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APLICAÇÃO DE LASERS DE MICROSSEGUNDOS E FEMTOSSEGUNDOS PARA O CONDICIONAMENTO DE TECIDOS DUROS DENTAIS

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Tese apresentada ao Colegiado do Programa de Pós-Graduação em Odontologia do Centro de Ciências da Saúde da Universidade Federal de Pernambuco como requisito parcial para obtenção do grau de Doutor em Odontologia área de concentração em Clínica Integrada

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RESUMO

A utilização de diferentes LASERs na área odontológica tem se difundido rapidamente, sendo os LASERs de alta potência utilizados como uma alternativa para promover alterações morfológicas e químicas nas superfícies dentárias. O objetivo geral deste trabalho foi analisar o efeito da irradiação do substrato dental com dois LASERs de alta intensidade e com duração temporal diferentes (µs e fs), na resistência de união a compostos resinosos. Em um primeiro momento, avaliou-se diferentes parâmetros do LASER de Er,Cr:YSGG no esmalte, utilizando-se dois diferentes tipos de sistema adesivo (autocondicionante de dois passos e um adesivo universal), e seus efeitos na resistência de união através de testes de microcisalhamento. Os efeitos do LASER no esmalte foram avaliados através de microscopia eletrônica de varredura e a análise do padrão de fratura foi realizada com o auxílio da tomografia por coerência óptica. Não houve diferença significativa entre o grupo controle e o grupo tratado com 44 J/cm^2. A análise pelo MEV revelou alterações morfológicas significativas da superfície do esmalte irradiado com laser, mostrando áreas de hidroxiapatita fundida e recristalizada nos grupos de 66 J/cm^2. Concluiu-se que o uso do LASER com 44 J/cm^2 foi estatisticamente tão eficaz quanto o uso do ácido fosfórico e não pareceu influenciar a ação do adesivo autocondicionante. Em um segundo momento analisou-se o efeito da irradiação com LASER de femtossegundo Ti:Safira na dentina sadia e erodida, através de microscopia eletrônica de varredura, microscopia de força atômica e tomografia por coerência óptica, bem como seus efeitos na resistência de união através de testes de microcisalhamento utilizando-se um adesivo autocondicionante de dois passos. A resistência de união máxima foi registrada pelo grupo SD240 (16,42 MPa) e a resistência de união mínima foi registrada pelo grupo ED (8,89 MPa). O teste de Mann Whitney demonstrou diferenças entre ED e todos os outros grupos. Pode-se concluir que o LASER de femtossegundo Ti:Safira foi capaz de ablacionar o tecido dentinário sadio, proporcionando a abertura dos túbulos dentinários e uma certa rugosidade superficial. Entretanto não houve melhora significativa na adesão. A irradiação com LASER de femtossegundo promoveu maior ablação nas amostras de dentina erodida e melhora na adesão de forma significativa.

Palavras-chave: Lasers. Dentina. Esmalte Dentário. Corrosão Dentária.

ABSTRACT

The use of different lasers in dentistry has been diffused rapidly, with high power lasers being used as an alternative to promote chemical/morphological changes on the tooth surface. The main objective of this work was to analyze the effect of dental substrate irradiation with two high intensity lasers with different temporal duration (μs and fs) on bond strength to resinous compounds. At first, different parameters of the Er,Cr:YSGG laser were evaluated in enamel using two different types of adhesive system (two-step self-etching and universal adhesive), and their effects on bond strength through micro-shear tests. The laser effects on the enamel were evaluated by scanning electron microscopy and the analysis of the fracture pattern was performed with the aid of optical coherence tomography. There was no statistically significant difference between the control group and the treated group with 44J/cm^2. SEM analysis revealed significant morphological alterations of the laser-irradiated enamel surface, showing areas of melted and recrystalized hydroxyapatite in the 66 J/cm^2 groups. It was concluded that the laser use with 44J/cm^2 was statistically as effective as the use of phosphoric acid and did not appear to influence the action of the self-etching adhesive. In a second moment, the effect of femtosecond laser irradiation with the Ti:Sa laser was analyzed in sound and eroded dentin, by scanning electron microscopy, atomic force microscopy and optical coherence tomography, as well as their effects on bond strength through micro-shear tests using a two-step self-etching adhesive. The maximum bond strength was recorded for the SD240 (16.42 MPa) and the minimum bond strength was recorded for the ED (8.89 MPa). The Mann Whitney test demonstrated differences between ED and all the other groups. It can be concluded that the femtosecond Ti:Sa laser was able to ablate the sound dentin tissue, providing the opening of the dentinal tubules and a certain superficial roughness. However, there was no significant improvement in adhesion. Femtosecond laser irradiation promoted greater ablation in the eroded dentin samples and improved adhesion significantly.

Keywords: Lasers. Dentin. Dental Enamel. Dental Etching.

LISTA DE ABREVIATURAS E SIGLAS

AFM	-	Atomic Force Microscopy
CA	-	Califórnia
CAPES	-	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CETENE	-	Centro de Tecnologias Estratégicas do Nordeste
CSE	-	Clearfil SE (group)
ED	-	Eroded Dentin
Er,Cr:YSGG	-	Erbio, Chrome: Yttrium, Scandium, Gallium, Garnet
Er:YAG	-	Erbio:Yttrium, Aluminum, Garnet
et al.	-	E outros
FACEPE	-	Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco
FOP	-	Faculdade de Odontologia de Pernambuco
FOUSP	-	Faculdade de Odontologia da Universidade de São Paulo
IL	-	Illinois
IPEN	-	Instituto de Pesquisas Energéticas e Nucleares
JAVA	-	Linguagem de programação
LASER	-	L*ight Amplification by Stimulated Emission of Radiation*
LELO	-	Laboratório Especial de LASER em Odontologia
MEV	-	Microscopia Eletônica de Varredura
PR	-	Paraná
PVC	-	Polyvinyl Chloride
SBU	-	Single Bond Universal
SD	-	Sound Dentin
SDI	-	*Southern Dental Industries*
SD-OCT	-	*Spectral Domain Optical Coherence Tomography*
SLD	-	*Superluminescent Diode*
SPIE	-	The International Society for Optics and Photonics
TCO	-	Tomografia de Coerência Óptica
Ti:Safira	-	Titânio:Safira
UFPE	-	Universidade Federal de Pernambuco
UK	-	United Kingdon
UPE	-	Universidade de Pernambuco

SUMÁRIO

1 APRESENTAÇÃO

O LASER já não é apenas uma ferramenta terapêutica promissora, mas já pode sim ser considerado um dos avanços mais importantes na Odontologia nos últimos anos. Aos poucos o ceticismo quanto a sua efetividade foi sendo deixado de lado e ele se tornando um valioso e importante item do arsenal clínico. Tudo isso a partir de pesquisas como estas desta tese de doutorado, que puderam elucidar mecanismos de ação juntos aos tecidos e parâmetros ideais para a sua utilização.

Após curso de habilitação em LASERs em Odontologia, realizado em 2014 no Laboratório Especial de LASER em Odontologia (LELO/FOUSP), e contato com o Prof. Carlos de Paula Eduardo e Profa. Denise Maria Zezell (IPEN/USP), houve o incentivo para a realização de pesquisa com intuito de analisar o efeito do LASER de Er,Cr:YSGG no condicionamento dos tecidos duros dentais. Para isto, foram propostos diferentes parâmetros de irradiação com o LASER em esmalte para condicionamento do tecido na busca por uma maior força de adesão entre o substrato dental e um composto resinoso, em substituição ao uso do ácido fosfórico. O resultado parcial deste trabalho foi publicado sob o título “Evaluation of microshear bond strength of resin composites to enamel of dental adhesive systems associated with Er,Cr:YSGG Laser” no *Proceedings of SPIE*, qualis B3. Os dados e análises completas resultou no artigo intitulado “Effects of enamel etching by Er,Cr:YSGG Laser on microshear bond strength” submetido à revista *Lasers in Medical Science*, qualis A2.

Após este aprofundamento no universo dos LASERs, e conhecimento sobre o LASER de femtossegundo, um LASER laboratorial ainda pouco estudado na Odontologia, surge a ideia de avaliar o efeito deste no condicionamento dental. Através de estudos-piloto puderam-se estabelecer os melhores parâmetros a serem avaliados para o condicionamento de amostras de dentina sadia e erodida. Não é do nosso conhecimento, até o momento, nenhuma pesquisa na literatura realizada em dentina erodida e utilizando estes parâmetros. O resultado deste trabalho traz a literatura informações importantes a respeito da interação deste LASER com estes dois diferentes substratos dentinários e a influência do mesmo na adesão à estrutura dental. Os resultados serão divulgados através do artigo intitulado “Effects of femtosecond laser irradiation on the microshear bond strength to eroded and sound dentin” submetido à revista *Journal of Biophotonics*, qualis A1.

Nas próximas páginas o leitor poderá entender os pormenores do tema abordado, o detalhamento das metodologias utilizadas e os resultados, constantes nos manuscritos dos artigos provenientes desta tese. Espera-se com isto, contribuir para o entendimento do tema em questão e abrir portas para novos questionamentos, pois é através destes que há o avanço da ciência.

2 INTRODUÇÃO

2.1 A Odontologia adesiva

A Odontologia adesiva teve seu advento a partir Buonocore (1955) [1] que, baseado em um princípio industrial, desenvolveu a técnica do ataque ácido em esmalte para aumentar a união das resinas acrílicas, aumentando a receptividade do esmalte à adesão [2]. A partir de então surgiram diversas pesquisas na busca pelo aprimoramento da união entre a estrutura dentária e o material restaurador, proporcionando o surgimento de novas técnicas e materiais.

A adesão eficiente entre o material restaurador e o substrato dental, além de favorecer retenção necessária à restauração, evitaria o surgimento de lesões de cáries secundárias provenientes de microinfiltração, manchamento marginal e o desenvolvimento de sensibilidade pós-operatória [3]. Os sistemas adesivos tornaram-se então elementos fundamentais para a união do material restaurador às estruturas dentárias, sendo hoje o principal desafio desta técnica promover uma adesão igualmente eficiente para dois tecidos de naturezas tão distintas (esmalte/dentina) [4].

Atualmente estão disponíveis no mercado diferentes tipos de adesivos classificados de acordo com a estratégia de ação do condicionamento da estrutura dental: condicionamento ácido total (etch-and-rinse) ou autocondicionante (self-etch). Os sistemas adesivos do tipo etch-and-rinse ou convencionais utilizam o condicionamento ácido da estrutura dental previamente a sua aplicação, removendo por completo o *smear layer*. Podem ser de dois passos (condicionamento ácido e primer + agente adesivo em um único frasco) ou três passos (condicionamento ácido, primer e agente adesivo em frascos separados) [5]. Os sistemas adesivos autocondicionantes são divididos em adesivos self-etch de dois passos e em adesivos self-etch de passo único (all-in-one). Nos sistemas adesivos autocondicionantes de dois passos, o agente condicionador e o iniciador são colocados em um frasco e a resina adesiva em outro, enquanto que os adesivos de passo único combinam o iniciador autocondicionante e a resina hidrofóbica numa só aplicação, o que resulta numa simplificação do procedimento adesivo [6].

O esmalte dental é uma estrutura composta de 96% de material inorgânico na forma de cristas de hidroxiapatita e 4% de material orgânico e água. A adesão a essa estrutura é convencionalmente conseguida através do

condicionamento do substrato com ácido fosfórico na concentração de 30 a 37% por 15 a 30 segundos [7], sendo este capaz de promover uma dissolução seletiva dos prismas de esmalte, criando microporosidades que são infiltradas pelo adesivo [8], proporcionando uma adesão favorável.

Já a dentina é um tecido duro, elástico e avascular composto por aproximadamente 50% de material inorgânico formado por cristais de hidroxiapatita, 30% de material orgânico formado por fibrilas de colágeno e 20% de fluidos [8], caracterizando-se pela presença de múltiplos túbulos dentinários, preenchidos por fluido dentinário, que se estendem desde a junção amelodentinária até a polpa, tornando-a um substrato naturalmente úmido [9]. Assim, a heterogeneidade da estrutura dentinária e a presença de umidade neste substrato dificultam o procedimento adesivo. Quando do uso dos adesivos etch-and-rinse faz-se necessária a utilização do ácido fosfórico em dentina, o que causa uma desmineralização superficial do substrato dentinário, na faixa de micrômetros, suficiente para exposição da rede de fibrilas colágenas que será infiltrada pelo monômeros hidrofílicos do adesivo, formando a camada híbrida [10].

No entanto, uma das principais causas de falhas no processo de adesão à dentina é a discrepância entre a área desmineralizada pelo ácido fosfórico e a área infiltrada pelo agente de união [11]. Assim, com o intuito de simplificação dos procedimentos adesivos e de eliminação deste fator complicador, os adesivos autocondicionantes ou self-etch foram introduzidos no mercado, pois não requerem ataque ácido prévio, promovendo condicionamento e penetração do primer no substrato dental simultaneamente, por conter um monômero ácido em sua composição [2].

Os sistemas adesivos autocondicionantes dissolvem parcialmente a smear layer, desmineralizam e infiltram simultaneamente a dentina subjacente, incorporando a smear layer à interface de união, evitando o colapso das fibrilas de colágeno pela secagem com ar e também, a ocorrência de fibrilas desprotegidas pela resina aplicada [12]. Estes adesivos têm sido amplamente aceitos na literatura por apresentarem forças de adesão previsíveis e favoráveis à dentina. No entanto, há relatos controversos quando se trata do esmalte [13,14]. Neste substrato, a profundidade do condicionamento desempenha um papel importante na qualidade final da adesão [15], porém os adesivos autocondicionantes não atacam o esmalte com a mesma profundidade que o ácido fosfórico. Um padrão de desmineralização

mais fraco é produzido, devido à baixa acidez dos primers autocondicionantes, razão da fraca adesão ao esmalte [14]. Por causa disso, um condicionamento seletivo do esmalte com ácido fosfórico é recomendado anteriormente à aplicação do adesivo autocondicionante [16].

Nos últimos anos, um novo grupo de adesivos dentais conhecidos como Universais ou Multimodo foi introduzido no mercado. Estes adesivos são primordialmente um adesivo self-etch de um passo, mas que pode ser usado como um sistema convencional etch-and-rinse [17]. No entanto, uma redução da eficácia da ligação ao esmalte é observada quando os adesivos universais são aplicados como agente autocondicionante [18], sendo também recomendado o condicionamento com ácido fosfórico prévio [19].

2.2 A erosão dentária x Adesão

O conhecimento acerca do desenvolvimento da cárie, suas formas de prevenção e tratamento, fizeram com que a incidência da doença declinasse com o passar dos anos [20], favorecendo, assim, a manutenção dos dentes na cavidade bucal por mais tempo, predispondo-os a outras lesões dentárias como a erosão. A erosão dental é um acometimento bucal que vem tomando a cena e que não deve ser subestimado por ser uma condição multifatorial, na qual fatores nutricionais e ligados ao indivíduo podem desencadeá-la [21]. De acordo com Jaeggi e Lussi (2006) [22], há evidências de que a prevalência da erosão dental vem crescendo firmemente.

A erosão dentária é definida como uma perda irreversível do tecido duro dental, devido a um processo químico, sem envolvimento de microrganismos [23]. O desenvolvimento da erosão depende do tempo de duração e intensidade da exposição ao ácido podendo ocorrer em qualquer superfície do dente. No entanto, a distribuição da erosão na dentição é diretamente influenciada por fatores como a taxa do fluxo salivar e a capacidade tampão da saliva que podem modificar o processo erosivo. Quando a dentina é exposta a ácidos com força e concentração clinicamente relevantes, o componente mineral prontamente se dissolve enquanto a porção orgânica é retida, formando uma fina rede de colágeno. Esta zona de desmineralização aumenta com o aumento do tempo de exposição aos ácidos, formando uma zona totalmente desmineralizada, abaixo da qual se encontra uma dentina parcialmente desmineralizada e em seguida a dentina sadia [21].

No entanto, a erosão pode afetar negativamente a resistência de união dos adesivos a estrutura dentária uma vez que a presença de colágeno desnaturado pode interferir com as propriedades de ligação destes materiais [18]. Em uma dentina profundamente erodida, após a penetração do adesivo, formam-se camadas híbridas mais espessas e estruturalmente imperfeitas do que as da dentina sadia. Porosidades e zonas desmineralizadas sem reforço de resina estão presentes, uma vez que os monômeros de resina podem não penetrar tão profundamente quanto o ácido [24]. Porém, os sistemas adesivos autocondicionantes têm um iniciador ácido, que promove a desmineralização e a infiltração de monômeros simultaneamente, formando uma camada híbrida mais homogênea, sendo teoricamente mais indicados estes casos.

2.3 LASERs de microssegundos e femtossegundos x Adesão

2.3.1 LASER de Er,Cr:YSGG e o esmalte

A utilização de diferentes LASERs na área odontológica tem sido investigada há décadas e, desde então, tem se difundido rapidamente devido à imensa gama de aplicações da luz LASER, tanto em tecidos moles como em tecidos duros. Os primeiros estudos com a utilização do LASER na odontologia foram realizados a partir de 1964 por Goldman et al. [25], analisando os componentes inorgânicos de tecidos calcificados irradiados com LASER de rubi, e por Stern e Sognnaes (1964) [26], que observaram fusão e vitrificação do esmalte e a formação de crateras bem definidas com indícios de carbonização na dentina.

Os LASERs pulsados de alta potência (acima de 1W) têm sido utilizados como uma alternativa para promover alterações químicas/morfológicas na superfície do dente [27], com o intuito de melhora na adesão. Os LASERs de Erbio (Er:YAG e Er,Cr:YSGG), LASERs pulsados no regime de microssegundos, foram considerados as fontes fotônicas mais promissoras para serem usadas em tecidos mineralizados, pois seus comprimentos de onda (2940 nm e 2780 nm, respectivamente) mostram alta absorção por água e hidroxiapatita [28]. Hibst et al. (1988) [29], Hibst e Keller (1989) [30] e Keller e Hibst (1989) [31] realizaram os primeiros estudos utilizando o LASER de Er:YAG em tecidos duros dentais, observando a efetividade da ablação tanto em tecidos sadios como cariados.

Quando utilizado em doses apropriadas, LASERs de érbio conseguem remover cristais de hidroxiapatita, presentes no esmalte, de forma seletiva, através de micro-explosões durante a ablação que são capazes de ejetar partículas de tecido duro. A água ligada quimicamente a estrutura e a água livre, que é adicionada ao sistema, são aquecidas até seu ponto de ebulição. A água é então vaporizada proporcionando um aumento de volume. Quando esta expansão volumétrica ultrapassa o limite de resistência dos cristais de hidroxiapatita estes são ejetados explosivamente da superfície (Ablação Explosiva Termo-Mecânica), criando crateras irregulares, e promovendo uma superfície de padrão irregular sem produção de smear layer [32.33], o que pode potencialmente melhorar a retenção micromecânica de sistemas adesivos.

Ainda não há consenso na literatura quanto à eficácia da adesão à resina após o condicionamento do esmalte com o LASER de Er,Cr:YSGG. Adebayo et al. (2012) [34] não revelaram diferenças significativas na resistência de união entre o esmalte desgastado por broca diamantada e o esmalte irradiado com o LASER de Er,Cr:YSGG para o adesivo autocondicionante de dois passos (Clearfil SE Bond) e dois adesivos do tipo "all-in-one". Já Başaran et al. (2011) [35] avaliaram diferentes parâmetros do LASER de Er,Cr:YSGG para condicionamento do esmalte como alternativa ao uso do ácido fosfórico em ensaios de adesão a cimentos resinosos e puderam concluir que o LASER de Er,Cr:YSGG apresentou forças de adesão adequadas.

No entanto, alguns estudos indicam que o condicionamento realizado pelo LASER produz uma adesão efetivamente menor que o convencional condicionamento com ácido fosfórico [36-39]. Há relatos na literatura de que o LASER modifica a proporção cálcio/fosfato e também reduz a proporção carbonato/fosfato tornando, portanto, a superfície irradiada mais mineralizada, estável e resistente a ácidos [40]. Estas alterações na composição química podem afetar a adesão [41] podendo, a superfície se tornar resistente ao condicionamento pelos ácidos fracos de um adesivo autocondicionante [42].

2.3.2 LASER pulsado de Ti:Safira e a dentina

Recentemente, LASERs de pulso ultracurto têm sido testados como uma potencial ferramenta alternativa para o condicionamento da superfície dentária. Cristais de safira dopados com titânio (Ti:Safira) são os principais meios ativos

usados para produzir estes LASERs. Um LASER de femtossegundo é um LASER pulsado ultracurto com duração de pulso menor que 1ps (1 picosegundo = 10^{-12} segundos), sendo um valor típico em LASERs comerciais de 50-150fs (1 femtosegundo = 10^{-15} segundos), que produz energia suficiente para transformar em plasma a estrutura dental sem tempo para difusão térmica [43,44]. Estes LASERs pulsados, amplificados em energia na ordem de milijoules e focalizados, permitem a ablação de camadas finas (~micrômetros) com excelente precisão e reprodutibilidade, o que pode resultar em menos danos colaterais para as áreas adjacentes do que qualquer outro processo mecânico, térmico ou químico.

A ablação no regime de pulsos ultracurtos se dá através do mecanismo mediado por plasma. O material é aquecido em uma escala de tempo de picossegundos (ps), ocorrendo uma transição direta da fase sólida para o plasma (mistura de íons e elétrons livres) e em seguida uma rápida expansão hidrodinâmica e ablação. Este processo ocorre mais rapidamente que a difusão térmica ao tecido adjacente, preservando a estrutura e propriedades do material irradiado [45].

A superfície tratada com o LASER de femtossegundo apresenta uma aparência irregular, sem a presença de smear layer e túbulos dentinários abertos [43,46] e uma superfície com ausência completa de danos térmicos e mecânicos sem sinais de microfissuras, fusão ou carbonização [43,47]. Além disso, a composição da dentina parece não ser significativamente modificada pelo LASER [48]. Estas observações nos levam a acreditar que o substrato de dentina irradiada com o LASER de femtossegundo seria ideal para a adesão. Gerhardt-Szep et al. (2011) [49] observaram que o procedimento adesivo foi facilitado em dentina irradiada pelo uso do LASER de femtossegundo, dispensando o uso do primer, sem afetar a força de união. No entanto, Portillo et al. (2015) [50] verificaram que a eficácia adesiva foi diminuída quando do uso do LASER de femtossegundo em dentina, diminuindo a força de união entre resina e substrato quando do uso de adesivos etch-and-rinse de dois passos e self-etch de dois passos.

O objetivo geral deste trabalho foi analisar o efeito da irradiação do substrato dental com dois diferentes LASERs pulsados e de alta potência, na resistência de união a compostos resinosos. Em um primeiro momento, avaliou-se a ação do LASER de Er,Cr:YSGG no esmalte, utilizando-se dois diferentes tipos de sistema adesivo, e seus efeitos na resistência de união através de testes de microcisalhamento. Os efeitos do LASER no esmalte foram avaliados através de

microscopia eletrônica de varredura e a análise do padrão de fratura foi realizada com o auxílio da tomografia por coerência óptica. Em um segundo momento analisou-se o efeito do LASER de femtossegundo Ti:Safira na dentina sadia e erodida através de microscopia eletrônica de varredura, microscopia de força atômica e tomografia por coerência óptica, e seus efeitos na resistência de união através de testes de microcisalhamento.

3 METODOLOGIA

Foi realizado um estudo do tipo experimental em laboratório desenvolvido na Pós-graduação em Odontologia pertencente ao Departamento de Prótese e Cirurgia Buco-facial, no Departamento de Física da Universidade Federal de Pernambuco – UFPE, no Instituto de Pesquisas Energéticas e Nucleares - IPEN/USP e na Faculdade de Odontologia de Pernambuco - FOP/UPE, no Departamento de Engenharia Mecânica da Universidade Federal de Pernambuco – UFPE e no Centro de Tecnologias Estratégicas do Nordeste - CETENE.

A presente pesquisa foi submetida à apreciação pelo Comitê de Ética em Pesquisa da Universidade Federal de Pernambuco e aprovada sob o protocolo de número #1.735.580.

3.1 Resistência de União ao esmalte irradiado com Er,Cr:YSGG

3.1.1 Seleção e Preparo das amostras

Para o desenvolvimento do experimento foram utilizados 30 molares permanentes, livres de cárie, fraturas e trincas, e extraídos nos últimos seis meses, preservados em solução de cloramina T 0,5%, e obtidos junto ao Banco de Dentes da UFPE. Os dentes foram limpos com ultrassom (Jet Sonic, Gnatus), escovas tipo Robinson e taças de borracha com pedra pomes e água para a remoção de resíduos, cálculo e película adquirida. Os espécimes foram então seccionados aproximadamente 2 mm abaixo da junção cemento-esmalte para eliminação das raízes com disco diamantado em baixa velocidade (Isomet, Buehler Ltd., IL, USA). Da mesma forma, cortes no sentindo mésio-distal e vestíbulo-lingual foram realizados, obtendo-se fatias da superfície vestibular, lingual e proximal do esmalte dos elementos. Estas fatias foram incluídas para a realização da irradiação com o LASER Er,Cr:YSGG de modo que o esmalte (área menos convexa) ficasse exposto, em tubos de PVC com 2 cm de altura e 2 cm de diâmetro com resina acrílica autopolimerizável.

Todas as amostras tiveram a superfície de esmalte exposta planificada e polida com lixas de carbeto de silício de granulação 400, 600 em máquina politriz a fim de se produzir uma camada de smear layer padronizada. As amostras foram

então armazenadas em água destilada até a divisão dos grupos e início do experimento.

3.1.2 Irradiação das amostras

As amostras de esmalte foram divididas aleatoriamente em seis grupos (n = 15) em função do tipo de condicionamento e do sistema adesivo utilizado (Tabela 1).

Tabela 1 – Grupos, sistemas adesivos e protocolos de condicionamento.

Grupo	Sistema Adesivo	Protocolo de condicionamento
Controle CSE	Clearfil SE Bond (Kuraray, Noritake Dental Inc., Kurashiki, Okayama, Japan)	Ácido fosfórico 37% por 15 segundos
Controle SBU	Adper Single Bond Universal (3M, Deutschland GmbH, Seefeld, Germany)	Ácido fosfórico 37% por 15 segundos
CSE 66 J/cm^2	Clearfil SE Bond	Er, Cr:YSGG - 5 Hz, 0.5 W, 66 J/cm^2
SBU 66 J/cm^2	Adper Single Bond Universal	Er, Cr:YSGG - 5Hz, 0.5 W, 66 J/cm^2
CSE 44 J/cm^2	Clearfil SE Bond	Er, Cr:YSGG - 15 Hz, 1.25 W, 44 j/cm^2
SBU 44 J/cm^2	Adper Single Bond Universal	Er, Cr:YSGG - 15 Hz, 1.25 W, 44 j/cm^2

As amostras receberam a irradiação com LASER de Er,Cr:YSGG (Waterlase Millenium, Biolase, San Clemente, CA, EUA), que consiste em um LASER em estado sólido, cujo meio ativo é um cristal de YSGG (YTTRIUM, SCANDIUM, GALLIUM, GARNET – Ítrio, Escândio, Gálio, Granada). Este LASER emite um comprimento de onda de 2,78 µm, duração de pulso 60 µs, taxa de repetição de até 100 Hz e potência média de 0,1 a 6 W. Por emitir radiação no espectro do infravermelho próximo, necessita de uma luz guia (visível) para sua focalização.

O LASER foi utilizado de forma focalizada, utilizando suporte especialmente desenvolvido para padronização da distância. A ponta do LASER (tip Z6-600, d = 0,6 mm) foi posicionado a 1 mm (90°) da superfície do esmalte (Figura 1) sob refrigeração (ar 50% e água 50%) para todos os grupos irradiados. Foi considerado um diâmetro útil do spot do LASER de 63,2% do diâmetro do “tip” (d = 0,379 mm). Com o auxílio de um motor de passo (ESP300, Newport, Irvine, CA,

EUA) as amostras foram irradiadas de forma a não haver sobreposição de pulsos (Figura 2). Para isto utilizou-se uma velocidade de 1,9 mm/s para as amostras irradiadas com a frequência de 5 Hz e de 5,7 mm/s para as amostras irradiadas com 15 Hz.

As amostras dos grupos controle (CSE e SBU) foram condicionadas com ácido fosfórico 37% em forma de gel, por 15 segundos, lavados com jatos de água por 60 segundos e secos por leves jatos de ar.

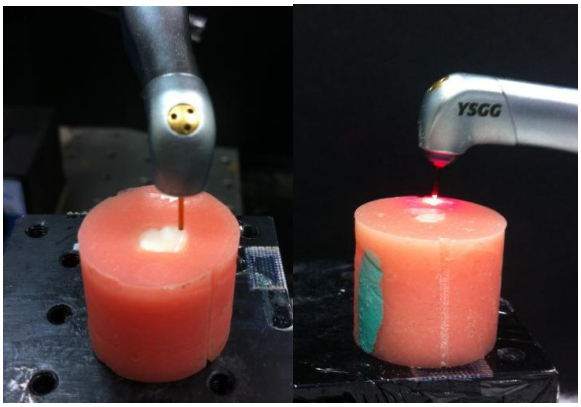

Figura 1 – Amostra sendo irradiada pelo LASER de Er,Cr:YSGG

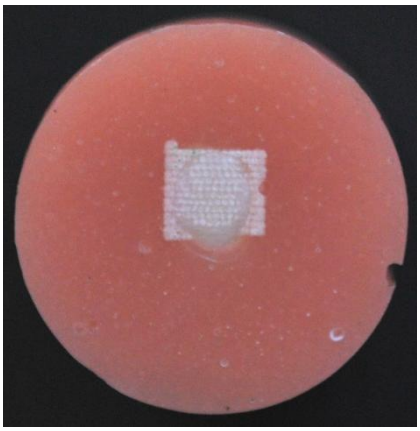

Figura 2 – Superfície da amostra irradiada pelo LASER de Er,Cr:YSGG

3.1.3 Preparo dos Corpos de Prova

Um dispositivo cilíndrico metálico desenvolvido pela USP foi utilizado para confecção das matrizes para a resina composta. Um tubo transparente de polietileno foi posicionado dentro de um orifício tubular, presente na área central deste dispositivo, com finalidade de corte. Com auxílio de uma lâmina de bisturi nº15c foram confeccionadas matrizes individualizadas com altura de 1 mm.

Uma área de 2 mm de diâmetro foi demarcada com fita adesiva dupla face. O sistema adesivo foi aplicado por um pincel (Microbrush, KG, Cotia, São Paulo, Brasil) de acordo com instruções do fabricante. Em seguida, a matriz (Ø = 0,9 mm, h = 1 mm) foi inserida sobre a área e, logo após, o adesivo foi polimerizado por 20 segundos com o LED Radii-cal (SDI, Bayswater, Victoria, Australia), emitindo luz na faixa de intensidade média de 1200 mW/cm^2, segundo o fabricante, e conferido com radiômetro de cura analógico de alta precisão.

A resina composta Tetric N Ceram A3 (Ivoclar vivadent, Schaan, Liechtenstein) foi inserida com espátula não aderente (Duflex) e o incremento foi polimerizado por 20 segundos. Todos os corpos de prova foram armazenados em água destilada em estufa a 36ºC por 1 hora, para que só então as matrizes fossem removidas cuidadosamente. Com o auxílio de uma lâmina de bisturi 15c as matrizes e a fita dupla face foram cuidadosamente removidas para expor os cilindros de resina. Após esta etapa, todos os corpos de prova foram estocados por 24 horas em água destilada em estufa a 36ºC. As amostras foram examinadas ao microscópio estereoscópico (ampliação x10) para observar possíveis defeitos para só então iniciar-se os testes de resistência ao cisalhamento.

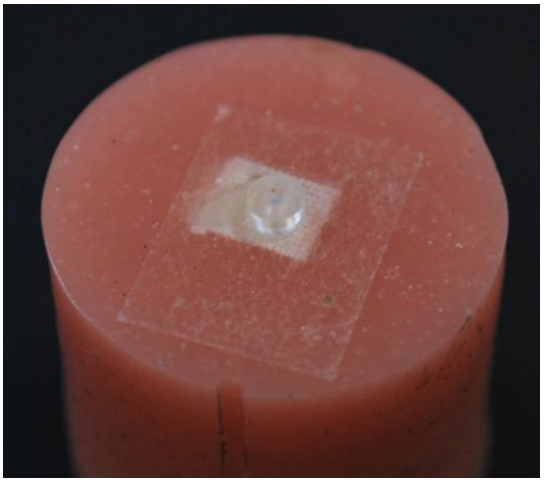

Figura 3 - Matriz transparente de polietileno inserido sobre fita dupla face perfurada.

3.1.4 Ensaio de microcisalhamento

Todos os corpos de prova de cada grupo foram submetidos ao teste individualmente. Cada amostra contendo os corpos de prova foi adaptada à máquina de ensaio universal Kratos e submetidas a um carregamento de cisalhamento feito através de um fio ortodôntico de 0,2 mm de diâmetro, a uma velocidade de 0,5

mm/min até o rompimento da união (Figura 4). A resistência de união ao microcisalhamento foi calculada e expressa em MPa.

Figura 4 – Corpo de prova (grupo controle) em máquina de ensaios universal.

3.1.5 Análise do padrão de fratura

A análise do padrão de fratura foi realizada com um microscópio óptico em aumentos de 5 x, 10 x, 20 x e 50 x (Olympus BX51, Tóquio, Japão). Imagens 2D e 3D de Tomografia de Coerência Óptica (TCO) foram realizadas para confirmar os resultados em um equipamento no domínio espectral SD-OCT (Sistema Callisto Spectral Domain OCT, Thorlabs Inc, New Jersey, EUA). Nesta montagem, a fonte de luz consiste num diodo superluminescente (SLD) com comprimento de onda central de 930 nm. Após a secagem dos espécimes, os mesmos foram posicionados na mesa de trabalho montada com três parafusos micrométricos (eixos X, Y e Z) (Figura 5).

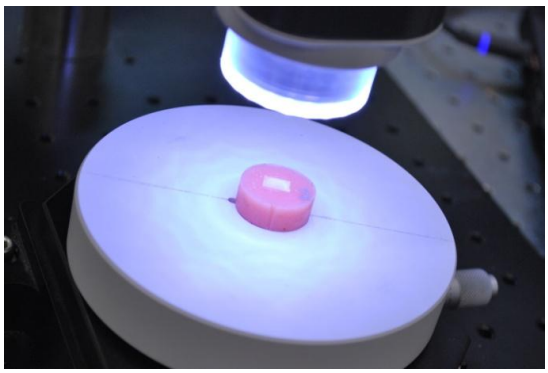

Figura 5 – Amostra posicionada em mesa de trabalho para análise do padrão de fratura por Tomografia de coerência óptica.

Foram realizadas varreduras da superfície do espécime, obtendo-se imagens 2D (1024 x 512 pixels / 4 x 0,5 mm) da área irradiada/referência. Da mesma

forma, foram obtidas imagens em 3D (1024 x 512 pixels / 3 X 3 X 0,5 mm) da superfície adesiva. Os tipos de falha foram classificadas em três categorias, com base no percentual de material livre de substrato: Adesiva (falha da interface resina / esmalte); Coesiva (exclusivamente no esmalte ou resina composta); Mista (falha na interface resina-esmalte que incluem falha coesiva dos substratos vizinhos).

3.1.6 Microscopia Eletrônica de Varredura (MEV)

Fotomicrorafias de áreas representativas das superfícies irradiadas foram obtidas. Para esta avaliação, foram preparadas duas amostras adicionais para cada grupo. As amostras foram revestidas com ouro e analisadas no MEV (Mira 3, Tescan, Cambridge, UK) a 5 kV com 100 x e 2000 x de aumento.

3.1.7 Analise estatística

Os dados foram organizados em planilha Excel (Microsoft Office 2007) e analisados usando SPSS 13.0 (Statistical Package for Social Sciences, Chicago, IL, EUA) para Windows. Estatísticas descritivas foram obtidas e o teste de Kruskal Wallis foi realizado para comparar os resultados entre os grupos. No caso de diferenças significativas, o teste de Mann Whitney foi realizado por comparação aos pares entre os grupos (p = 0,05).

3.2 Resistência de União à dentina irradiada com Ti:Safira

3.2.1 Seleção e Preparo das amostras

Foram utilizados 20 molares permanentes, livres de cárie, fraturas e trincas, e extraídos nos últimos seis meses, preservados em solução de cloramina T 0,5%, e obtidos junto ao Banco de Dentes da UFPE. Os dentes foram limpos com ultrassom (Jet Sonic, Gnatus), escovas tipo Robinson e taças de borracha com pedra pomes e água para a remoção de resíduos, cálculo e película adquirida. Os espécimes foram então seccionados aproximadamente 2 mm abaixo da junção cemento-esmalte para eliminação das raízes com disco diamantado em baixa velocidade (Isomet, Buehler Ltd., IL, USA). A face oclusal foi removida de forma a deixar exposta a dentina da região e estes espécimes foram incluídos em resina

acrílica autopolimerizável com o auxílio de canos de PVC com 2 cm de altura e 2 cm de diâmetro para a realização da irradiação com o LASER Ti:Safira.

3.2.2 Ciclagem erosiva

Dez amostras foram submetidas a ciclagem erosiva de pH que foi realizada durante 5 dias por imersão em ácido cítrico 0,05 M (pH 2,3, 10 min, 6 × / dia). Entre os ataques de ácido, as amostras foram imersas durante 60 minutos em solução supersaturada (pH 7,0) que consiste de 1,5 mmol / L de CaCl2, 1,0 mmol / L de KH2PO4, e 50 mmol / L de NaCl [51], à temperatura ambiente (25 ° C), sob agitação constante (30 rpm) num agitador. Durante o tempo restante da ciclagem erosiva, as amostras foram também armazenadas em solução supersaturada, até o início da experiência no dia seguinte. As soluções foram renovadas todos os dias, e o pH das soluções foi verificada no início e no final de cada dia. Este modelo de ciclagem erosiva foi proposto por Ganss et al. (2001) [52].

3.2.3 Irradiação das amostras

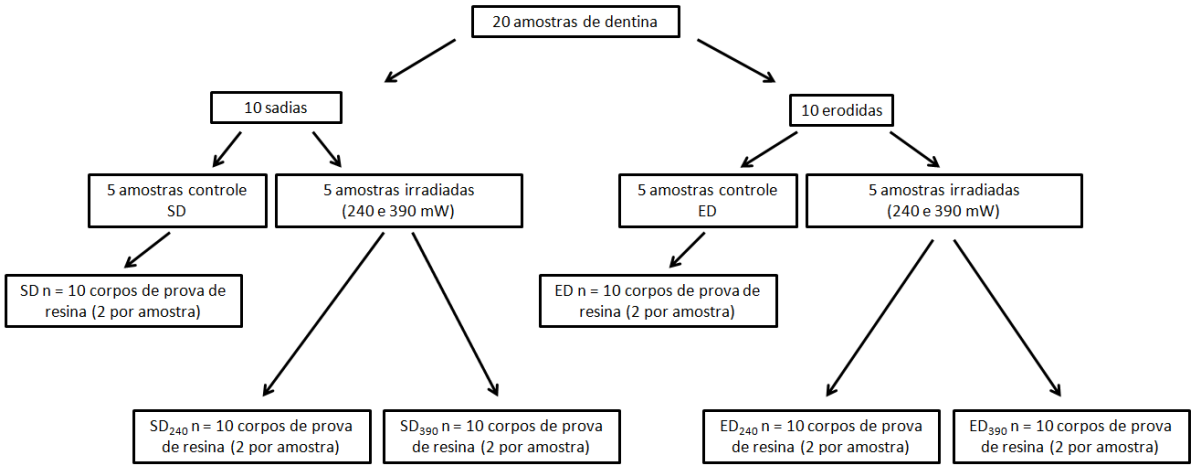

Figura 6 – Desenho esquemático da distribuição das amostras e grupos.

Para esta pesquisa foram utilizadas vinte amostras de dentina, sendo dez submetidas a um ensaio erosivo e dez mantidas sadias. Dentre as 10 amostras erodidas, foram separas cinco amostras para o grupo controle (SD) e cinco amostras foram irradiadas (condicionadas com LASER de Ti:Safira com 240 mW (SD_{240}) e com 390 mW (SD_{390}) em áreas distintas). Da mesma forma, dentre as amostras consideradas sadias, foram separas cinco amostras para o grupo controle (SD) e cinco amostras foram irradiadas (condicionadas com LASER de Ti:Safira com 240

mW (SD_{240}) e com 390 mW (SD_{390}) em áreas distintas). Formados os quatro conjuntos de amostras, foram separados seis grupos contendo dez corpos de prova de resina cada. Cada amostra de dentina dos grupos controle (SD) recebeu dois corpos de prova de resina, e cada amostra de dentina (sadia ou erodida) condicionada com LASER de Ti:Safira recebeu quatro corpos de prova, sendo dois por área de irradiação. Esta distribuição está resumida em um desenho esquemático (Figura 6).

A irradiação com LASER foi realizada utilizando um LASER Ti:Safira de 70 fs, Q-Switched e Mode-Locked (Libra®, Coherent, EUA). Duas potências de LASER foram utilizadas neste estudo (240 mW e 390 mW), produzindo duas energias de pulso diferentes (240 e 390 μJ). O LASER foi direcionado para a superfície usando uma lente objetiva (20x, 0,3 de abertura numérica), a fim de promover ablação mediada por plasma (Figura 7). As amostras foram conectadas a uma montagem XYZ com precisão micrométrica, conectada a um motor de passo (PM500-C, Newport, Irvine, Ca, EUA) controlado por uma rotina LabVIEW®. Para aumentar a largura das linhas de varredura, diminuir os danos térmicos e mecânicos e promover a remoção superficial da camada de esfregaço, realizou-se a varredura a LASER com as amostras deslocadas a 200 μm do foco na direção de incidência (eixo-Z), produzindo linhas com aproximadamente 90 μm de diâmetro. O suporte XYZ foi programado para se deslocar 2,5 cm na direção horizontal (eixo X) de modo a percorrer toda a amostra. Na direção vertical (eixo Y) foram produzidas 20 linhas, igualmente espaçadas de 90 μm, produzindo uma área de irradiação com aproximadamente 1,8 mm de diâmetro total (Figura 8). A velocidade de varrimento da amostra foi fixada em 30 mm/s. Estes parâmetros foram cuidadosamente testados em um estudo piloto anterior.

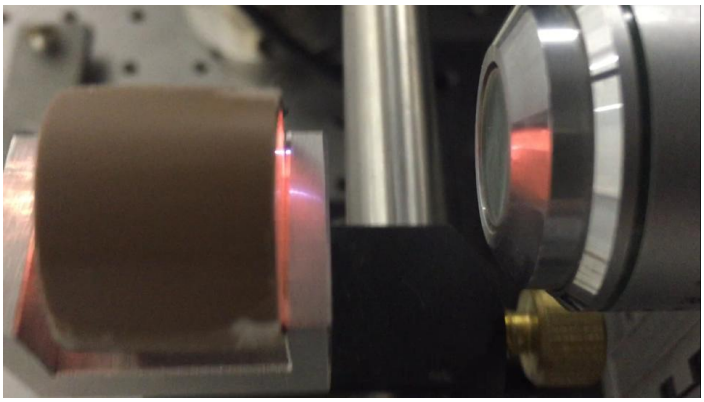

Figura 7 – Amostra sendo irradiada por LASER de Ti:Safira.

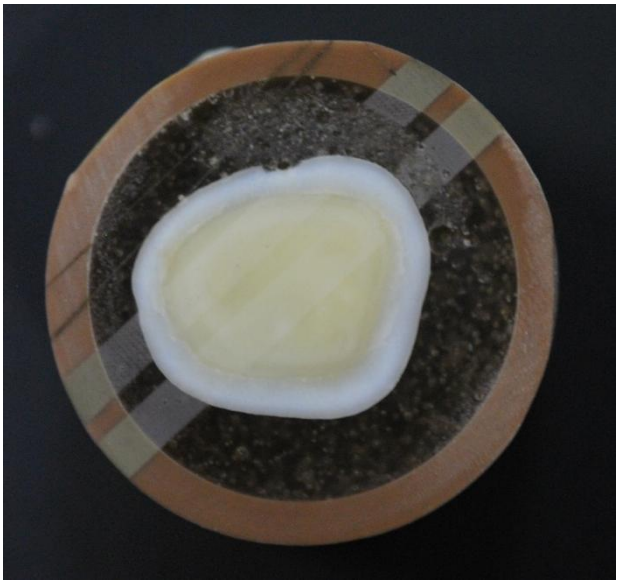

Figura 8 – Superfície da amostra irradiada pelo LASER de Ti:Safira.

3.2.4 Análise por Tomografia de Coerência Óptica (TCO)

A profundidade de condicionamento após a irradiação de cada amostra irradiada foi cuidadosamente analisada por meio de imagens 2D e 3D de um equipamento de Tomografia por Corência Óptica (TCO) no domínio espectral SD-OCT (Sistema Callisto Spectral Domain OCT, Thorlabs Inc, New Jersey, EUA) (Figura 9). Nesta montagem, a fonte de luz consiste num diodo superluminescente (SLD) com comprimento de onda central de 930 nm.

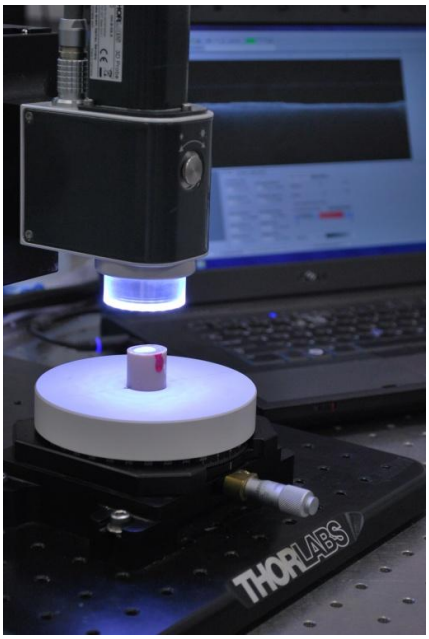

Figura 9 - Amostra posicionada em mesa de trabalho para análise da profundidade de condicionamento realizado pelo LASER através de Tomografia de coerência óptica.

Após a secagem dos espécimes, os mesmos serão posicionados na mesa de trabalho montada com três parafusos micrométricos (eixos X, Y e Z). Foram realizadas varreduras da superfície do espécime, obtendo-se imagens 2D (1024 x 512 pixels / 4 x 0,5 mm) da área irradiada/referência. Da mesma forma, foram obtidas imagens em 3D (1024 x 512 pixels / 3 X 3 X 0,5 mm) das superfícies (irradiada e referência). A discrepância entre a altura da área de referência e a superfície irradiada foi medida utilizando o programa ImageJ (JAVA, de domínio público).

3.2.5 Preparo dos Corpos de Prova

Uma área de 2 mm de diâmetro foi demarcada com fita adesiva dupla face. O sistema adesivo foi aplicado por um pincel (Microbrush, KG, Cotia, São Paulo, Brasil) de acordo com instruções do fabricante. Em seguida, o tubo transparente de polietileno (Ø = 0,9 mm, h = 1 mm) foi posicionado sobre a área e, logo após, o adesivo foi polimerizado por 20 segundos com o LED Radii-cal (SDI, Bayswater, Victoria, Australia), emitindo luz na faixa de intensidade média de 1200 mW/cm^2, segundo o fabricante, e conferido com radiômetro de cura analógico de alta precisão.

A resina composta Tetric N Ceram A3 (Ivoclar vivadent, Schaan, Liechtenstein) foi inserida com espátula não aderente (Duflex) polimerizando o incremento por 20 segundos. Os espécimes foram mantidos em água destilada durante vinte e quatro horas a 37 ºC. O tubo e a fita dupla face foram cuidadosamente removidos com uma lâmina de bisturi 15C, expondo os cilindros de resina composta. As amostras foram examinadas ao microscópio estereoscópico (ampliação x10) para observar possíveis defeitos.

3.2.6 Ensaio de microcisalhamento

Os espécimes foram testados em uma máquina de ensaios universal (EMIC, DL 10000, São José dos Pinhais-PR, Brasil). Cada amostra contendo os corpos de prova foi adaptada à máquina e submetidas a um carregamento de cisalhamento feito através de um fio ortodôntico de 0,2 mm de diâmetro, a uma velocidade de 0,5 mm/min até o rompimento da união (Figura 10). Os valores de

resistência de união foram registrados por um computador, usando o software Tesc 3,04 (EMIC, São José dos Pinhais-PR, Brasil).

Figura 10 – Corpos de prova em máquina de ensaios universal.

3.2.7 Análise do padrão de fratura

A análise do padrão de fratura foi realizada com um microscópio óptico em aumentos de 5 x, 10 x, 20 x e 50 x (Olympus BX51, Tóquio, Japão). Imagens 2D e 3D de Tomografia de Coerência Óptica (TCO) foram realizadas para confirmar os resultados. Após a secagem dos espécimes, os mesmos foram posicionados na mesa de trabalho montada com três parafusos micrométricos (eixos X, Y e Z). Foram realizadas varreduras da superfície do espécime, obtendo-se imagens 2D (1024 x 512 pixels / 4 x 0,5 mm) da área irradiada/referência. Da mesma forma, foram obtidas imagens em 3D (1024 x 512 pixels / 3 X 3 X 0,5 mm) da superfície adesiva. Os tipos de falha foram classificadas em três categorias, com base no percentual de material livre de substrato: Adesiva (falha da interface resina / esmalte); Coesiva (exclusivamente no esmalte ou resina composta); Mista (falha na interface resina-esmalte que incluem falha coesiva dos substratos vizinhos).

3.2.8 Microscopia Eletrônica de Varredura (MEV)

Fotomicrografias de áreas representativas das superfícies irradiadas foram obtidas. Para esta avaliação, foram preparadas duas amostras adicionais para

cada grupo. As amostras foram revestidas com ouro e analisadas por MEV (Mira 3, Tescan, Cambridge, UK) a 10 kV com 5000 x.

3.2.9 Microscopia de Força Atômica

Uma amostra de cada grupo foi analisada utilizando microscopia de força atômica (AFM, Alpha 300 RA, WITec, Ulm, Alemanha). A morfologia da superfície do bloco foi através varredura em modo contato com o auxílio de um cantilever com 0,2 N / m. Imagens 30 x 30 µm com resolução de 256 x 256 pixels e ponto de operação de 0,5 V foram coletadas a uma taxa de varredura muito baixa para obter detalhes da estrutura da dentina e para evitar danificar a ponta.

3.2.10 Análise estatística

Os dados foram organizados em uma planilha do Excel (Microsoft Office 2007) e analisados usando o SPSS 13.0 (Statistical Package for the Social Sciences, Chicago, IL, EUA) para Windows. Foram obtidas estatísticas descritivas e o teste de Kruskal Wallis foi realizado para comparar os resultados entre os grupos. Em caso de diferenças significativas, o teste de Mann Whitney foi realizado para comparação entre pares ($p = 0,05$).

ARTIGO 1

Estudo-piloto do uso do LASER de Er,Cr:YSGG
para condicionamento do esmalte e seu efeito na adesão.
Publicado no *Proceedings of SPIE* (qualis B3).

Evaluation of Microshear Bond Strength of Resin Composites to Enamel of Dental Adhesive Systems Associated with Er,Cr:YSGG Laser

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ABSTRACT

The aim of this in vitro study was to evaluate the microshear bond strength (μSBS) of resin composite to enamel etching by Er,Cr:YSGG laser with the use of two differents adhesives systems. Fifty freshly extracted human molars halves were embedded in acrylic resin before preparation for the study, making a total of up to 100 available samples. The specimens were randomly assigned into six groups (n=10) according to substrate pre-treatment and adhesive system on the enamel. A two-step self-etching primer system (Clearfil SE Bond) and a universal adhesive used as an etch-and-rinse adhesive (Adper Single Bond Universal) were applied to the nonirradiated enamel surface according to manufacturer's instructions, as control groups (Control CF and Control SB, respectively). For the other groups, enamel surfaces were previously irradiated with the Er,Cr:YSGG laser with 0.5 W, 75 mJ and 66 J/cm^2 (CF 5 Hz and SB 5 Hz) and 1.25 W, 50 mJ and 44 J/cm^2 (CF 15 Hz and SB 15 Hz). Irradiation was performed under air (50%) and water (50%) cooling. An independent *t*-test was performed to compare the adhesive systems. Mean μSBS ± sd (MPa) for each group was 16.857 ± 2.61, 17.87 ± 5.83, 12.23 ± 2.02, 9.88 ± 2.26, 15.94 ± 1.98, 17.62 ± 2.10, respectively. The control groups and the 50 mJ laser groups showed no statistically significant differences, regardless of the adhesive system used. The results obtained lead us to affirm that the bonding interaction of adhesives to enamel depends not only on the morphological aspects of the dental surface, but also on the characteristics of the adhesive employed and the parameters of the laser.

Keywords: Microshear bond strength. Er,Cr:YSGG laser. Adhesive system.

1. INTRODUCTION

Since the introduction of the acid etching technique by Buonocore in 1955, a standard protocol to remove the smear layer for successful bonding, different etching methods have been proposed to foment adhesion of composite resins to the dental structure. The self-etching primer systems, which need not necessarily previous smear layer removal, were introduced to simplifying adhesive procedures [1]. Self-etch systems are in turn divided in two-step self-etch and one-step self-etch (all-in-one) adhesives. In the two-step self-etch adhesives, the conditioning agent and the primer are placed in one bottle and the adhesive resin in another, whereas onestep adhesives combine self-etch primer and hydrophobic resin into one application, which results in simplification of the bonding procedure [2]. These systems simultaneously promote demineralization and resin infiltration through the demineralized dental surfaces.

Both types of self-etching systems have been reported to produce predictable bond strengths to dentin. However, controversial and unpredictable bonding values to enamel has been reported, with limited evidence suitability for replacing conventional phosphoric acid etching [3]. It was observed a reduction of enamel bonding effectiveness when universal adhesives were applied on enamel as self-etching adhesives [4]. Nevertheless, with the use of selective phosphoric acid etching, the performance of a one-step self-etch adhesives was significantly improved [5].

High power lasers have been introduced as an alternative to promote chemical/morphological changes on the tooth surface. Laser irradiation produces enamel and dentin surfaces that are free of smear layer, imbricate pattern, and crack formation. Erbium lasers (Er:YAG and Er,Cr:YSGG) have been considered the most promising lasers to be used on mineralized tissues because both wavelengths show high absorption by water and

hydroxyapatite. Erbium lasers are able to cause thermomechanical ablation process on hard tissues, like the etching process, forming a rough structure in dental tissue [6]. The use of both laser and acid together has been reported to enhance the strength of bonding to hard tooth surfaces relative to those exposed to acid alone [7]. Meanwhile, there are not well-designed studies comparing acid etching followed by laser ablation or laser ablation followed by acid etching.

The aim of this in vitro study was to evaluate the microshear bond strength (μSBS) of resin composite to enamel etching by Er,Cr:YSGG laser with the use of two differents adhesives systems.

2. MATERIALS AND METHODS

2.1 Specimen preparation

A total of 50 extracted human molars were visually examined to confirm the absence of physical damage such as deep grooves or cracks. The specimens were cleaned with distilled water and brushes, and then disinfected in 0.5% chloramine for 2 weeks. The roots were sectioned 2 mm from the enamel–cementum junction, and the crowns were divided into halves with a slow-speed diamond saw in a sectioning machine. To facilitate handling of the specimens throughout the experiment, each half was embedded in acrylic resin. The outer surface of the enamel specimens was then ground flat with water-cooled sandpaper of decreasing grit (400, 600) in order to produce a clinical relevant and standardize smear layer.

2.2 Experimental Design

The enamel specimens (n=10) were randomly assigned into six groups acording to etching treatment and adhesive system, as summarized in Table 1. The control groups were submitted to polishing only and the enamel surfaces were etching with phosphoric acid and treated with: Control CF - a two-step self-etching primer system (Clearfil SE Bond, Kuraray Medical Inc., Kurashiki, Japan) and Control SB - a universal adhesive used as a two-step etch-and-rinse adhesive (Adper Single Bond Universal, 3M ESPE, St. Paul, MN).

For the other groups, enamel surfaces were irradiated with the Er,Cr:YSGG laser (Waterlase Millenium, Biolase, San Clemente, CA) that works at 2780 nm. The tip was positioned at 1mm (90º) from the enamel surface. In Groups CF 5 Hz and SB 5 Hz the output power was 0.5 W with 75 mJ and 66 J/cm^2. For Groups CF 15 Hz and SB 15 Hz the output Power was 1,25 W with 50 mJ and 44 J/cm^2. Irradiation was performed under air (50%) and water (50%) cooling for all groups. The same adhesives were applied on Er,Cr:YSGG laser-irradiated enamels.

Table 1 – Groups and etching protocols.

Group	Adhesive system	Etching protocol
Control CF	Clearfil SE Bond	Phosphoric acid (according to the manufacturer's instructions)
Control SB	Adper Single Bond Universal	Phosphoric acid (according to the manufacturer's instructions)
CF 5 Hz	Clearfil SE Bond	Er, Cr:YSGG - 5 Hz, 0.5 W, 66 J/cm^2
SB 5 Hz	Adper Single Bond Universal	Er, Cr:YSGG - 5Hz, 0.5 W, 66 J/cm^2
CF 15 Hz	Clearfil SE Bond	Er, Cr:YSGG - 15 Hz, 1.25 W, 44 j/cm^2
SB 15 Hz	Adper Single Bond Universal	Er, Cr:YSGG - 15 Hz, 1.25 W, 44 j/cm^2

2.3 Restorative Procedure

The tested adhesive systems were applied to the enamel surfaces according to manufacturer's instructions. Transparent polyethylene tubes with 0.9 mm for the internal diameter and 1 mm in heigth were positioned over a double-faced perfurated tapes and then resin composite (Tetric N-Ceram,) was carefully packed inside the tubes. The resin composite was light-cured for 20 s using a LED light-curing with 1200 mW/cm^2 (Radii-cal, SDI, Bayswater, Victoria, Australia).

The specimens were kept in distilled water for one hour at 37 ºC and then the tubes and the double-faced tapes were removed with a blade, exposing the cylinders of resin composite. Right after, the specimens

were storaged in distilled water for a week at 37 ºC. All specimens were examined under a stereomicroscope at x10 magnification to observe possible defects.

2.4 Microshear Bond Strength Test

The specimens were tested in a universal testing machine (Kratos, Kratos Equipamentos Industriais Ltda, Cotia, SP, Brazil). A thin orthodontic wire (0.2 mm) was looped around the cylinder aligned with the setup to ensure the correct orientation of the forces. The crosshead speed was set at 0.5 mm/min until failure.

2.5 Statistical analysis

Data were organized into an Excel spreadsheet (Microsoft Office 2007) and analyzed using SPSS 13.0 (Statistical Package for the Social Sciences, Chicago, IL, USA) for Windows. Statistical measures were obtained and the Komogorov-Smirnov test was used to assess the normality of the data. An independent *t*-test was performed to compare the adhesive systems. All tests were applied with 95% confidence.

3. RESULTS

Figure 1 shows the box plots of microshear bond strength, displaying the interquartile range and the standard deviation. The small square inside the boxs represent the median, the parallel line inside the boxes represents the mean. Table 2 shows the mean shear bond strengths values (in MPa) of two-step self-etch (Clearfil SE Bond) and one-step self-etch (all-in-one) (Adper Single Bond Universal) adhesive bonded to enamel. Microshear bond strength values obtained for control groups on enamel were in average of 16.857 ± 2.61 MPa for Control CF and 11.875 ± 5.83 MPa for Control SB. The maximum bond strength was recorded for the SB 15 Hz group and the minimum bond strength was recorded for the SB 5 Hz group.

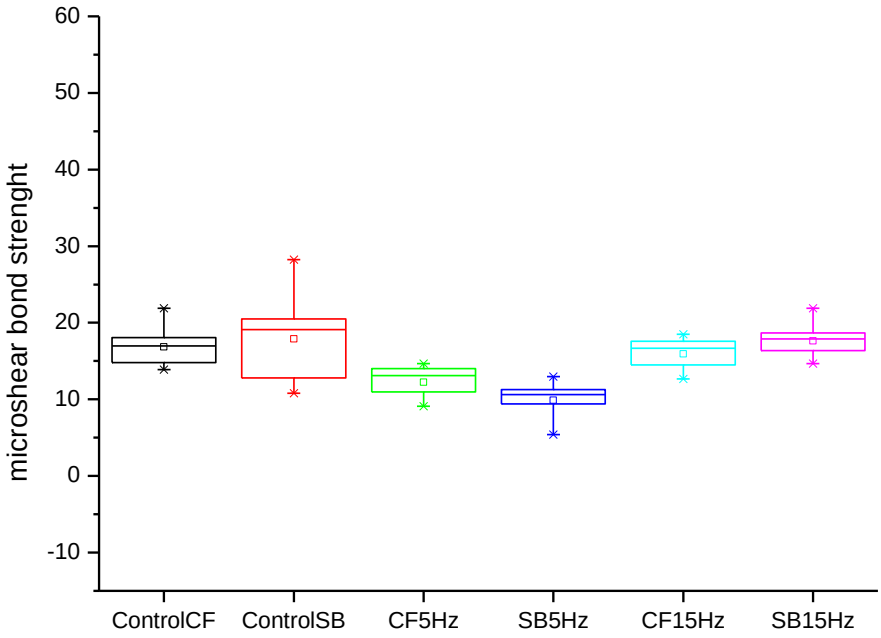

Figure 1 - Box plots of microshear bond strength display the interquartile range and the standard deviation.. The small square inside the boxs represent the median, the parallel line inside the boxes represents the mean.

Table 2 - Microshear bond strengths (MPa) and standard deviation (± sd) of the tested adhesives to enamel. Different superscript letters indicate statistical differences ($p < 0.05$)

	Clearfil SE Bond	*Adper Single Bond Universal*
Control	16.857 ± 2.61^{a}	11.875 ± 5.83^{b}
5 Hz	12.230 ± 2.02^{d}	9.886 ± 2.26^{e}
15 Hz	$15.947 \pm 1.98^{a,c}$	$17.628 \pm 2.10^{b,c}$

An independent *t*-test was performed to compare the effect of Clearfil SE Bond and Adper Single Bond Universal adhesive systems on Bond strength. The results of t-Test showed no statistical differences between the control groups ($p = 0.62095$). Comparison of mean values of μSBS of the groups ($n = 10$) is shown in Table 2.

4. DISCUSSION

The bond strength of adhesive systems is one of the main factors to be considered in composite resin restorations. For years the standard protocol to remove the smear layer for successful bonding has been acid etching, that appears to improve retention by selectively eroding certain hydroxyapatite formations, characterized by surface irregularities and demineralization areas, facilitating penetration by the development of resin tags [8].

New bonding agents have been introduced to the market as self-etch primer and single-bottle systems to simplifying adhesive procedures. These systems simultaneously promote demineralization and resin infiltration through the demineralized dental surfaces. However, a reduction of enamel bonding effectiveness when universal adhesives were applied on enamel as self-etch adhesives has been recently reported [9]. Their etching capacity has been shown to be more restricted, as they present low reactivity with the mineral component, lower availability of H^+ ions, and high molecular weight, compared with phosphoric acid, promoting etching that is not as deep and retentive on dental enamel [10]. Therefore, the use of selective phosphoric acid etching has been recommended. Meantime, because of the demineralization areas acid-induced, enamel becomes more susceptible to caries attack [11].

Laser etching has become an alternative to acid etching of enamel. Laser can promote physical changes such as melting and recrystallization in the enamel [12]. Laser irradiation laser ablation are able to eject hard tissue particles, resulting in a typical imbricate patterned surface with an evidently rough aspect without smearlayer [13]. These morphological features seem to be enough for adhesion, since the interaction of self-etch adhesives with dental substrates is expected to be better in the absence of smear debris [14].

Laser irradiation of the enamel modifies the calcium-phosphate ratio and leads to the formation of more stable and less acid-soluble compounds [11]. Increased resistance to acid etching of Er:YAG-irradiated enamel has been reported [15]. However, a more mineralized, acid resistant enamel surface may resist etching by the weak acid of a self-etching primer adhesive [16], which would be a limitation. Besides that, on enamel, surface roughness may influence bond strengths for self-etching primer adhesives [17]. Variability in bonding effectiveness of this group of adhesives may be attributed in part to their pH and etching aggressiveness on the enamel and dentin substrate and to the particular enamel-dentin adhesive used [18].

Conflicting reports have described the effectiveness of resin bonding following laser tooth preparation. Some studies have found significant lowering of enamel bond strengths with a two-step adhesive, a one-step self-etching primer adhesive, and a total-etch adhesive following Er,Cr:YSGG [12]. Part of the results of our study confirmed these findings, since the use of Er,Cr:YAGG laser with 5 Hz, 0.5 W, 75 mJ and 66 J/cm2 reduced the bond strength to enamel, regardless of the adhesive system used. The occurrence of thermal alterations on the irradiated enamel, such as melting and chemical changes, could render the enamel less receptive for adhesion according Cardoso et al. (2008) [12]. Another reported explanation is that Er,Cr:YSGG-laser irradiation can reduce the acid dissolution of dental hard tissues that could hamper the acid conditioning of enamel, especially considering the limited etching potential of "mild" self-etch systems [19].

Differently, Basaran et al. (2007) [8] found that bond strength obtained with Er,Cr:YSGG laser (operated at 1 W or 2 W for 15 seconds) was comparable to that obtained with acid etching. In our study, when the laser parameters 15 Hz, 1.25 W, 50 mJ and 44 J/cm^2 were used, the results of bond strength were similar to those in the control groups. According to Hossain et al. (2003) [20] laser-irradiated enamel shows a significant increase in calcium in its composition. The 10-MDP molecule, present in the used adhesives, can partially

decalcify dental hard tissues, penetrate the demineralized substrate and chemically bond to the remaining calcium [19], which could explain these results.

5. CONCLUSIONS

The results obtained lead us to affirm that the bonding interaction of adhesives to enamel depends not only on the morphological aspects of the dental surface, but also on the characteristics of the adhesive employed and the parameters of the laser. The highest value obtained for the Microshear bond strengths was 17.628 ± 2.10 Mpa, using Adper Single Bond Universal with the laser operating at 15Hz, 1.25 W, 44 j/cm^2.

6. ACKNOWLEDGEMENTS

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7. CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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ARTIGO II

Trabalho completo sobre o uso do LASER de Er,Cr:YSGG para condicionamento do esmalte e seu efeito na adesão.
Submetido à revista *Lasers in Medical Science* (qualis A2).

Effects of enamel etching by Er,Cr:YSGG Laser on microshear bond strength

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ABSTRACT

Purpose: The aim of this *in vitro* study was to evaluate enamel microshear bond strength (μSBS) after Er,Cr:YSGG laser etching followed by an acidic primer (Clearfil SE Bond), and an universal bonding agent (Single Bond Universal).

Methods: Fifty freshly extracted human molars were used for the study. Crowns were seccioned in mesio-distal direction with a low-speed diamond disc under refrigeration. Each slice was embedded in acrylic resin through the dentinal portion. Exposed enamel was then ground flat with water-cooled sandpaper of decreasing grit (#400, 600) in order to produce a clinically relevant and standardized smear layer. The enamel specimens were randomly assigned into six groups (n=15). Enamel surfaces were irradiated with the Er,Cr:YSGG laser at 5 Hz, 0.5 W, 66 J/cm^2 (CSE_{66J/cm^2} and SBU_{66J/cm^2}) and at 15 Hz, 1.25 W, 44 J/cm^2 (CSE_{44J/cm^2} and SBU_{44J/cm^2}). Irradiation was performed under air and water-cooling (50/50%). Control groups consisted of non-irradiated specimens etched with 37% phosphoric acid for 15s according to manufacturer's instructions (CSE and SBU). Data were statistically analyzed using Kruskal Wallis and Mann Whitney test (p=0.05).

Results: There was no statistically significant difference between the control group and the treated group with 44J/cm^2. SEM analysis revealed significant morphological alterations of the laser-irradiated enamel surface, showing areas of melted and recrystalized hydroxyapatite in the 66 J/cm^2 groups.

Conclusion: The use of laser with 15 Hz (44J/cm^2) was statistically as effective as the use of phosporic acid and did not appear to influence the self-etching adhesive action.

Keywords:
Enamel bonding
Adhesion
Self-etch
Universal Adhesives
Er,Cr:YSGG Laser

INTRODUCTION

The current adhesive systems have gone through several changes in an attempt to ensure technique simplification, without decreasing bonding effectiveness [1, 2]. Self-etching adhesive systems (SE) are able to simultaneously promote demineralization and resin infiltration through the demineralized dental surfaces, due to acidic monomers present in the composition [3]. The self-etch adhesives are divided in two-step, when the conditioning agent and the primer are placed in one bottle separated of the adhesive resin; and one-step (all-in-one) adhesives that combine self-etch primer and hydrophobic resin into one single bottle, which results in simplification of the bonding procedure [4].

Self-etching systems have been widely accepted with predictable bond strengths to dentin. However, controversial and unforeseeable bonding values to enamel has been reported [5, 6]. Bonding to the enamel is based on a micromechanical binding resin monomers into microporosities in the enamel created by chemical dissolution of hydroxyapatite using phosphoric acid [2]. The depth of the etching pattern plays an important role in the final quality of the adhesion [7]. Meanwhile, self-etch adhesives do not etch enamel to the same depth as phosporic acid does. Shallower demineralization pattern produced by mild acidity of self-etch primers may be the reason for their weakness in enamel adhesion, making its use to raise some concerns [6]. Because of that, selective etching of enamel has been recommended prior to the application of self-etch adhesives [8].

In the past years, a new group of dental adhesives known as Universal or Multimode were introduced into the market. These adhesives are primarily one-step SE adhesive that may be used as a conventional etch and rinse system [9]. Reduced enamel bonding effectiveness is observed when universal adhesives are applied on enamel as a self-etching agent [2]. Therefore, prior enamel selective phosphoric acid etching is recommended [10].

High power lasers have been used as an alternative to promote chemical/morphological changes on the tooth surface. Erbium-doped lasers (Er:YAG and Er,Cr:YSGG) have been considered the most promising photonic sources to be used on mineralized tissues because their wavelengths (2940 nm and 2780 nm, respectively) show high absorption by water and hydroxyapatite [11]. Laser produces micro-explosions during ablation that are able to eject hard tissue particles, promoting a typical rough patterned surface without smear layer production [12]. This aspect is quite favorable for adhesion, especially when it comes to self-etch adhesives that is better in the absence of debris [13]. However, it has been reported that laser modifies the calcium-to-phosphate ratio and reduces the carbonate-to-phosphate ratio as well. The surface etched by laser is more mineralized, stable and acid resistant [14] and may resist etching by the weak acid of a self-etching primer adhesives [15].

The aim of this *in vitro* study was to evaluate the microshear bond strength (μSBS) to enamel etched with Er,Cr:YSGG laser with a two-step self-etching primer adhesive and one universal adhesive system. The null hypothesis tested was that there is no difference between the bonding agents tested for bond strength when used in enamel irradiated by the Er,Cr:YSGG laser, regardless of the parameter used.

METHODS

This study was approved by the ethics committee under the protocol #1.735.580.

Specimen preparation

Thirty sound extracted human molars were selected for this study. Teeth were visually examined to confirm the absence of physical damage such as deep grooves or cracks. The specimens were cleaned with distilled water and brushes with Robinson brushes, and then

disinfected in 0.5% chloramine for 2 weeks. The roots were sectioned 2 mm from the enamel–cementum junction, and the crowns were divided into halves with a slow-speed diamond saw in a sectioning machine under refrigeration. To facilitate handling of the specimens throughout the experiment, each half was embedded in acrylic resin. The outer surface of the enamel specimens was then ground flat with water-cooled sandpaper of decreasing grit (400, 600) in order to produce a clinical relevant and standardized smear layer.

Experimental Design

The enamel specimens were randomly assigned into six groups (n=15) acording to etching treatment and adhesive system. Two bonding agents were used in this study (Table 1).

Table 1 – Groups and etching protocols.

Bonding agent #Batch n.	**Composition**	**Group**	**Etching protocol**
Clearfil SE Bond [1] #051552	Two-step self-etching primer system. Primer: 10-Methacryloyloxydecyl dihydrogen phosphate (MDP), 2-Hydroxyethyl methacrylate (HEMA), Hydrophilic aliphatic dimethacrylate, dl-Camphorquinone, N,N-Diethanol-p-toluidine, Water. Bond: 10-Methacryloyloxydecyl dihydrogen phosphate (MDP), Bisphenol A diglycidylmethacrylate (Bis-GMA), 2-Hydroxyethyl methacrylate (HEMA), Hydrophilic aliphatic dimethacrylate, dl-Camphorquinone, N,N-Diethanol-p-toluidine, Colloidal silica.	CSE	Enamel etching with 37% phosphoric acid for 15 sec, wash with water, and then dry.
		CSE_{66J/cm^2}	Etching with Er, Cr:YSGG laser at 5 Hz, 0.5 W, 66 J/cm^2 under water/air cooling.
		CSE_{44J/cm^2}	Etching with Er, Cr:YSGG laser at 15 Hz, 1.25 W, 44 J/cm2 under water/air cooling.
Single Bond Universal [2] #1435300232	Used as all-in-one self-etching adhesive. MDP-Phosphate Monomer, Dimethacrylate resins, HEMA, Vitrebond^{TM} Copolymer, Filler, Ethanol, Water, Initiators, Silane.	SBU	Enamel etching with 37% phosphoric acid for 15 sec, wash with water, and then dry.
		SBU_{66J/cm^2}	Etching with Er, Cr:YSGG laser at 5 Hz, 0.5 W, 66 J/cm^2 under water/air cooling.
		SBU_{44J/cm^2}	Etching with Er, Cr:YSGG laser at 15 Hz, 1.25 W, 44 J/cm2 under water/air cooling.

[1] Kuraray Noritake Dental Inc., Kurashiki, Okayama, Japan; [2] 3M Deutschland GmbH, Seefeld, Germany

Laser irradiation was performed with the Er,Cr:YSGG laser (Waterlase Millenium, Biolase, San Clemente, CA) that works at 2780 nm. The laser tip was positioned at 1mm (90º)

from the enamel surface under air (50%) and water (50%) cooling for all groups. With the aid of a step motor (ESP300, Newport, Irvine, CA, USA) the samples were irradiated in such a way that there was no overlap of pulses. For this, a rate of 1.9 mm/s was used for samples irradiated at the frequency of 5 Hz and of 5.7 mm/s for samples irradiated with 15 Hz.

Restorative Procedure

Bonding agents were applied to the enamel surfaces according to manufacturer's instructions. Transparent polyethylene tubes (Ø=0.9 mm, h=1 mm) were positioned over double-faced perforated tapes. Resin composite (Tetric N-Ceram A3, Ivoclar Vivadent, Schaan, Liechtenstein) was carefully packed inside the tubes and light-cured for 20 s using a LED light-curing with 1200 mW/cm^2 (Radii-cal, SDI, Bayswater, Victoria, Australia).

Specimens were kept in distilled water for one hour at 37 ºC (± 1). The tube and the double-faced tape was carefully removed with a blade n. 15, exposing the resin composite cylinders. Specimens were water stored (37 ^{o}C ± 1) for another 7 days. After this period, specimens were examined under a stereomicroscope (X 10) to observe possible defects.

Microshear Bond Strength Test

The specimens were tested in a universal testing machine (Kratos Equipamentos Industriais Ltda, Cotia, SP, Brazil). A thin orthodontic wire (0.2 mm) was looped around the cylinder aligned with the setup to ensure the correct orientation of the applied forces. The crosshead speed was set at 0.5 mm/min until failure.

Failure Mode Examination

Failure mode analysis was performed with an optical microscope at X 5, X 10, X 20 and X 50 magnification (Olympus BX51, Tokyo, Japan). Further images were obtained using Optical Coherence Tomography (OCT) to confirm and illustrate the microscopy results. OCT is an imaging diagnostic modality widely used in several medical and non-medical areas,

including dentistry [16]. The method is based on an interferometric technique exploiting the low coherence of broadband optical sources, which when associated with a Michelson interferometer and a detection system forms the basic setup.

Samples from each group were carefully analyzed through 2D images and 3D images over the entire area (2 X 2 mm) of each sample using a spectral domain SD-OCT (Callisto Spectral Domain OCT System, Thorlabs Inc, New Jersey, USA). This type of OCT incorporates a broadband light source with a high-speed spectrometer to provide depth profiles that can be added to cross-sectional images, which can then be used for tridimensional reconstructions.

The light source used is a SLD (Super Luminescent Diode) with central wavelength of 930 nm, spectral bandwidth of 100 nm and the maximum output power is 5 mW. Images generated by this system present axial resolution of 7/5.3 μm (air/water), lateral resolution of 8 μm and maximum imaging depth at 1.3 mm. The axial scan rate of the system is 1.2 kHz, capturing two frames per second with 105 dB of sensitivity.

The failure modes were classified into three categories, based on the percentage of substrate-free material in: Adhesive (failure at the resin/enamel interface); Cohesive (failure exclusively within enamel or resin composite); Mixed (failure at the resin-enamel interface that include cohesive failure of the neighboring substrates) [20].

Scanning electron microscopy

Photomicrographs of representative areas of the irradiatiated surfaces were evaluated under SEM. For this assessment, two additional samples were prepared for each group. Specimens were sputter-coated with gold and analyzed under SEM (Mira 3, Tescan, Cambridge, UK) at 5 kV with x 100 and x 2000.

Statistical analysis

Data were organized into an Excel spreadsheet (Microsoft Office 2007) and analyzed using SPSS 13.0 (Statistical Package for the Social Sciences, Chicago, IL, USA) for Windows. Descriptive statistics were obtained and the Kruskal Wallis test was performed to compare results between groups. In case of significant differences, Mann Whitney test was performed for pairwise comparison between groups ($p = 0.05$).

RESULTS

The results for the μSBS are shown on Table 2. The maximum bond strength was recorded for the SBU control (19.916 MPa) and the minimum for the SBU_{5Hz} (9.223 MPa). Kruskal Wallis revealed significant differences between groups ($p<0.01$). The Mann Whitney test demonstrated differences between 66 J/cm^{2}groups and all the other groups.

Table 2 – Mean μSBS (MPa) and standard deviation ( ) of the tested adhesives according to the type of enamel pre treatment. Different superscript letters indicate statistical differences ($p < 0.05$)

	μSBS
CSE	17.788 ± 4.07 ^{A}
CSE_{66J/cm^2}	11.752 ± 2.81 ^{B}
CSE_{44J/cm^2}	15.629 ± 2.85 ^{A}
SBU	19.916 ± 6.68 ^{A}
SBU_{66J/cm^2}	9.223 ± 3.08 ^{B}
SBU_{44J/cm^2}	17.613 ± 3.61 ^{A}

Representative SEM images of the etching areas are shown in Fig. 1 and Fig. 2. The higher energy density laser protocol (5Hz, 0.5 W, 66 J/cm^2) showed significant morphological alterations of the laser-irradiated enamel surface, with areas of melted and recrystalized hydroxyapatite (Fig. 1a). On the other hand, the lower incident energy density (15 Hz, 1.25 W, 44 mJ/cm^2) showed a much smoother profile (Fig. 1b).

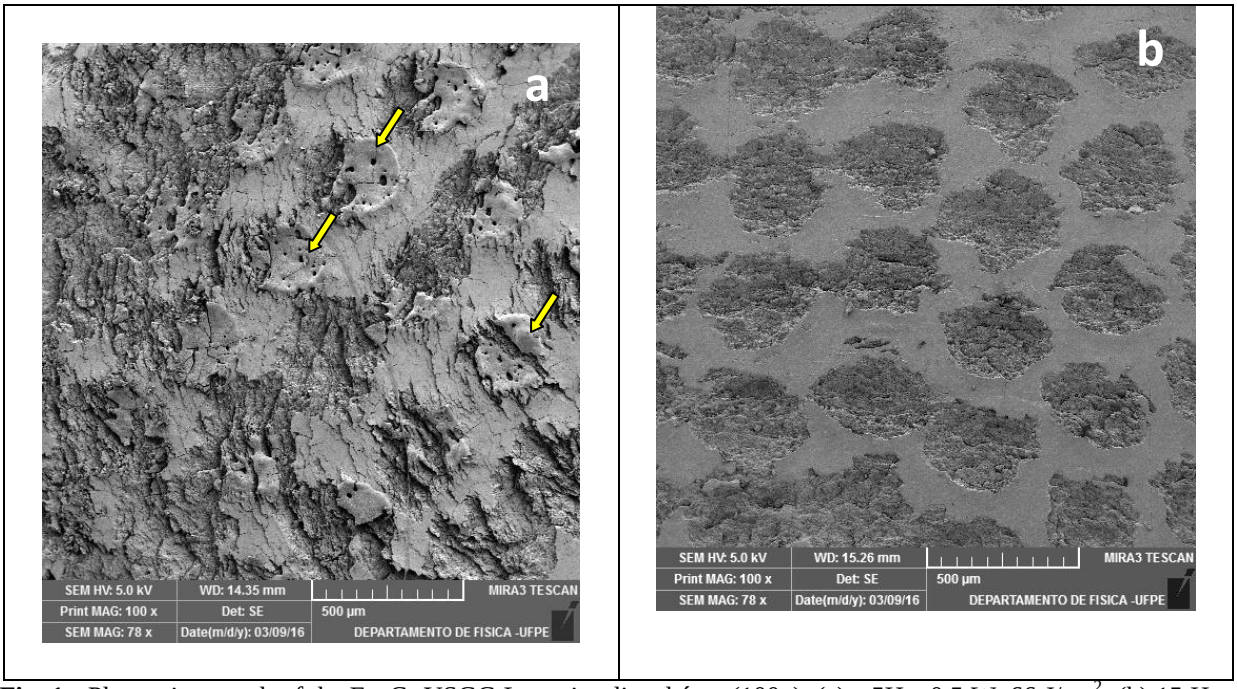

Fig. 1 - Photomicrograph of the Er, Cr:YSGG Laser irradiated área (100x). (a) - 5Hz, 0.5 W, 66 J/cm^2; (b) 15 Hz, 1.25 W, 44 J/cm^2. Arrows indicate areas of melted and recrystalized hydroxyapatite.

The etching pattern was also evaluated according to the regular rough surface and spaces described by Silverstone et al. [17]. A type I acid-etched pattern was observed in the control group, as observed the honeycomb image aspect because of dissolved central area of the enamel prisms (Fig. 2a). A 66mJ/cm^2 irradiation produced a pattern similar to type II, with a pebble or cobblestone appearance image (Fig. 2b). However, a 44mJ/cm^2 irradiation mainly produced a type III etching pattern, a mixture of type 1 and type 2 images (Fig. 2c). Cracks were observed in the irradiated enamel structure (arrows Fig. 2b, Fig. 2c).

Failure modes were analyzed initially comparing the microscopy image with OCT images (Fig. 3). Similar features can be readily seen. Fig. 4 shows 2D and 3D OCT images from samples used to analyze the fracture modes.

Following the failure mode examination described before, the percentage of samples in the three assigned modes are presented in Table 3. It was observed a decrease in the number of adhesive fractures in the irradiated groups when compared to the control group. These results are discussed in the following sections.

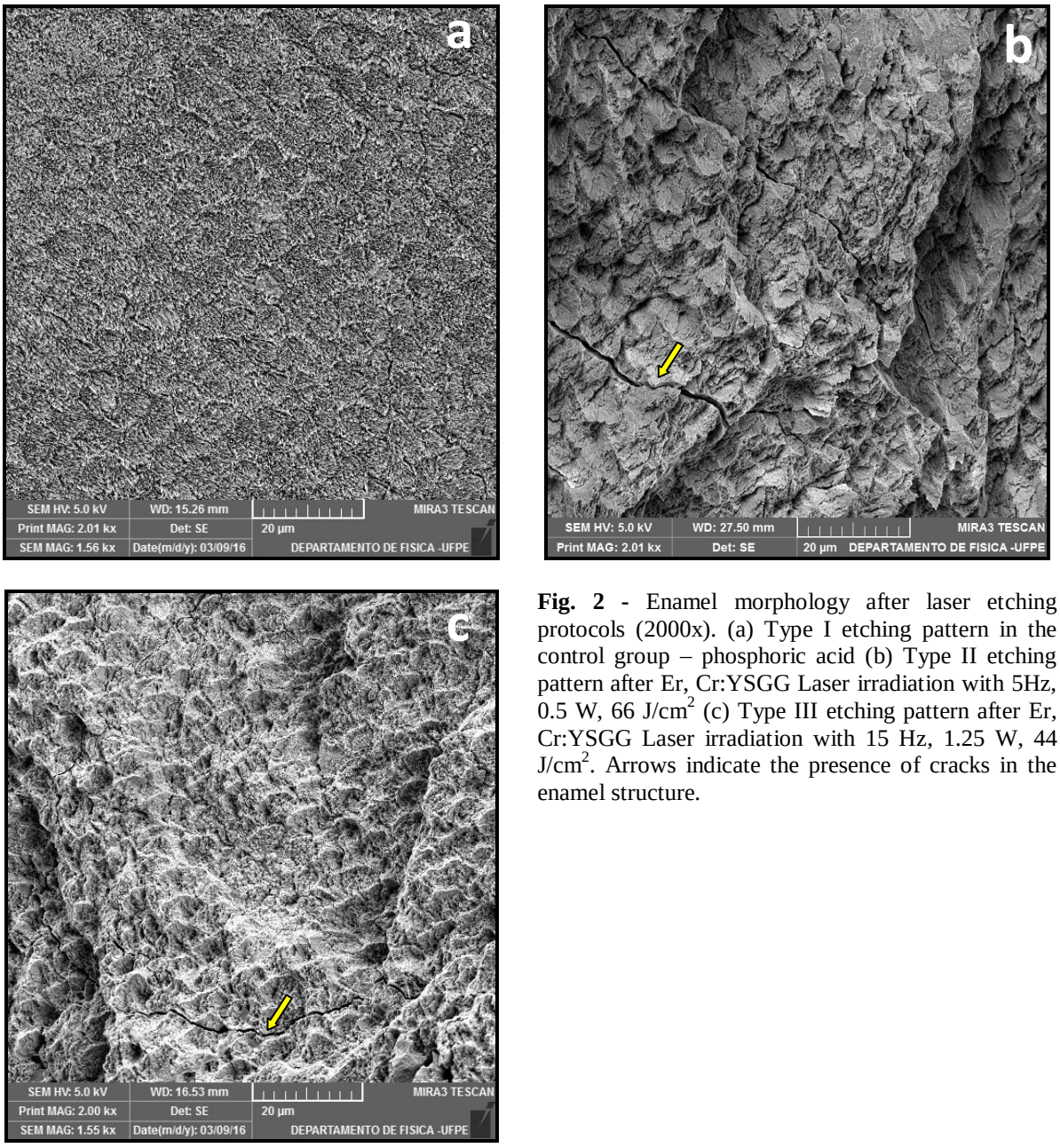

Fig. 2 - Enamel morphology after laser etching protocols (2000x). (a) Type I etching pattern in the control group – phosphoric acid (b) Type II etching pattern after Er, Cr:YSGG Laser irradiation with 5Hz, 0.5 W, 66 J/cm^2 (c) Type III etching pattern after Er, Cr:YSGG Laser irradiation with 15 Hz, 1.25 W, 44 J/cm^2. Arrows indicate the presence of cracks in the enamel structure.

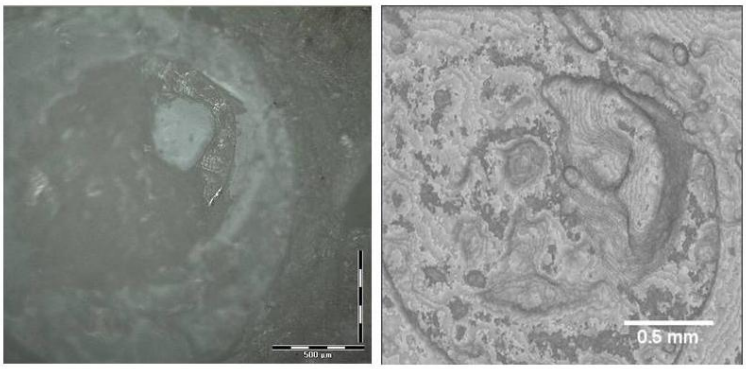

Fig. 3 - (a) Optical microscope image of a mixed failure; (b) 3D OCT *en-face* image of the same sample

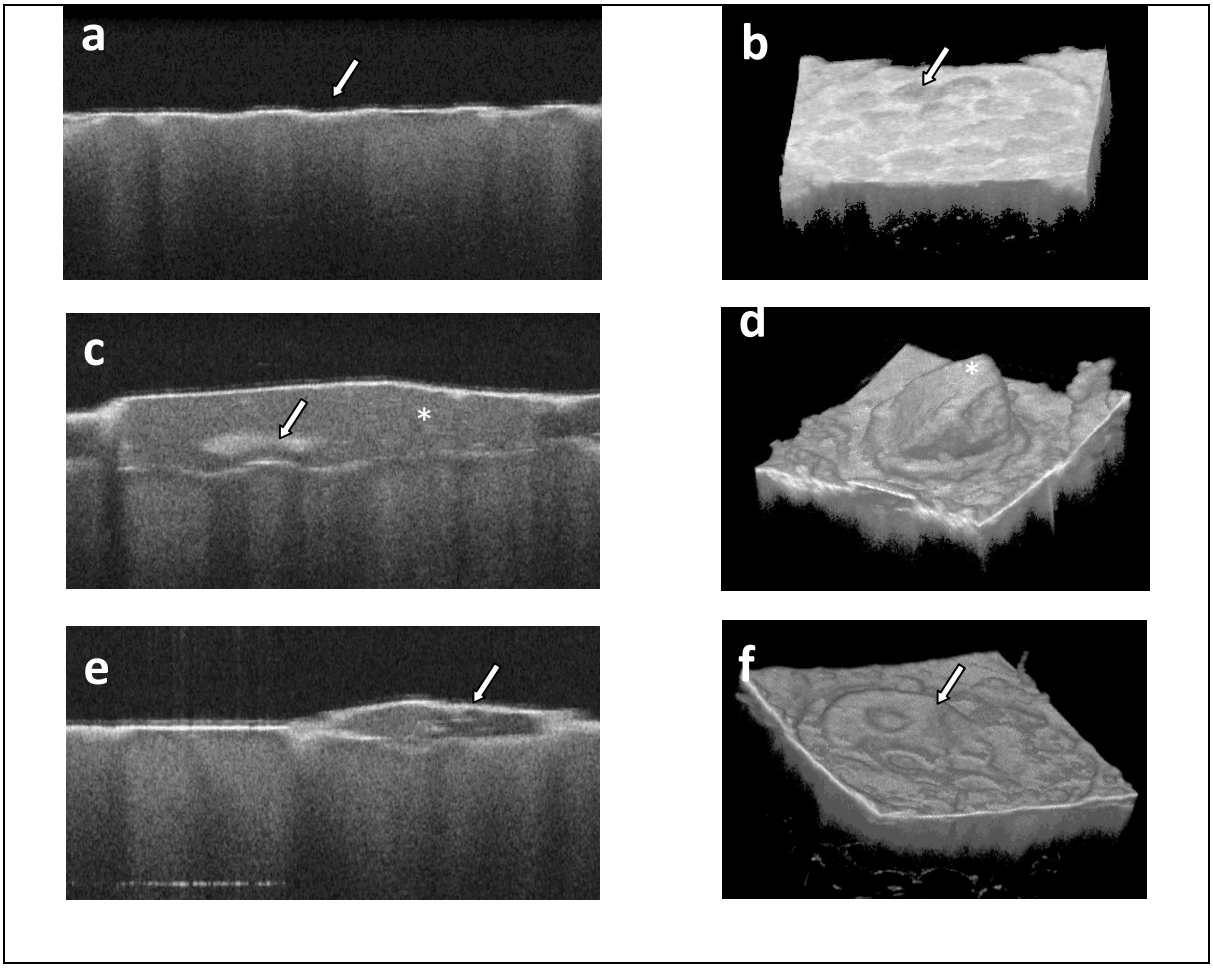

Fig. 4 - Failure mode under OCT. ADHESIVE FAILURE MODE (a) 2D image, the arrow indicates the absence of composite resin; (b) 3D image, the arrow indicates the spots of laser irradiation; COHESIVE FAILURE MODE (c) 2D image; (d) 3D image. The asterisk indicates the presence of a residual resin composite and the arrow indicates an initial failure at the adhesive interface). MIXED FAILURE MODE (e) 2D image; (f) 3D image. Arrows indicates a remant resin adhered to dental substrate.

Table 3 – Distribuition (%) of the fracture mode for all experimental groups.

	Adhesive	*Cohesive*	*Mixed*
CSE	46.7	6.6	46.7
CSE_{66J/cm^2}	33.4	6.6	60.0
CSE_{44J/cm^2}	26.7	20	53.3
SBU	66.7	13.3	20.0
SBU_{66J/cm^2}	6.6	26.7	66.7
SBU_{44J/cm^2}	26.7	33.3	40.0

DISCUSSION

The results of the current experiment require the accept of the null hypothesis, as differences among the bonding agents were not confirmed.

A reduction of enamel bonding effectiveness of one-step self-etching adhesives and universal or multi-mode adhesives applied as self-etching adhesive has been reported [2, 7, 18]. Their etching capacity has been shown to be more restricted, as they present low reactivity with the mineral component, lower availability of H^+ ions, and high molecular weight, compared with phosphoric acid, promoting etching that is not as deep and retentive on dental enamel [19]. Compared with the mild etching pattern of the SE primer, phosphoric acid etching promotes the porosity of the demineralized enamel, resulting in increased resin interlocking and micromechanical retention [20]. Therefore, the use of selective phosphoric acid etching has been recommended [10, 18]. In this study, both adhesives were used after a selective phosporic acid etching as has been recommended by the most of literature in the non-irradiated groups. It is already proven that prior application of phosporic acid with multi-mode adhesives improved the bond strength to enamel [21, 22].

Laser etching has become an alternative to acid etching of enamel. Er,Cr:YSGG-laser irradiation can reduce the acid dissolution of dental hard tissues, playing an important role in the prevention of secondary caries, unlike this, the decalcification of the enamel surface caused by acid phosphoric etching can facilitates the caries attack [23, 24]. Laser irradiation are able to produce a typical imbricate patterned surface with a clearly rough aspect without smearlayer [23]. The absence of smear layer seems to be quite favorable for adhesion, since the interaction of “mild” self-etch adhesives with enamel substrate is expected to be better in the absence of smear debris [13]. However, laser irradiation of the enamel modifies the calcium-phosphate ratio and leads to the formation of more stable and less acid-soluble compounds [25]. According to Hossain et al. [26] laser-irradiated enamel shows a significant

increase in calcium in its composition. However, a more mineralized, acid resistant enamel surface may resist etching by the weak acid of a self-etching primer adhesive [15], which would be a limitation.

Conflicting reports have described the effectiveness of resin bonding following laser tooth preparation. Adebayo et al. [27] revealed no significant differences in bond strength on diamond bur–cut enamel and Er,Cr:YSGG lased enamel (171,5 J/cm^2) for the two-step self-etching primer adhesive (Clearfil SE Bond) and two ‘‘all-in-one’’ adhesives. Instead, Cardoso et al. [23] have found significant lowering of enamel bond strengths with the same two-step self-etching adhesive, a one-step self-etching primer adhesive, and a total-etch adhesive following Er,Cr:YSGG (107,1 J/cm^2). Different energy density parameters were used in these studies, but in both the laser provoke marked superficial changes in enamel. In order to minimize these effects, in our study the parameters used were carefully evaluated in a previous pilot study (data not shown).

In our study, when the laser parameters 15 Hz, 1.25 W, 50 mJ and 44 J/cm^2 were used, the results of bond strength of both adhesives were similar to those in the control groups. CSE is a 10-MDP-based adhesive (10-methacryloyloxydecyl dihydrogen phosphate) that was chosen because has a high success percentage between the self-etching adhesives and presents a clinical perfrmance similar to the etch-and-rinse adhesives [28]. The 10-MDP molecule has the ability to partially decalcify dental hard tissues, penetrate the demineralized substrate and chemically bond to the remaining calcium [29]. CSE is applied in two steps with a prior priming procedure, unlike SBU adhesive, applied as a single step adhesive. According Cardoso et al. [23], some components of the primer may hinder the penetration of some important adhesive components, such as MDP, Bis-GMA and dimethacrylates, impairing adhesion to the substrate. On the other hand, the all-in-one universal adhesive (SBU) not requiring a previous priming step, which could make it more effective in penetrating and

sealing the micro-cracks induced by laser irradiation, but there was no difference between the bonding agents. Furthermore, sample irradiation was performed under air (50%) and water (50%) cooling for all irradiated groups. The Er,Cr:YSGG laser delivers photons through the air-water spray matrix directed straight into the target tissue. Water plays an important role during the Er,Cr:YSGG laser irradiation by suppressing the temperature and improving the cutting efficiency. The water molecules on the dental surface absorb the radiation, causing a sudden warming and water evaporation. A high-stream pressure occurs, inducing a controlled micro-expansion and the ejection of dental hard tissues [12].

Despite the use of constant water cooling during laser-irradiation, zones of melting and recrystallization were clearly observed in the CSE_{5Hz} and SBU_{5Hz} groups (Fig1a). It may be caused by the higher energy density (66 J/ cm^2) was used in these groups. The use of higher values of energy density has also been related to a more harmful thermal effect on dental hard tissues [30]. Micro-cracks throughout the irradiated area was also observed in all irradiated groups. These findings has been reported in other studies as a consequence of laser ablation and diffusion of heat to adjacent areas [23, 31, 32].

The occurrence of thermal alterations on the irradiated enamel, such as melting and chemical changes, could render the enamel less receptive for adhesion [23]. Part of the results of our study confirmed these findings, since the use of Er,Cr:YSGG laser with 5 Hz, 0.5 W, 75 mJ and 66 J/cm^2 reduced the bond strength to enamel, regardless of the adhesive system used.

In addition to bond strength, failure pattern analysis is an important outcome to be considered in adhesion methods [30]. Optical coherence tomography and optical microscope were performed after microshear bond strength test to observe the failure mode. In general, optical microscopes are employed for this task. However, the optical images in this case are limited to in-plane images, without showing in depth or surface relief features. OCT, on the

other hand, can generate in depth and surface relief images, due to its imaging generation processing and 3D capabilities. Although OCT has not been widely exploited so far for failure mode analysis, it can be show here that the surface images of OCT are comparable to the optical microscope. This feature can be further explored to quantify the amount of dental material left over the tooth surface.

It can be observed a decrease in the number of adhesive fractures in the irradiated groups when compared to the control groups, and an increase in the number of cohesive and mainly mixed failures (Table 3). It can be suggest that the surface roughness caused by the laser irradiation would enhance the micromechanical retention. The retention of part of material seems to be positive, since complete debonding at the interface seems to be related to an ineffective surface treatment or failure to infiltrate the adhesive system [30]. However, even with an increase in surface roughness and an increase in the number of cohesive and mixed fractures, there was a decrease in bonding strength values in the groups irradiated with 5 Hz, 0.5 W, 75 mJ and 66 J/cm^2. In these groups it can be believe that the superficial changes suffered by the enamel (melting e recrystallization) had greater influence on the final result of the bond strength. In the 15 Hz, 1.25 W, 50 mJ and 44 J/cm^2 groups we could observe an even greater increase in roughness, but without superficial changes, which coud explain the absence of statistical difference with control groups.

CONCLUSIONS

It can be concluded that the use of a Er,Cr:YSGG laser operating at 15 Hz, 1.25 W, 44 J/cm^2 was as effective as the use of phosporic acid on the microshear bond strength of enamel surfacce. It did not appear to influence the self-etching adhesive action. Optical coherence tomography was used to evaluate the fracture pattern, opening up new possibilities of analysis for this parameter.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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ARTIGO III

Trabalho sobre o condicionamento de dentina sadia e erodida com o laser de femtossegundo e seu efeito na adesão.
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Effects of femtosecond laser irradiation on the microshear bond strength of eroded and sound dentin

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Abstract

The aim of this *in vitro* study was to assess the bond strength of an adhesive system to sound and eroded dentin after irradiation with femtosecond laser. Twenty slices of human molar dentin were properly treated for the study, and half of the specimens were subjected to erosive cycle. The samples were randomly assigned into six groups (n=10): sound dentin (SD), sound dentin etched with the Ti: Saphire laser (795 nm, 70 fs, 240 mW, 1 kHz) eroded dentin (ED), and fs laser etched eroded dentin. Irradiated specimens were analyzed through complementary imaging techniques including 2D and 3D OCT images, Scanning Electron Microscopy and Atomic Force Microscopy. The adhesive system (Clearfil SE Bond) was applied to the dentin surface and a resin composite (Tetric N-Ceram) was light-cured to bond the treated surfaces. Micro-shear bond strength was measured while fracture analysis was performed by optical microscope. Data were statistically analyzed using Mann Whitney test (p=0.05). It can be concluded that the Ti:Sapphire femtosecond laser successfully ablated the sound dentin tissue, providing the opening of the dentinal tubules and increasing the surface roughness with higher adhesion values observed. However, the most significant improvement in bond strength was observed in the eroded dentin.

Abstract figure and short legend: Irradiation of the sample with Ti:sapphire laser; 3D OCT image and MEV of irradiated surface (sound dentin).

Short Title: Cassimiro-Silva et al.: Femtosecond laser on eroded and sound dentin

Key words: Femtosecond Laser, Dentin bonding agents, Tooth erosion, Optical coherence tomography, Atomic force microscopy

Introduction

Dental erosion is defined as the tissue loss, chemically removed from the tooth surface by acid or chelating substances, without bacterial involvement [1]. It is related to nutrition and individual factors, and in more advanced stages the dentin structure is involved. As a consequence, the structural integrity of the tooth and its aesthetics, are compromised, making restorative treatments necessary [2].

Despite the great advances in adhesive dentistry, adhesion to tooth structure remains a challenge, especially to dentin. Its continuous exposure to acids can result in complex histological changes, which is a problem for the quality of adhesion to the substrate [3]. The erosive challenge leads to the dissolution of hydroxyapatite crystals, the increase of the tubules' diameter and the exposure of highly mechanically resistant collagen fibrils network [2, 4, 5]. This scenario impairs the permeability of adhesive systems and the formation of hybrid layers [6].

Therefore, new techniques have been reported to improve the adhesion and to simplify the clinical procedure. Lasers have been used as an alternative to promote chemical/morphological changes on the tooth surface, which could positively influence the dental adhesion [7]. However, lasers used to ablate dental hard tissues can cause collateral damage to dental structure like microcracks, carbonization and recrystallizations that can compromise the adhesion to the dental substrate [8-10]. In this context, a laser source that promotes low-damage tissue ablation is recommended and the exploration of more adequate irradiation parameters is needed.

Femtosecond laser (fs-laser) is emerging as a promising tool to ablate dental hard tissues with high degree of accuracy and negligible heat damage to the surrounding tissue [10, 11]. A fs-laser is an ultrashort pulsed laser with pulse durations in the femtosecond range (10^{-}

^{15} seconds) that produce peak powers and energy enough to ionize the dental substrate into plasma. In this ultrafast pulse duration regime, there is no time for thermal diffusion [12, 13]. Furthermore, this ablation process ejects most of the dental matter into the air, preventing smear layer production. Therefore, dentinal tubules remain open [14] facilitating, for instance, the adhesive penetration.

Femtosecond lasers have been employed in dentistry for different applications, including multiphoton imaging of the dentine-enamel junction [15], influence on shear bond strength in orthodontics [16, 17] and Zirconia based materials [18, 19]. Ablation rates and efficiencies have also been studied using fs sources [10, 13]. Some studies in the literature have reported the use of the femtosecond lasers for the dentin treatment prior to adhesive procedures [11, 20]. Additionally, studies on the adhesion to eroded substrate, following the pretreatment of the dentin with femtosecond lasers, have not been reported to the best of our knowledge. Therefore, the aim of this *in vitro* study was to assess the micro-shear bond strength of an adhesive system to sound and eroded dentin after irradiation with femtosecond laser. Scanning Electron Microscopy (SEM), Atomic Force Microscopy (AFM) and Optical Coherence Tomography (OCT), all imaging based complementary techniques with different spatial resolutions, were used to assess the aspects of the dentin sufaces after the pretreatment as means to explain the differences observed among the experimental groups.

Materials and Methods

Specimen preparation

This study was approved by the ethics committee of the Federal University of Pernambuco under the protocol #1.735.580.

Twenty freshly extracted human molars were selected for this study. Teeth were visually examined to confirm the absence of physical damage, such as deep grooves or cracks.

The specimens were cleaned with distilled water and brushes, and further disinfected in 0.5% chloramine for 2 weeks.

The roots were sectioned at a 2 mm distance from the enamel–cementum junction and the oclusal enamel was removed with a low-speed diamond saw (Isomet, Buehler Ltd., IL, USA) to expose superficial dentin surfaces. To facilitate the specimens handling, each specimen was embedded in an acrylic resin (Jet, Clássico, São Paulo, Brazil). The superficial dentin surfaces were submitted to metallographic polishing (Biopdi, SP, Brazil) with 400 and 600-grit water-cooled sandpaper for 60s each to remove remaining enamel, planify the target surface, and to produce standardized and clinically relevant smear layers.

Erosive Cycle

Ten specimens were submited to an erosive pH cycling that was performed during 5 days by immersion in 0.05 M citric acid (pH 2.3, 10 min, 6×/day). Between acid attacks, the specimens were immersed for 60 min in supersaturated solution (pH 7.0) consisting of 1.5 mmol/L $CaCl_2$, 1.0 mmol/L KH_2PO_4, and 50 mmol/L NaCl [21], at room temperature (25 °C), under constant agitation (30 rpm) on a shaker. During the remaining time of the erosive cycling, the specimens were stored in supersaturated solution until the beginning of the experiment at the following day. The solutions were renewed everyday, and the pH of the solutions was checked at the beginning and at the end of each experimental day. This model of erosive cycling was proposed by Ganss et al. [23].

Experimental Design

The twenty specimens of dentin were randomly divided into experimental groups according to the state of the substrate (eroded or not) and the performed laser conditioning. Five specimens were assigned to the control group of sound dentin without conditioning (SD), and five specimens were assigned to the control group of eroded dentin without conditioning (ED). In each specimen, two different regions of assessment were designated, resulting in a

number n=10 of measurements per group. The specimens subjected to laser etching were irradiated in two different regions, each with a different laser power (see below for more details of the laser and laser etching setup). Five specimens of sound dentin were etched with a pulsed Ti:Sapphire fs laser at average powers of 240 mW (SD240) and 390 mW (SD390), corresponding to energies of 240 μJ and 390 μJ. The remaining five specimens of eroded dentin were also etched with the same source and same characteristics. In each irradiated region, two resin samples were bonded, resulting in a number n=10 of measurements per group.

Laser irradiation was performed using a a pulsed Ti:sapphire laser (802 nm, 100 fs, 1 kHz, pulse energy up to 1 mJ, Libra®, Coherent, USA). Two different average powers were used in this study (240 mW and 390 mW), producing two different pulse energies (240 and 390 μJ, respetively). The laser was directed onto the surface by using an objective lens (20x, 0.3 numerical apperture) in order to promote plasma-mediated ablation. The samples were attached to a XYZ mount with micrometer precision, connected to a stepper motor controlled by a LabVIEW® routine. In order to increase the width of the scanning lines, decrease the thermal and mechanical damage, and to promote superficial removal of the smear layer, the laser scanning was performed with the samples moved 200 μm away from the focus in the incidence direction (Z-axis), producing lines with 90 μm width. The XYZ mount was programmed to move 2,5 cm in the horizontal direction (X-axis) in order to run over the whole sample. In the vertical direction (Y-axis), 20 scanning lines were produced, equally spaced by 90 μm, producing an etching area with 1.8 mm of total width. The scanning velocity was fixed of 30 mm/s. Considering this value (90 μm) to be the beam diameter at the sample, the calculated fluences for the pulse energies employed here, at 1kHz and the scanning speeds, are ~11J/cm^2 and ~18J/cm^2, for the lowest and highest energies employed. This value is 4-5 times above typical ablation threshold cited in the literature for equivalent

lasers systems [12,13,22].

Restorative Procedure

The tested adhesive system (Clearfil SE Bond, Kuraray Noritake Dental Inc., Kurashiki, Okayama, Japan) were applied to the dentin surfaces according to manufacturer's instructions. Transparent polyethylene tubes (Ø=0.9 mm, h=1 mm) were positioned over double-faced perforated tapes. Resin composite (Tetric N-Ceram A3, Ivoclar vivadent, Schaan, Liechtenstein) was carefully packed inside the tubes and was light-cured for 20 s using a LED light-curing with 1200 mW/cm^2 (Radii-cal, SDI, Bayswater, Victoria, Australia).

The samples were kept in distilled water for 24 hours at 37 ºC. The tube and the double-faced tape were carefully removed with a blade, exposing the resin composite cylinders. Samples were examined under a stereomicroscope (x10 magnification) to observe possible defects.

Optical Coherence Tomography

Optical Coherence Tomography is an interferometric imaging technique exploiting the low coherence of broadband optical sources, which when associated with a Michelson interferometer and a detection system forms the basic setup. Specimens from each irradiated group were carefully analyzed through 2D and 3D images using a spectral domain SD-OCT (Callisto Spectral Domain OCT System, Thorlabs Inc, New Jersey, USA). The OCT light source is a SLD (Super Luminescent Diode) with central wavelength of 930 nm, spectral bandwidth of 100 nm and maximum output power of 5 mW. Images generated by this system present axial resolution of 7/5.3 μm (air/water), lateral resolution of 8 μm and maximum imaging depth at 1.3 mm. The axial scan rate of the system is 1.2 kHz, capturing two frames per second with 105 dB of sensitivity. This system provides depth profiles that, added to

cross-sectional images, generate tridimensional reconstructions. Specimens were positioned on a manual stage that allows XYZ translation with micrometer precision. All surfaces were analyzed by scanning lines starting from the reference area to the irradiated area, passing by the interface. The SD-OCT system generated cross-sectional 2D images with 1024 X 512 pixels (4 X 0.5 mm) and 3D images with 1024 X 512 pixels (3 X 3 mm). The discrepancy between the height of the reference area and the irradiated surface was measured using the ImageJ program (JAVA, public domain).

Microshear Bond Strength Test (μSBS)

The specimens were tested in a universal testing machine (EMIC, DL 10000, São José dos Pinhais-PR, Brazil). A thin orthodontic wire (0.2 mm) was looped around the cylinder aligned with the setup to ensure the correct orientation of the applied forces. The crosshead speed was set at 0.5 mm/min until failure. The computer software Tesc 3.04 (EMIC, São José dos Pinhais-PR, Brazil) registered the bond strength values.

Failure Mode Examination

Failure mode analysis was performed with an optical microscope at 5 x, 10 x, 20 x and 50 x magnification (Olympus BX51, Tokyo, Japan). Optical Coherence Tomography (OCT) system was performed to get 2D and 3D images to confirm the results. The failure modes were classified into three categories, based on the percentage of substrate-free material in: Adhesive (failure at the resin/enamel interface); Cohesive (failure exclusively within enamel or resin composite); Mixed (failure at the resin-enamel interface that include cohesive failure of the neighboring substrates).

Scanning Electron Microscopy

Photomicrographs of representative areas of the irradiatiated surfaces were obtained under SEM. For this evaluation, two additional samples were prepared. Specimens were

sputter-coated with gold and analyzed under SEM (Mira 3, Tescan, Cambridge, UK) at 10 kV with x 5000.

Atomic Force Microccopy

Two additional samples were prepared for atomic force microscopy analysis (AFM, Alpha 300 RA, WITec, Ulm, Germany). The block surface morphology was probed in 'contact mode'. Imaging was performed with an Al-coated cantilever and probed with 0.2 N/m. Images 30 x 30 Mm with resolution of 256 x 256 pixels and operating point of 0.5 V were collected at a very low scan rate to obtain details of the dentin structure and to avoid damaging the tip.

Statistical analysis

Data were organized into an Excel spreadsheet (Microsoft Office 2007) and analyzed using SPSS 13.0 (Statistical Package for the Social Sciences, Chicago, IL, USA) for Windows. Descriptive statistics were obtained and the Kruskal Wallis test was performed to compare results between groups. In case of significant differences, Mann Whitney test was performed for pairwise comparison between groups ($p = 0.05$).

Results

Representative cross sectional OCT images of the four treatment groups, observed on dentin surface after laser irradiation, are shown in Figure 1. The images show a wear pattern performed by the laser, which is deeper and more homogeneous in the groups of eroded dentin. The results for the tissue loss of the irradiated region with respect to the adjacent area are presented in Table 1.

Table 1 – Mean tissue loss (μm) in all irradiated groups measured by OCT. Different superscript letters indicate statistical differences between groups ($p < 0.05$)

	Tissue loss (μm)
ED_{240}	22.20 ± 3.11^{A}
ED_{390}	22.80 ± 1.30^{A}

SD_{240}	11.80 ± 0.84 ^{B}
SD_{390}	13.20 ± 1.48 ^{B}

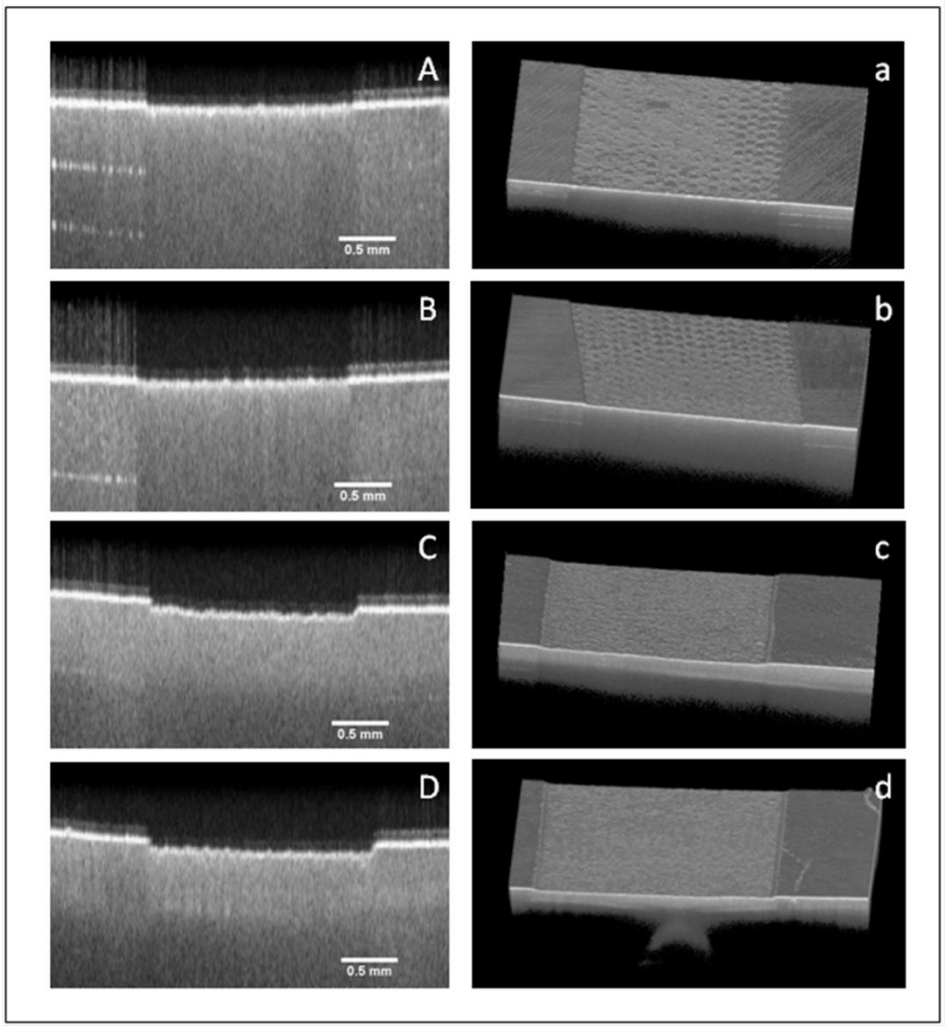

Figure 1 – Comparison of OCT images of the dentin surface reference and irradiated area. Images identified with lowercase represent 3D OCT and images by uppercase cross-sectional 2D OCT. (A/a) SD_{240}, (B/b) SD_{390}, (C/c) ED_{240} and (D/d) ED_{390}. Scale bar corresponding to 0.5 mm.

The results for the μSBS analysis are show in Figure 2. The maximum bond strength was recorded for the SD_{240} (16.42 MPa) and the minimum bond strength was recorded for the ED (8.89 MPa). Kruskal Wallis revealed significant differences between groups ($p < 0.01$). The Mann Whitney test demonstrated differences between ED and all the other groups.

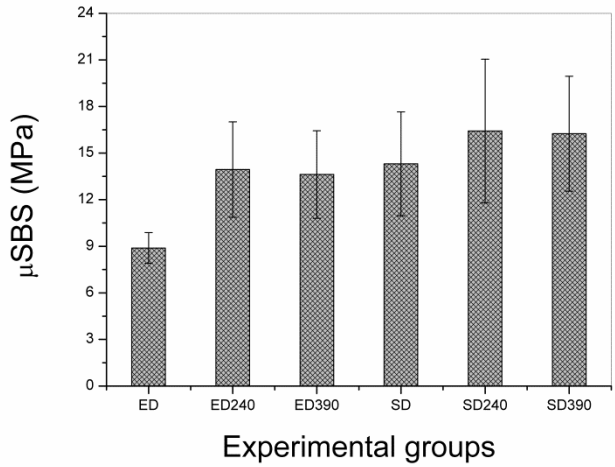

Figure 2 – Mean µSBS and standard deviation of the tested adhesives according to the type of dentin pretreatment.

Figure 3a shows sound dentin surface with dentin tubules completely covered by the smear layer; Figure 3b shows dentin surface after erosive cycle. It may be noted the complete opening of the dentinal tubules demineralized peritubular dentin, and a rough intertubular dentin; Figures 3c-f show the morphorlogy of the dentin surface after the femtosecond laser irradiation. The sound dentin irradiated groups presented an irregular and rough appearance (Figure 3c,e). Some dentinal tubules are exposed and open (Figure 3c). SEM analysis revealed a smoother profile with morphological alterations of the laser-irradiated eroded dentin surface, such as areas of melted and recrystalized hydroxyapatite (Figure 3d,f).

AFM micrograph of SD group showed dentin surfaces with dentin tubules completely covered by the smear layer, and ripples created by the action of abrasives were visible (Figure 4a). However, in ED group, it is clearly possible to identify the openings of the dentin tubules, indicating that the smear layer was completely removed from the dentin surfaces after the erosive cycle (Figure 4b). In irradiated groups (Figures 4c-f) the presence of peaks and valleys formed by the laser action was also visible.

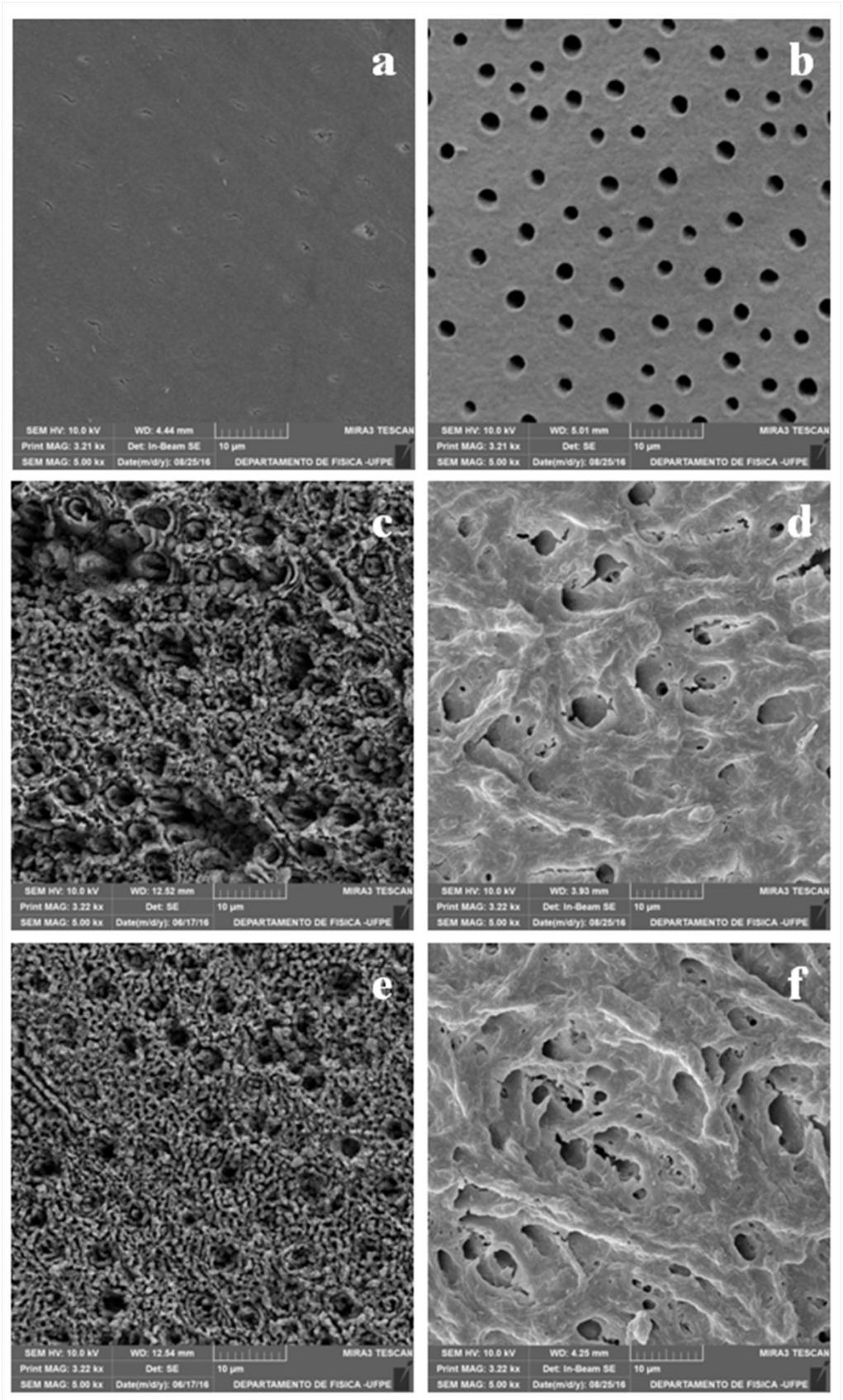

Figure 3 - Representative scanning electron microscope (SEM) images (5000x) of (a) sound dentin surface prepared with 400 and 600-grit water-cooled sandpaper only, (b) eroded dentin, Ti:Sapphire Laser irradiated areas (c) SD_{240}, (d) ED_{240}, (e) SD_{390} and (f) ED_{390}.

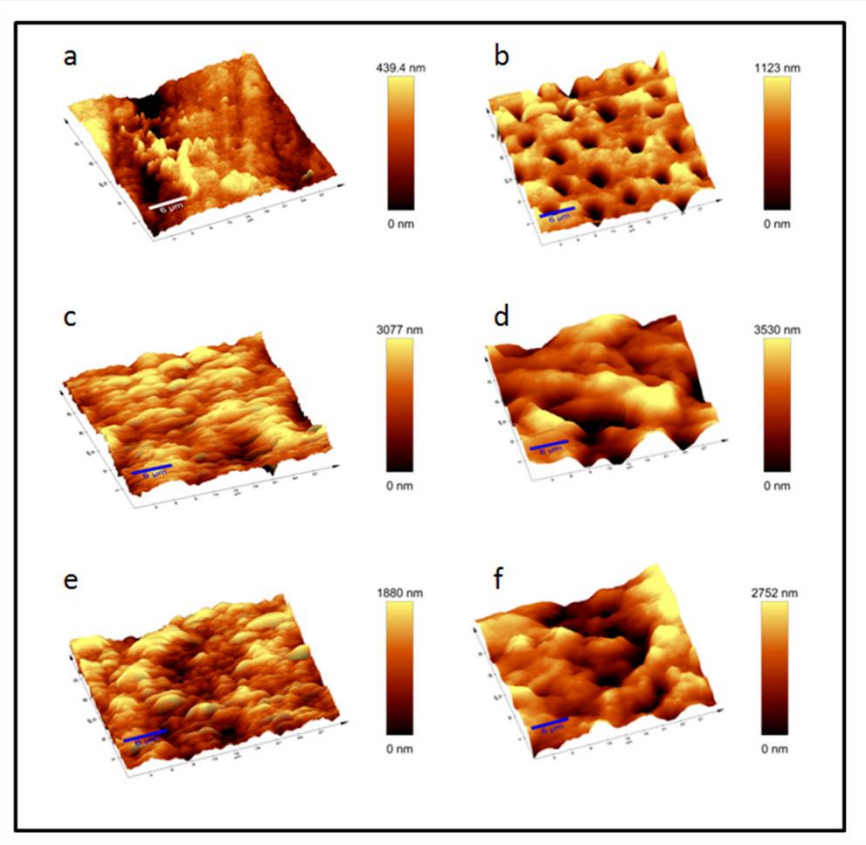

Figure 4 - AFM image of sound dentin surface (a), after erosive challenge (b), Ti:Sapphire Laser irradiated area SD_{240} (c), ED_{240} (d), SD_{390} (e) and ED_{390} (f).

Following the failure mode examination described before, the percentage of samples in the three assigned modes are presented in Table 2. We observed a decrease in the number of adhesive fractures in the sound irradiated groups when compared to the sound control group, and an increase in the number of mixed failures. All presented results are discussed in the following sections.

Table 2 – Percentage of samples (%) according to fracture mode for all experimental groups.

Group	Fracture Pattern (μSBS)		
	Adhesive	Cohesive	Mixed
SD	80	0	20
SD_{240}	60	0	40
SD_{390}	70	0	30
ED	40	10	50
ED_{240}	20	0	80
ED_{390}	20	10	70

Discussion

The pH cycling to simulate erosion *in vitro* used in this study has been proposed previously by Ganss et al. [23]. This erosive challenge could remove the dentinal plugs and demineralize inter- and peritubular dentin, increasing the tubule's diameter and exposing its organic matrix composed by a collagen fibrils network as described by Prati et al. [4]. It is already reported in the literature that erosion can negatively affect the bond strength since the presence of denatured collagen might interfere with the bonding properties of adhesive materials [24]. This is evidenced when comparing the μSBS values of the SD and ED control groups in Figure 2.

In a deeply eroded dentin, after the penetration of the adhesive, thicker and structurally imperfect hybrid layers are formed than those of sound dentin. Porosities and demineralized zones without resin reinforcement are present, since resin monomers may not penetrate as deeply as acid [25]. However, self-etch adhesive systems have an acid primer, which promotes demineralization and monomer infiltration simultaneously, promoting a more homogeneous hybrid layer and preventing discover of collagen layer. Zimmerli et al. [26] demonstrated that the self-etch adhesive system (Clearfil SE Bond) showed better results in eroded dentin than etch-and-rinse adhesive system. For this reason we chose to use this type of adhesive in our research.

Despite the large number of researchs on dentin ablation by femtosecond laser [10, 12, 13, 22, 27-29], little is known about its effects on eroded dentin surfaces. To our knowledge, our experiment is the first to evaluate adhesion to eroded dentin after the pretreatment with femtosecond laser. For this, the irradiation parameters used were lower than those found in the literature and the laser was used in a defocused way, at a distance of 200μm from the surface. This choice was made to remove mainly the smear layer, promoting greater preservation of the dentin and its natural properties, and in turn enhancing the bond strength.

The first contrasting outcome of the effect of femtosecond laser irradiation on sound and eroded dentin is the promoted tissue loss, shown in Table 1. For the same laser power, the tissue loss in eroded dentin presents an almost twofold increase in the tissue loss when compared to sound dentin. However, no significant difference between samples in the same condition with respect to the laser power is observed. Demineralized dentin has a lower ablation threshold than healthy dentin and, consequently, the same laser power is expected to promote higher ablation rates in those samples, as already reported by other authors [14, 31,32].

The laser-treated surface of sound dentin specimens had a rough and irregular appearance without smear layer and open dentinal tubules [12], as can be observed in the results of our groups of sound dentin. This was observed in the 3D OCT images, as well as during MEV and AFM analysis. These findings also corroborate with Alves et al. [22] who states that the composition of dentin does not appear to be significantly modified by the laser treatment. These statements lead us to believe that the sound dentin substrate irradiated with the femtosecond laser would be ideal to adhesion. This is supported by the fact that mean μSBS values on SD samples were always greater than their ED counterpart were. Femtosecond lasers have already been used to increase the adhesion of bonding agents with dentin [11, 20, 30]. As expected, in our study there was an improvement in the bond strength of irradiated sound dentin samples when compared to non-irradiated samples, although it was not statistically significant.

The bond strength evaluation revealed a deficiency on adhesion of ED substrate compared with sound dentin. These findings corroborate with other studies in the literature [3, 6, 24, 26, 33]. It is possible that the roughness increase, seen as an increase in the total effective area available to the adhesive composite, generated a greater adhesion force per unit

area in the tubules. Together with the preservation of the collagen fibrils network, it contributed to the overall μSBS in comparison to non-irradiated samples.

The loss of dentin tissue after laser irradiation was measured by the use of OCT. It is an imaging diagnostic modality widely used in medical and non-medical areas, including dentistry [34]. With this analysis we aimed to observe which laser parameter would ablate a greater amount of tissue, according to its previous erosion condition. We could observe a greater mineral loss after the laser irradiation in the previously eroded samples, irrespective of the used laser parameter. This is explained by the greater ease in removing the softened outer dentin layer caused by erosive cycling. A considerable amount of tissue was removed in both types of substrate even when using a defocused laser. It is an expected result because the generated plasma is the one responsible for the ablation process. Its farther particles possess less energy than those in the focus, but do not change in nature by only defocusing the sample.

Previous investigations [10, 12, 13, 22, 27, 28, 35] showed that sound dentin does not suffer thermal or mechanical damages when irradiated with femtosecond lasers. This is expected when the laser is scanned over the sample, decreasing the delivered number of pulses in each point, as observed in our study. However, it should be noted that even using femtosecond lasers, a large number of pulses delivered in the same point could increase thermal loading and the plasma extent, generating microcraks, melting and carbonization [29].

In contrast, it was not what we could observe in the groups of eroded dentin. SEM images revealed morphological alterations of the laser-irradiated eroded dentin surface, such as areas which appear to have undergone melting and recrystallization, which theoretically could hinder adherence to the structure. This is related to the chemical and morphological alterations on the dentin surface after the erosive cycle [36-39]. The fusion point of the exposed organic matrix is extremely lower than the hydroxyapatite, causing the local thermal

loading to melt the demineralized tissue. However, even with apparently unsatisfactory conditions for adhesion, irradiated eroded samples had better μSBS values than eroded/non-irradiated ones with significant statistical difference. Moreover, the adhesion was not statistically different from the irradiated sound samples, regardless of the incident laser power. To explore the fundamental reasons for such behavior, further investigation is needed.

Regarding the observed fracture modes, we can suggest that the opening of the dentinal tubules and the surface roughness caused by the laser irradiation would enhance the micromechanical retention. According to Guedes et al. [40] the retention of part of material seems to be positive, since complete debonding at the interface seems to be related to an ineffective surface treatment or failure to infiltrate the adhesive system. No cohesive failure was observed in the groups of sound dentin. There was a 50% decrease in the number of adhesive failures in irradiated eroded dentin groups. Again, this can be explained by the removal of the softened outer dentin layer after laser irradiation, exposing deeper dentin tissue that did not undergo the erosive process, which would be more conducive to adhesion.

Finally, it is worth to compare our results for shear bonding strength with fs laser with those of microtensile bond strength measurements also performed in dentin and with fs laser, as reported by Portillo et al. [20], particularly for the Clearfil adhesive employed (same as in our work). These authors observed that femtosecond laser irradiation reduced the bonding effectiveness when this two-step self-etching adhesive was used, contrary to our results. The different laser parameters used could explain these results, besides the different bond strength tests since it has been reported in the literature that microshear testing is a more accurate method than microtensile [41, 42].

Conclusion

It can be concluded that the Ti:Sapphire femtosecond laser successfully ablated the sound dentin tissue, provided the opening of the dentinal tubules and increased the surface

roughness, characteristics that were responsible to an improved adhesion. The higher adhesion values was observed in sound irradiated dentin, although the most significant improvement was observed in eroded dentin, when compared to control group samples. The femtosecond laser irradiation promoted a greater ablation in the eroded dentin specimens, as well as melting and recrystallization of hydroxyapatite. Therefore, optimal lasing parameters for eroded dentin still deserve further investigation.

Acknowledgments

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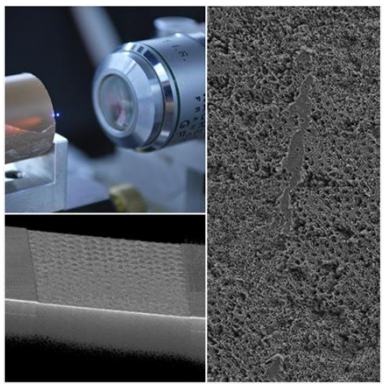
Graphical Abstract:

Ti:Sapphire femtosecond laser was applied to etch sound and eroded dentin tissue to promote better bond strength to composite resin. The laser successfully ablated the sound dentin tissue, providing the opening of the dentinal tubules. Significant improvement in bond strength was observed in the eroded dentin.



CONCLUSÃO

Pode-se concluir que os dois tipos de LASERs utilizados nesta pesquisa foram capazes de ablacionar o tecido duro dental de forma a promover uma adesão eficaz. O LASER de Er,Cr:YSGG com uma densidade de energia de 44 J/cm^2 foi estatisticamente tão efetivo quanto o uso do ácido fosfórico e não pareceu influenciar a ação do adesivo autocondicionante. Já o LASER de Ti:Safira operando no regime de femtossegundo foi capaz de ablacionar o tecido dentinário sadio, proporcionando a abertura dos túbulos dentinários e uma certa rugosidade superficial. Entretanto, não houve melhora significativa na adesão. Por outro lado, irradiação com LASER de femtossegundo promoveu maior ablação nas amostras de dentina erodida e melhora na adesão de forma significativa.

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