and presents evidence of ablation; the irradiation pattern is not homogeneous; some areas seen not be irradiated (cross) (original magnification 500X, bar represents 200 μm); (**B**) the irradiated surface presents evidence of ablation (star) (original magnification 2,000X, bar represents 50 μm).

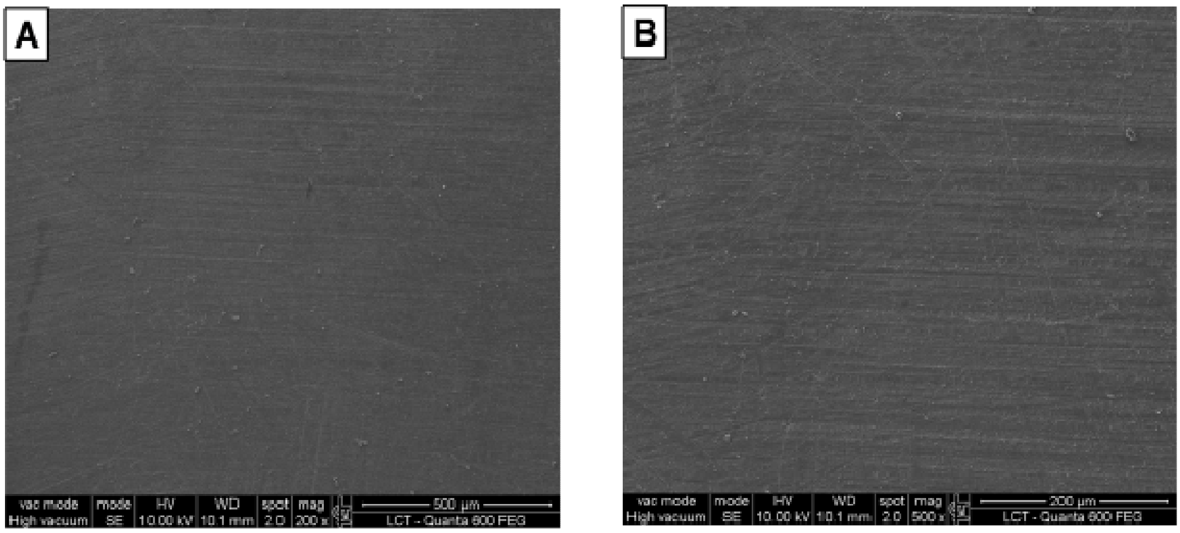

Figure 7. Scanning electron microphotographs (SEM) of the non bleached and non irradiated enamel (Group 6). (**A**) A flatten surface can be noted (original magnification

500X, bar represents 500 μm); (**B**) flatten surface with smear layer (original magnification 2,000X, bar represents 200 μm).

3.2 CAPÍTULO 2

Microtensile bond strength of resin composite to dentin treated with Er:YAG laser of bleached teeth.

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Abstract

The objective of this study was to evaluate the influence of Er:YAG laser (λ= 2.94µm) on microtensile bond strenght (µTBS) and morphology of bovine dentin bleached with 16% carbamide peroxide. Forty bovine teeth blocks (7x3x3mm^3) were randomly assigned to four groups: G1- bleached and irradiated with Er:YAG laser (Kavo Key Laser II – KaVo, Biberach, German) with energy density of 25.52 J/cm^2 (focused mode); G2 - bleached; G3 - non-bleached and irradiated with Er:YAG laser (25.52 J/cm^2); G4 - control, non-treated. G1 and G2 were bleached with 16% carbamide peroxide for 6h during 21 days. After that, all blocks were abraded with 320-600grit abrasive papers. G1 and G3 were laser irradiated. Blocks were immediately restored with 4mm high composite resin (Adper Single Bond 2, Z-250-3M/ESPE). After 24hs of storage at 37^{o}C in relative humidity, the restored blocks (n=9) were sectioned and trimmed to an hour-glass shape of approximately 1 mm^2 at the bonded interface area, that were tested in tension with a cross-head speed of 1mm/min. µTBS data were analyzed by two-way ANOVA and Tukey test (α=0.05). Failure mode was determined at a magnification of 100X using a stereomicroscope. One block of each group was selected for scanning electron microscope (SEM) analysis. Results: Mean bond strengths (SD) in MPa were: G1- 32.7 (5.9)^A; G2- 31.1 (6.3)^A; G3- 25.2 (8.3)^B; G4- 36.7 (9.9).^{A} Groups with different uppercase letters were significantly different from each other (*P*<.05). Conclusions: Bleaching procedure did not affect µTBS values for dentin adhesion. Er:YAG laser irradiation with 25.52 J/cm^2 prior to adhesive procedure of bleached teeth did not affect µTBS at dentin and promoted a dentin surface with no smear layer and opened dentin tubules observed under SEM. On the other hand, Er:YAG laser irradiation prior to adhesive procedure of non-bleached dentin surface impaired µTBS compared with control group.

KEY WORDS

Er:YAG Laser, Scanning electron microscope, Microtensile bond strength, bleached dentin

INTRODUCTION

Aesthetic dentistry usually combines bleaching treatments and replacement of resin composite restorations.[1] It has been reported the effects of carbamide peroxide gel on reducting bond strength of composite resin to dentin [2] and enamel surfaces.[3,4] The bond strength values can come back to normal value in 1 to 3 weeks after bleaching treatment with residual bleaching radicals release.[4,5] The residual oxygen in dental tissue may inhibit the polymerization process.[6] The mechanism is triggered by the inhibition of the adhesive system and resin composite polymerization.[1]

Er:YAG laser was approved by the Food and Drug Administration (FDA) for dental hard tissue and do not cause thermal damages under safe and studies protocols.[7,8] The Er:YAG laser has a wavelength of 2.94µm, which coincides with the water absorption spectrum, and results in maximum absorption and complete transformation into thermal energy.[9] Erbium lasers promote ablation of dental structure effectively because of their high absorption of water with a shallow thermal penetration.[9] The water evaporation occurs when temperature achieves 100 to 650^{o}C together with the beginning of carbonate loss.[10] The wavelength of Er:YAG laser modificates hard dental tissue once it acts on their properties, including permeability, microhardness, and acid resistance.[11] The released heat by Er:YAG laser in the superficial hard dental creates an increased pressure within the irradiated tissue. The strength of the substrate is surpassed and ablation occurs.[12]

Er:YAG laser at low-energy setting can modify dentin surface.[1] Er:YAG laser acts on dentin surface creating a smear layer free area with open tubules with certain low density energy output.[13,14] Since dentin substrate is fulfilled by residual bleaching agents after dental bleaching, and these agents are free radicals which are very unstable, the heat generated by Er:YAG laser at low-energy densities may accelerate the release of free radicals and modify dentin into a better substrate to receive the adhesion procedures.

There is a lack of information about the effect of Er:YAG laser prior to dentin adhesion of bleached teeth with 16% carbamide peroxide. So, the objective of this study was to evaluate the effect Er:YAG laser irradiation on the adhesion of bleached dentin surface.

MATERIAL AND METHODS

Forty extracted bovine incisors stored in 0.1% thymol solution at 4°C were used in this study. Blocks with 7x3x3 mm^3 were sectioned from the buccal surface with double-faced diamond disks (n° 7020; KG Sorensen, Barueri, SP, Brazil) used at a low speed (Kavo, Joinville, SC, Brazil).

Bovine Dental Fragments Bleaching

Dental bleaching has been performed during 21 days on enamel surface. An individual tray for each specimen of groups 1 to 4 was manufactured using 0.4mm thick ethyl vinyl acetate (EVA, Bio Art Equipaments Odontológicos Ltda., São Carlos, Brazil) polymer in a vacuum forming machine. The bleaching agent used was 16% carbamide peroxide (Whiteness Perfect 16%, FGM, Denstcare Ltda, Joinville, Brazil) was applied for 6 hours a day by covering the dental block with 0.04ml of bleaching material and the individual custom tray. The blocks were stored in 14 ml of artificial at 37°C saliva that was changed daily. The artificial saliva contained calcium and phosphate in a known degree of saturation to mimic the remineralizing properties of natural saliva (50 mmol/l KCl, 1.5 mmol/l Ca, 0.9mmol/l PO4, 20 mmol/l tri-hydroxymethil-aminomethan, pH 7.0).[15,16]

Laser Irradiation

After bleaching procedure the fragments were polished with 320, 400 and 600-grit silicon carbide sandpaper disks (Carburundum Abrasivos Ltda, Vinhedo, Brasil) using polishing machine Teclago (PL02 RB LAB Com. Técnica Ltda, São Paulo, Brasil). These procedures were conducted to obtain flat standardized dentin surfaces for microtensile bond strength test. Samples were randomly assigned to four experimental groups according to the surface treatment performed (Table 1).

The Er:YAG laser (KaVo KEY Laser II – KaVo, Biberach, German), presents a wavelength of 2.94 µm, and a pulsed duration of 250-400 µs. The equipment was used with the handpiece no. 2051, with beam diameter of 0.63 mm, input power of 100 mJ, repetition rate fixed at 2 Hz without refrigeration in all specimens. The output power was 82mJ measured with a power meter (Coherent, Newport, United States). All area of dentin was irradiated uniformly by hand in a grid pattern for 40s by a single operator.

In order to standardize the working distance of 12mm of the handpiece from target, an endodontic K-file was fixed to the laser hand-piece. The irradiation was performed at surface area of G1 and G3 (Table 1) on focused mode. The calculated energy density was 25.62 J/cm^2).

Table 1- Experimental groups.

Groups	Bleaching	Dentin (n=10)
G1	Yes	Er:YAG laser
G2	Yes	-
G3	No	Er:YAG laser
G4	No	-

After surface treatment Adper Single Bond 2 (3M/ESPE) was used according to the manufacturers' instructions. It is an etch-and-rinse adhesive system. Table 2 lists composition, lot number, and application instructions of the selected materials. The adhesive system was applied with microbrush disposable tips to avoid excess and pooling of adhesive at teeth surface and air dried gently for 5s. After bonding procedure, restorations were made at 4-mm height with micro-hybrid resin composite Filtek Z250 – A2 (3M/ESPE Dental Products). The activation of adhesive and resin composite was performed with quartz-tungsten halogen lamp with a 700mW/cm^2 output (Optilux, Demetron/Kerr, Danbury, CT, USA) according to manufacturers' instructions.

Table 2. Composition, lot number and application mode of the selected materials

Product	Composition	Directions for use
Adper Single Bond 2 (3M-ESPE, Irvine, CA, USA) Lot: 9WBBR	HEMA, bis-GMA, DMAs, methacrylate functional, copolymer of polyacrylic and polyitaconic acids, water, ethanol, nanofilller, photoinitiator	Two coats were consecutively applied, gently air-dried and light-cured for 10 seconds
Z250 – A2 (3M-ESPE, Irvine, CA, USA) Lot: 9EP	Bis-GMA, Bis-EMA, UDMA e camphorquinone. Fillers: Zirconia-sílica	Light activate for 20 seconds each increment

Abbreviations: bis-GMA=bisphenol glycidyl methacrylate; Bis-EMA= bisphenol A polyethylene glycol diether dimethacrylate; UDMA=urethane dimethacrylate DMA=dimethacrylates; HEMA=2-hydroxyethyl methacrylate

The specimens were sectioned to serial slabs using a diamond saw (IsoMet 1000; Buehler Ltd, Lake Bluff, IL, USA) and trimmed at the interface to an hour-glass shape (‘trimmed’micro-specimens) with approximately 1 mm^2 of bonded area which was subjected to µTBS test using a universal testing machine (EZ Test, Shimadzu, Kyoto, Japan) at a cross-head speed of 1mm/min.[7] The micro-specimens were attached to a microtensile apparatus grip, using a cyanoacrylate adhesive (Loctite Super Bond Gel; Henkel, Düsseldorf, Germany). Dimensions of each side of the debonded specimen were measured using a digital micrometer (Mitutoyo Co., Tokyo, Japan) and the µTBS values in MPa were calculated for each specimen by the ratio of the fractured load and measured surface area. The mean of five µTBS values of each specimen was used as the specimen microtensile bond value.

The failure mode was observed at a magnification of 100X using a stereomicroscope (PanTec, Panambra Ind. e Técnica SA, São Paulo, Brazil) and scored according to standardized criteria into 1 of 3 types: cohesive failure in dentin (CD), adhesive failure between dentin and adhesive (AD) and cohesive failure in resin composite (CR). Instead of classifying failure as mixed, the area percentage of each type of failure in each type of failure was recorded. The failure modes were given as percentage.[1]

Scanning electron microscopy analysis of the dentin surface

One treated slab of each group was kept for SEM evaluations and one representative failure type was selected during failure mode analysis. The specimens were mounted on aluminum stubs and platinum-sputter coated prior to viewing at different magnifications. Representative failure modes were selected among fractured micro specimens and adhesive surface were examined with a scanning electron microscope (SEM) (FEI; Quanta 600F, Nederland, NE). The entire surface was scanned and the most representative areas were photographed at 200, 500 and 2,000X magnifications.

Statistical Analysis

Two-way ANOVA method was used to evaluate the statistical significance of mean µTBS (MPa) data and was compared by Tukey interval calculated at the 95% level of confidence. The debonded sample analysis was described by percentage.

RESULTS

As data presented normal distribution they were submitted to two-way ANOVA and Tukey's test (α=0.05) considering the factors of bleaching and laser treatment and their interaction. Table 3 presents the mean µTBS values of each experimental group and studied factors, the standard deviation and the results of Tukey test and pre-test failures.

No statistically significant differences were observed for bleaching (p=0.74) or laser treatment (p=0.07). There was statistically significant difference for the interaction bleaching and laser treatment (p=0.02, Table 3).

Table 3. Mean Microtensile bond strength values (MPa) of each experimental group and studied factors, standard deviation (±SD), the results of Tukey test and pre-test failures [%pf].

Irradiation	Er:YAG laser	No Er:YAG laser
Bleached	32.7 (±5.9) Aa [22%]	31.1 (±6.3) Aa [0%]
No Bleached	25.2 (±8.3) Ba [0%]	36.4 (±9.9) Aa [0%]

Means followed by the same *Capital* letters in the row or *Lower case* letters in the column indicate no statistical difference (p>0.05), n= number of samples.

The distribution of failure modes among adhesive interface is shown in Figure 1.

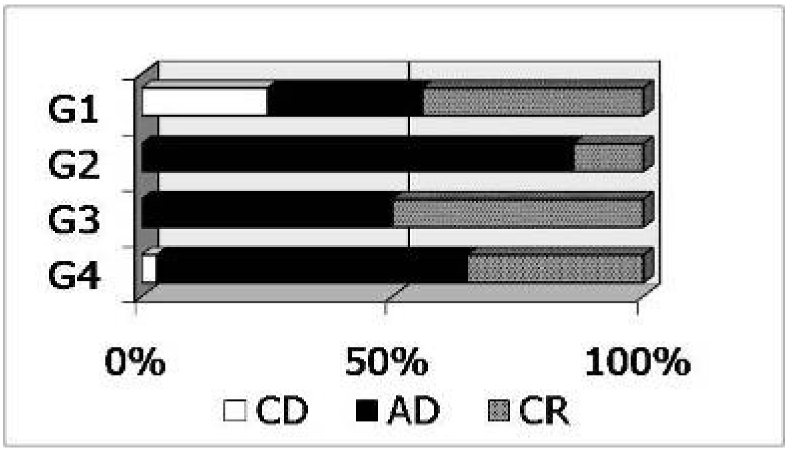

Figure 1. Distribution of failure modes within groups. CD, cohesive failure in dentin; AD, adhesive failure between dentin and resin; CR, cohesive failure in resin composite.

The predominant failure mode in all groups was adhesive failure with exception of G1, which recorded high cohesive failure in dentine (25%).

The pre-test failure recorded was G1-22%, G2- 0%, G3- 0% and G4- 0%.

Scanning electron microscopy evaluation

A representative SEM image of each failure mode is shown in Figure 2. Figure 2A is a typical SEM image of cohesive failure in dentin (CD); Figure 2B is the SEM image of adhesive failure between resin and dentin (AD) and Figure 2C is the SEM image of 70% cohesive failure in resin (CR) and 30% adhesive failure.

The SEM appearances of each group of dentin treatment are present in Figures 3 to 5. Er:YAG laser irradiation with 25.56 J/cm^2 promoted ablation of dentin surface observed under SEM for bleached slabs (Figure 3). The irradiation pattern was not homogeneous. The Er:YAG laser created a surface with absence of smear layer and opened dentinal tubules. There is no evidence of thermal damage and signs of burning, cracks and melting. The intertubular dentin was removed more accentuated than the peritubular dentin. The peritubular dentin is more evident around dentinal tubules entrance.

There were no morphological alterations on the dentin surface of bleached teeth (Figure 4) compared with control (Figure 5), that were covered by smear layer.

DISCUSSION

Vital dental bleaching with carbamide peroxide is a conservative and safe procedure for tooth discoloration.[17,18] The clinical use of carbamide peroxide has been first described by Haywood and Heymann (1989) and is widely used in modern dentistry.[19]

Carbamide peroxide is effective for bleaching procedure at 10% but the use of increased concentration has been developed for faster results.[1,20] Thus, it has been suggested that the higher concentration and frequency of hydrogen peroxide application the quicker the bleaching result and side effects due to peroxide cumulation.[21-23]

It was related that the residual peroxide might impair resin bonding to enamel and dentin if a restoration need replacement to achieve optimal shade matching. This undesirable effect may occur by the presence of residual hydrogen peroxide and its free radicals, and due to less mineral content and more organic matrix the dentin substrate may be more compromised.

Some studies revealed that bleached dentin showed reduction in monomer penetration into dentin and interfered in adhesive/resin monomer conversion into polymer[6] resulting in reduction on shear bond strength values and microleakage around restoration.

Dental bur creates a flat surface in dentin which is recovered by smear layer[7,12] and this appearance was observed in control group (G4), dentin tubules could not be seen under the smear layer created by enamel abraded (Figure 5). Similar to other studies in non-bleached dentin, the Er:YAG laser irradiation removed the smear layer and the dentin surface presented opened dentin tubules with no evidence of thermal damage such as cracking or carbonization (Figure 3).[12,24,25]

Er:YAG laser has a high absorption rate by water.[26] According to Giachetti et al. (2004) the Er:YAG irradiation for dentin conditioning must have an output inferior to 200mJ to tissue ablation.[25] The irradiation produces microexplosions during tissue ablation that can remove dentin.[12] These microexplosions lead to the ejection of organic and inorganic tissue particles, that can be noted in the scanning electron microphotographs evaluations[14,27,28] and created irregularities on the irradiated surface without smear layer and opened dentinal tubules.

The adhesive system also plays an important role in irradiated dentin.[13] Esteves-Oliveira (2007) reported that the Er:YAG laser irradiation was more favorable to tensile bond strength values than Er, Cr:YSGG laser irradiation.[29] Although the irradiated dentin presents no smear layer,[27,13] an acid-etching procedure is necessary to improve bonding on this surface.[13,30] Therefore previous studies have shown the need of a acid dentin etching after laser treatment to improve its adhesion to adhesive/composite resin.[1,31]

On the other side, Ferreira et al. (2009)[30] presented lower µTBS values with acid conditioning during 15s after Er:YAG laser treatment similar to the results of group 3. Ceballos et al. (2002) showed with transmission electron microscopy of irradiated dentin bonded with Adper Single Bond 2 showed that laser-modified layer was absent after acid etching. However, partial denaturing of collagen fibrils within the subsurface intertubular dentin could still be observed, which results are related to morphological alterations in dentin.[31] On the other hand, Chousterman et al. (2009) related that there was a significant increase in µTBS for irradiated dentin after acid etching for 90s and this fact must be considered in future studies with adhesion of bleached teeth.[13]

In spite of good bond strength results to dentin of bleached teeth, the irradiation of non-bleached dentin showed a reduction on bond strength and statistically differed from control group (G4). It has been reported that Er:YAG focused mode (12mm) promotes irregular and scaly surface.[32] Also the thermal effect of laser irradiation can produce changes in dentin's organic component.[27]

Reis et al. (2004)[33] affirmed that bovine teeth are possible substitute for human teeth in dentin bond testing. Dental bleaching also has a deleterious dentin adhesive performance.[1,18,34] It has been suggested that these adverse effects are most pronounced when the bleaching agent is applied directly to dentin.[1,34] Although the results of this study for blocks bleached with 16% carbamide peroxide and abraded to dentin (Group 2) did not present higher values for µTBS values compared to control group (Group 4); the bleached and non-irradiated dentin recorded higher adhesive failure type (86%). The probable cause for this is due to the fact that peroxide passed freely through enamel and impaired adhesion.

By the comparison of G1 and G3 results it can be supposed that irradiation with Er:YAG of non-bleached dentin (G3) showed lower µTBS values to adhesive/resin, however, G1 showed a higher percentage of CD failures and lower AD failures than G3 which could be related to laser irradiation.

To the best of our knowledge, there are no comparable studies analyzing the influence of Er:YAG laser irradiation on the µTBS after teeth bleaching with 16% carbamide peroxide in dentin. In this study, the hypothesis that Er:YAG laser treatment could change dentin in order to increase adhesion immediately after dental bleaching was tested. Specimens bleached and irradiated (Group 1) had similar adhesion µTBS values that specimens only submitted to bleaching procedure but presented the highest pre-test failure (22%).

In conclusion, Er:YAG laser irradiation promotes superficial dentin modifications that achieves similar adhesion on the dentin of bleached teeth compared with control group. Further work is required to determine clinical implications because µTBS was similar to control group but adhesive failure was higher than control group.

CONCLUSIONS

Within the limits of this in vitro study, it was concluded that bleaching procedure did not affect µTBS values for dentin adhesion. Er:YAG laser irradiation prior to adhesive procedure of non-bleached dentin surface impaired µTBS compared with control group. Er:YAG laser irradiation with 25.52 J/cm^2 prior to adhesive procedure promoted ablation on dentin surface observed under SEM.

DISCLOSURE

The authors have no interest in any of the companies or products mentioned in this article.

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FIGURES

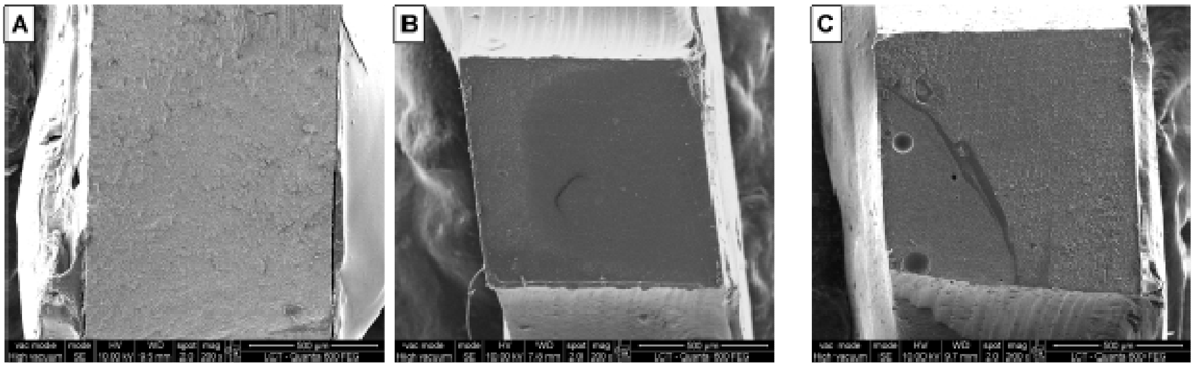


Figure 2. Scanning electron microscopic (SEM) photographs of the three representative failure type (**A**) cohesive failure in dentin; (**B**) adhesive failure between resin and dentin (**C**) 70% cohesive failure in resin and 30% adhesive failure (original magnification 200X, bar represents 500 µm).

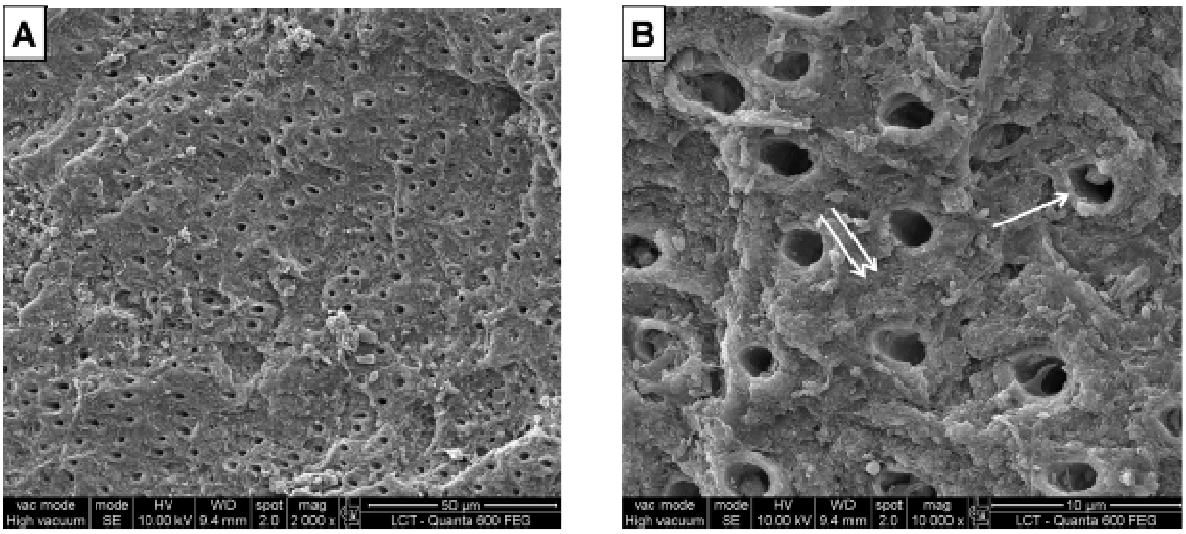


Figure 3. Scanning electron micrographs (SEM) of the dentin surface of bleached teeth irradiated with 25.56 J/cm^2 (Group 1). (**A**) The irradiation pattern was not homogeneous. The Er:YAG laser created a surface with absence of smear layer, opened dentinal tubules, irregular and microretentive morphological pattern (original magnification 2,000X, bar represents 50 µm); (**B**) there is no evidence of thermal damage and signs of burning, cracks and melting. The intertubular dentin (double arrow) was removed more acentuated than the peritubular dentin (continuum arrow). The peritubular dentin is more evident around dentinal tubules entrance (original magnification 10,000X, bar represents 10 µm).

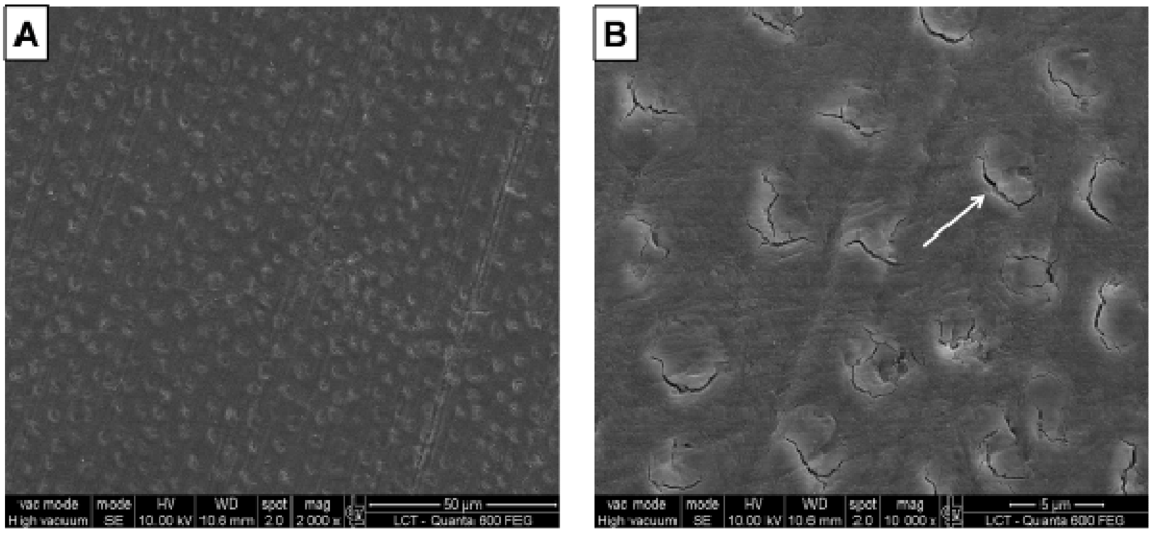
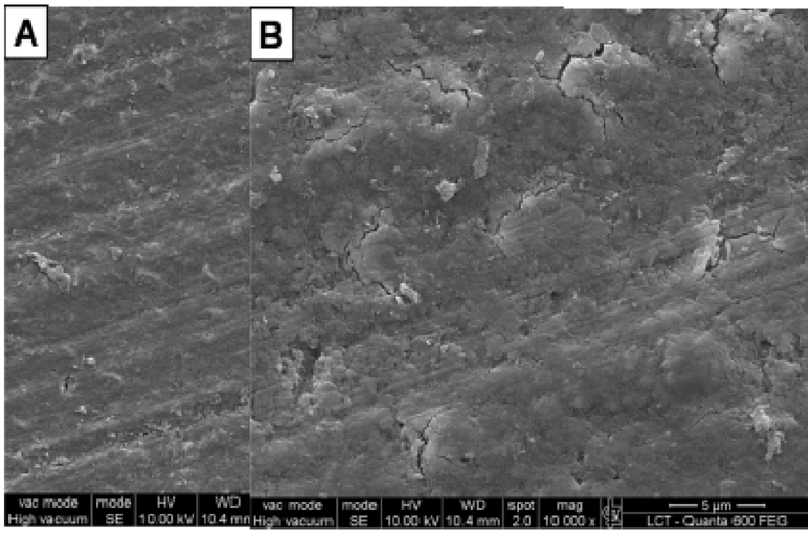


Figure 4. Scanning electron microscopic (SEM) photographs of the dentin surfaces of bleached teeth (Group 2). (**A**) A smear layer is all over dentin surface and occluded dentin tubules may be observed (original magnification 2,000X, bar represents 50 µm); (**B**) smear layer occluded dentin tubules (arrow) (original magnification 10,000X, bar represents 5 µm).

Figure 5. Scanning electron microscopic (SEM) photographs of the dentin surface of unbleached and unlased enamel surface (Group 4). (**A**) Smear layer (original magnification 2,000X, bar represents 50 µm); (**B**) smear layer (original magnification 10,000X, bar represents 10 µm).



4. CONCLUSÕES

-Através dos resultados obtidos e, considerando-se as condições experimentais deste estudo, pôde-se concluir que os parâmetros avaliados do laser de Er:YAG não favoreceram a adesão ao esmalte e à dentina bovina clareada com peróxido de carbamida 16%.

- A irradiação do esmalte bovino com laser de Er:YAG com densidade de energia de 25,56 J/cm^2 não influenciou os valores de resistência de união e promoveu ablação do esmalte observada sob microscopia eletrônica de varredura.

- A irradiação do esmalte bovino com laser de Er:YAG com densidade de energia de 4,42 J/cm^2 não influenciou os valores de resistência de união e não promoveu ablação do esmalte observada sob microscopia eletrônica de varredura.

- O procedimento clareador com peróxido de carbamida 16% não afetou os valores de resistência de união ao esmalte ou à dentina.

- A irradiação da dentina de dentes bovinos não clareados com laser de Er:YAG com densidade de energia de 25,52 J/cm^2 diminuiu os valores de resistência de união e promoveu ablação da dentina criando um padrão rugoso observado sob microscopia eletrônica de varredura.

- A irradiação da dentina de dentes bovinos clareados com laser de Er:YAG com densidade de energia de 25,52 J/cm^2 não influenciou os valores de resistência de união e promoveu ablação da dentina criando um padrão rugoso observado sob microscopia eletrônica de varredura.

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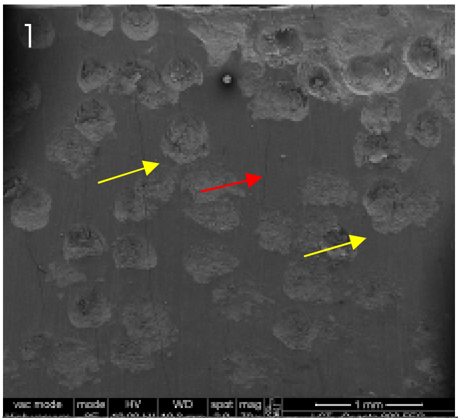
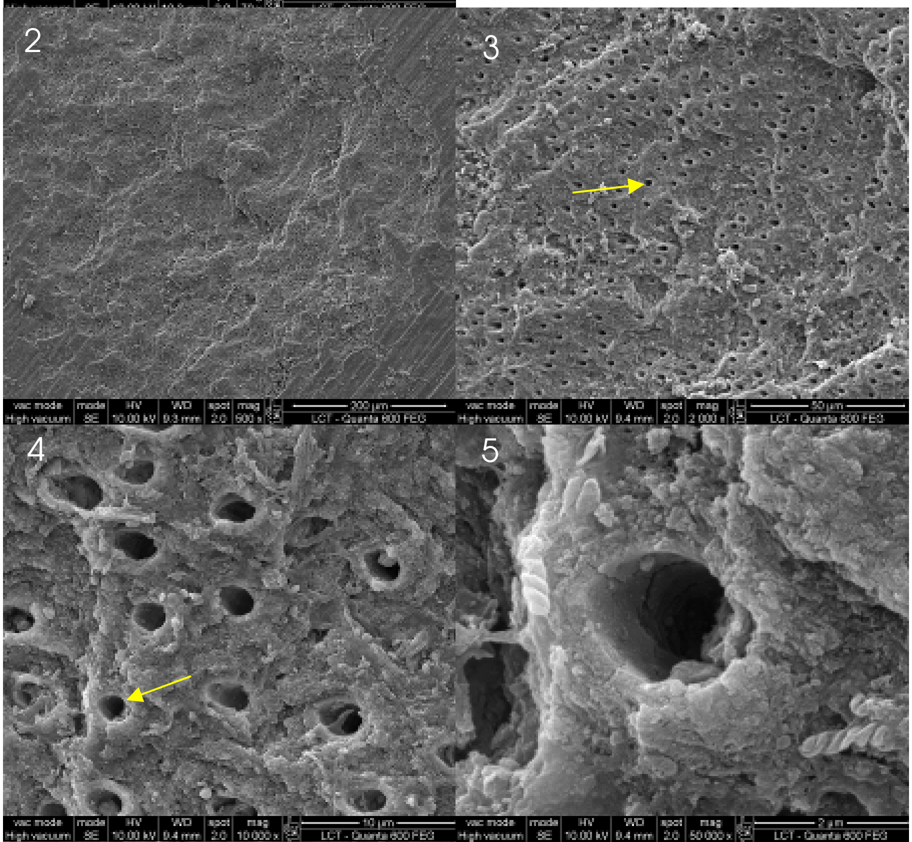
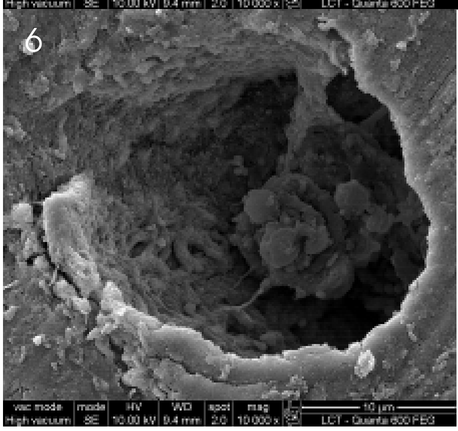
Anexos

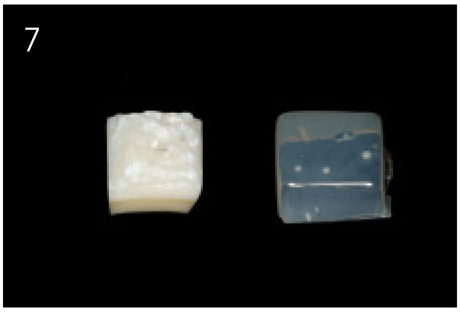


Anexo 1: Esmalte irradiado 25,52 J/cm^2 com magnitude de 70X. Setas amarelas - observam-se zonas pontuadas de ablação, formando micro cavidades. Seta vermelha - *Cracking* por desidratação do substrato





Anexo 2-6: Dentina irradiada com 25,52 J/cm^2 - apresentou superfície rugosa, sem a presença de *smear layer e com exposição dos* canalículos dentinários. A seta do anexo 3 aponta canalículo dentinário (2.000X). A seta do anexo 4 (10.000X) aponta o canalículo presente no anexo 5, ampliado a uma magnitude de 50.000X. O anexo 6 apresenta um túbulo dentinário “gigante”, típico de dentes bovinos, a uma magnitude de 10.000X.

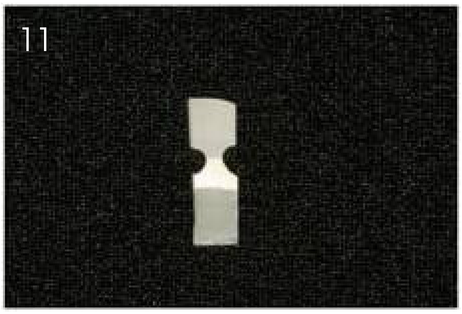


Anexo 7: Espécime com peróxido de carbamida 16% e moldeira individual

Anexo 8: Irradiação de laser Er:YAG em dentina

Anexo 9: Espécime sendo cortado na cortadeira metalográfica

Anexo 10: Espécime cortado em fatias de 1mm



Anexo 11 *Hourglass* confeccionado

Anexo 12: Kavo Key Laser II (Er:YAG)

Anexo 13: Máquina de ensaio de micro-tração

Anexo 14: Microscópio eletrônico de varredura (MEV) - FEI Quanta 600 F