

**UNIVERSIDADE DE UBERABA
MESTRADO ACADÊMICO EM ODONTOLOGIA**

BRENDA FERREIRA ARANTES

**INFLUÊNCIA DO LASER Er,Cr:YSGG, ASSOCIADO OU NÃO
A AGENTES DESSENSIBILIZANTES, NA PREVENÇÃO DA
EROSÃO ÁCIDA EM DENTINA RADICULAR BOVINA**

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Dissertação apresentada ao Programa de Pós-graduação em Odontologia - Mestrado Acadêmico da Universidade de Uberaba, como requisito parcial para a obtenção do título de Mestre em Odontologia, na área de concentração em Clínica Odontológica Integrada

Orientador: Prof. Dr. Cesar Penazzo Lepri

**UBERABA- MG
2018**

Catálogo elaborado pelo Setor de Reerência da Biblioteca Central UNIUBE

A14i Arantes, Brenda Ferreira.
Influência do laser Er:Cr:YSGG, associado ou não a agentes
dessensibilizantes, na prevenção da erosão ácida em dentina radicular
bovina / Brenda Ferreira Arantes. – Uberaba, 2018.
76 f. : il. color.

Dissertação (mestrado) – Universidade de Uberaba. Programa de
Mestrado em Odontologia. Área Clínica Odontológica Integrada.
Orientador: Prof. Dr. Cesar Penazzo Lepri.

1. Lasers em odontologia. 2. Dentes – Erosão. 3. Prevenção. 4.
Hipersensibilidade dentinária. I. Lepri, Cesar Penazzo. II.
Universidade de Uberaba. Programa de Mestrado em Odontologia.
Área Clínica Odontológica Integrada. III. Título.

CDD 617.063

BRENDA FERREIRA ARANTES

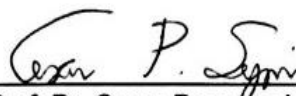
**“INFLUÊNCIA DO LASER Er:Cr:YSGG, ASSOCIADO OU NÃO A AGENTES
DESSESSIBILIZANTES, NA PREVENÇÃO DA EROÇÃO ÁCIDA EM
DENTINA RADICULAR BOVINA”**

Dissertação apresentada como parte dos requisitos para obtenção do título de Mestre em Odontologia do Programa de Pós-graduação em Odontologia – Mestrado da Universidade de Uberaba.

Área de concentração: Clínica Odontológica Integrada

Aprovado (a) em: 27/04/2018

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DEDICATÓRIA

À Deus,

Pelo dom da vida, por iluminar minha caminhada, por me proteger e me fortalecer em todos os momentos.

À família,

Aos meus amados pais, Paulo e Elisabete, pelo amor incondicional. Nunca mediram esforços para alcançar meus desejos e vontades. Exemplos de humildade, honestidade e integridade. Dedico essa vitória a vocês.

A minha querida irmã Milla, a qual sempre estive ao meu lado, me incentivando e apoiando em todos os momentos. Meu muito obrigado minha irmã.

A minha amada afilhada Lis, por trazer mais alegria a nossa família;

Ao Rafael, pelo seu amor, compreensão e companheirismo em todos os momentos. Muito obrigada.

A toda minha família e amigos que direta ou indiretamente contribuíram com essa grande conquista.

AGRADECIMENTO ESPECIAL

Ao meu orientador Prof. Dr. Cesar Penazzo Lepri,
exemplo de caráter, ética, humildade, profissionalismo e
competência.

Não tenho palavras para agradecer
o que me proporcionou desde a graduação,
até aqui.

Agradeço à Deus por ter conhecido
um exemplo de pessoa como você.

Muito obrigada pelos ensinamentos,
compreensão,
pela confiança depositada em mim
e principalmente pela paciência
em ensinar o que muitas vezes parecia
incompreensível aos meus olhos.

Você é uma pessoa admirável, e meu sentimento é de
gratidão.

AGRADECIMENTOS

À Universidade de Uberaba, através do Magnífico Reitor Dr. Marcelo Palmério;

À Pró-Reitoria de Pós-Graduação, Pesquisa e Extensão da Universidade de Uberaba, na pessoa do Pró-Reitor Prof. Dr. André Luís Teixeira Fernandes;

Ao diretor do Curso de Odontologia da Universidade de Uberaba, Porf. Dr. Luis Henrique Borges;

À CAPES, pela concessão do auxílio financeiro sob a forma de bolsa/taxa de estudos;

Às agências de fomento CNPq (PIBIC) e FAPEMIG (PIBIC) e ao PAPE-UNIUBE pela concessão de auxílio financeiro para o desenvolvimento do projeto;

Aos professores do mestrado por todo conhecimento e disposição em nos ensinar;

Aos professores da banca de qualificação Maria Angélica Hueb de Menezes Oliveira, Vinícius Rangel Geraldo Martins e Luis Henrique Borges pela contribuição;

À Laura Oliveira de Mendonça pela grande amizade e incentivo durante todo o mestrado;

À Michelle Gomides pela ajuda na fase laboratorial;

À Flávia, secretária do Curso de Pós-Graduação da Universidade de Uberaba, pela dedicação ao trabalho, competência e disponibilidade em esclarecer todas nossas dúvidas;

Aos meus colegas de Mestrado Júlio Cesar de Carvalho Alves, Júlio Cesar Lemos Duarte, Márcio Miranda Abdala, Rafael José Santos Rodrigues, Eleonora de Paula Amaral, Marco Antônio Maluf Curi, Sandro Cecilio Furiati pelos conhecimentos adquiridos durante esses dois anos e pelas alegrias proporcionadas;

Ao laboratório de Biomateriais de Universidade de Uberaba, ao técnico Marcelo e ao Martins, pela ajuda incessante durante todas as fases de execução do experimento;

À Denise Tornavoi de Castro pela disponibilidade na correção;

Ao laboratório de Laser em Odontologia do Departamento de Odontologia Restauradora da Faculdade de Odontologia de Ribeirão Preto da Universidade de São Paulo, pela disponibilização dos lasers utilizados neste estudo. Especialmente às Professoras Regina Guenka Palma Dibb e Juliana Jendiroba Faraoni Romano.

Agradeço a todos que direta ou indiretamente contribuíram para realização deste trabalho.

RESUMO

RESUMO –

O objetivo desse estudo *in vitro* foi avaliar a influência do *laser* Er,Cr:YSGG, associado ou não a agentes dessensibilizantes, na prevenção da erosão ácida em dentina radicular bovina. Foram obtidos 80 fragmentos de dentina (4mm x 4mm e 3mm), divididos aleatoriamente em 8 grupos (n=10). G1: controle negativo; G2: aplicação do verniz fluoretado a 5% (controle positivo); G3: aplicação do laser Er,Cr:YSGG (0,1W; 5Hz; sem refrigeração à água); G4: aplicação do verniz fluoretado 5% + laser Er,Cr:YSGG; G5: aplicação do oxalato de potássio 3%; G6: aplicação do oxalato de potássio 3% + laser Er,Cr:YSGG; G7: aplicação passiva do gel bifásico de silicato de cálcio/fosfato; G8: aplicação passiva do gel bifásico de silicato de cálcio/fosfato + laser Er,Cr:YSGG. Metade do fragmento foi devidamente isolada (região controle) e a outra metade recebeu um dos tratamentos propostos. A bebida erosiva utilizada foi um refrigerante a base de cola (pH=2,42 à 4°C), com duração de 5 minutos cada ciclo erosivo. Este procedimento foi realizado 2 vezes ao dia, com intervalos de 6 horas entre os desafios, por um período total de 14 dias. Avaliou-se a normalidade (Kolmogorov-Smirnov) e homogeneidade (Levene's) dos dados. Após estas análises, os dados de rugosidade superficial foram submetidos ao teste estatístico de Análise de Variância (ANOVA) e pós-teste de Tukey, para a diferenciação da média dos grupos. Para o perfil de desgaste, utilizou-se o teste Kruskal-Wallis e o pós-teste de Dunn. Após, realizou-se o teste de correlação de Spearman. Todos os testes estatísticos assumiram o nível de significância de 5% ($\alpha=0,05$). Observou-se que o G1 apresentou o maior valor de rugosidade superficial após o desafio erosivo ($3,586\mu\text{m}^2 \pm 0,205\mu\text{m}^2$) e o G7 apresentou o menor valor de rugosidade superficial após o desafio erosivo ($1,071\mu\text{m}^2 \pm 0,180\mu\text{m}^2$). Quanto à perda de volume, o G4 apresentou o menor percentual ($9,7\% \pm 0,9\%$) de volume perdido, enquanto o G1 apresentou o maior percentual ($41,8\% \pm 2,5\%$), ambos com ($p<0,05$). Foi observada fraca correlação entre as variáveis de resposta ($\rho=0,33$). Conclui-se que todos os grupos apresentaram menores valores de rugosidade superficial e de perda de volume quando comparados ao grupo controle negativo. Para a rugosidade superficial, o gel bifásico de silicato de cálcio/fosfato apresentou o melhor resultado. Para a perda de volume, a associação verniz fluoretado 5% + laser Er,Cr:YSGG mostrou os melhores resultados em comparação com os demais grupos.

Palavras-chave: Laser de YSGG, Erosão dental, Prevenção, Hipersensibilidade dentinária.

ABSTRACT

ABSTRACT –

The objective of this in vitro study was to evaluate the influence of the Er, Cr: YSGG laser, associated or not to desensitizing agents, in the prevention of acid erosion in bovine root dentin. Eighty dentin fragments were obtained, (4mm x 4mm and 3mm) randomly divided 8 groups (n=10). G1: negative control; G2: application of 5% fluoride varnish (positive control); G3: application of the Er, Cr: YSGG laser (0.1W; 5Hz; without water-cooling); G4: application of 5% fluoride varnish + Er, Cr: YSGG; G5: application of potassium oxalate 3%; G6: application of 3% potassium oxalate + Er, Cr: YSGG; G7: passive application of biphasic calcium silicate / phosphate gel; G8: passive application of biphasic calcium silicate / phosphate + Er, Cr: YSGG laser. Half of the fragment was properly isolated (control region) and the other half received one of the proposed treatments. The erosive drink used was a cola soft-drink (pH=2,42 at 4°C), lasting 5 minutes each erosive challenge. This procedure was performed twice a day, with 6-hour intervals between the challenges, for a total period of 14 days. Normality (Kolmogorov-Smirnov) and homogeneity (Levene's) of the data were evaluated. After these analyzes, the surface roughness data were submitted to the statistical analysis of Variance Analysis (ANOVA) and post-test of Tukey, to differentiate the mean of the groups. For the wear profile, the Kruskal-Wallis test and the Dunn post-test were used. Afterwards, the Spearman correlation test was performed. All statistical tests assumed a significance level of 5% ($\alpha = 0.05$). It was observed that the G1 presented the highest surface roughness value after the erosive challenge ($3,586 \mu\text{m}^2 \pm 0,205 \mu\text{m}^2$) and the G7 presented the lowest surface roughness value after the erosive challenge ($1,071 \mu\text{m}^2 \pm 0,180 \mu\text{m}^2$). As for volume loss, G4 presented the lowest percentage ($9.7\% \pm 0.9\%$) of lost volume, while G1 had the highest percentage ($41.8\% \pm 2.5\%$), both with ($p < 0.05$). There was a weak correlation between the response variables ($\rho = 0.33$). It was concluded that all groups presented lower values of surface roughness and loss of volume when compared to the negative control group. For the surface roughness, the biphasic calcium silicate / phosphate gel presented the best result. For volume loss, the 5% fluoride varnish + Er, Cr: YSGG laser showed the best results compared to the other groups.

Key words: YSGG laser, Dental erosion, Prevention, Dentinal hypersensitivity.

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LISTA DE ABREVIATURAS, SIGLAS E SÍMBOLOS

µm micrômetro

CO₂ dióxido de carbono

Er:YAG laser de érbio dopado com ítrio, alumínio, granada

Er,Cr:YSGG laser de érbio-cromo dopado com ítrio, scandium, gálio, granada

Nd:YAG laser de neodímio dopado com ítrio, alumínio, granada

He-Ne laser de hélio-neônio

et al. e colaboradores

F flúor

G grupo

HD hipersensibilidade dentinária

Hz hertz

J/cm² joule por centímetro quadrado

kV quilovolt(s)

mL mililitro(s)

mm milímetro(s)

°C grau Celsius

pH logaritmo negativo de concentração hidrogeniônica (-log[H⁺])

W watts

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1. INTRODUÇÃO

1. INTRODUÇÃO

A aplicação de métodos preventivos na Odontologia levou à diminuição do número de lesões cáries na população e, como consequência, houve redução na perda dos elementos dentais. Por outro lado, o maior tempo de permanência dos dentes na cavidade oral trouxe aumento na ocorrência de lesões cervicais não cáries, tais como erosão, abrasão, abfração e atrição (BARTLETT e SHAH, 2006; GRIPPO *et al.*, 2012; LOPES e ARANHA, 2013; HASHIM *et al.*, 2014).

O esmalte é o substrato mais comumente associado com a erosão. No entanto, a dentina radicular também pode ser afetada se for exposta ao meio bucal. O aumento da recessão gengival é fator importante que contribui para a exposição da dentina, que pode levar ao aparecimento da hipersensibilidade dentinária (HD). (HARA *et al.*, 2006).

A exposição da dentina ao meio bucal surge em decorrência da perda do esmalte e do cemento (TORWANE *et al.*, 2013). Essa perda é resultado de vários fatores, como: raspagem sub-gengival, apinhamento dental, recessão gengival ou pela associação de dois ou mais fatores. A associação destes fatores, como abrasão, abfração e erosão ácida também acarretam HD. A erosão ácida pode surgir através dos fatores extrínsecos (alimentos e bebidas ácidas, como frutas cítricas, café, refrigerantes, vinho e as demais bebidas alcoólicas) e os intrínsecos (anorexia, xerostomia, bulimia e refluxo gástrico), e até mesmo a força aplicada na escova dental pode ser um fator agravante da erosão (EHLEN *et al.*, 2008; MAGALHÃES *et al.*, 2009; NAIDU *et al.*, 2014).

A hipersensibilidade dentinária (HD) ou hiperalgesia é compreendida como sendo uma dor aguda, de curta duração, manifestando-se de maneira desconfortável para o paciente. Essa hiperalgesia ocorre devido à presença de túbulos dentinários abertos em uma superfície dentinária exposta (RIMONDINI *et al.*, 1995; REES e ADDY 2002; TORWANE *et al.*, 2013).

Conforme abordado no Canadian Advisory Board on Dentin Hypersensitivity, a hipersensibilidade dentinária tem sido definida como "uma dor derivada da dentina exposta, em resposta a agentes químicos, táteis, térmicos ou estímulos osmóticos que não podem ser explicados como resultante de qualquer outro defeito ou doença dental". É uma dor nítida, localizada e de curta duração, sem o envolvimento de bactérias (HASHIM *et al.*, 2014).

A erosão ácida tem sido apontada como um dos principais fatores desencadeadores da HD, podendo atuar isoladamente ou em associação com algumas situações clínicas. (SCHEUTZEL 1996; KELLEHER e BISHOP 1999; HE *et al.*, 2011).

A presença da erosão dental pode gerar hipersensibilidade dentinária, comprometer a estética do paciente ou levar a problemas de funcionalidade como, por exemplo, uma eventual exposição da polpa, em estágios avançados (SCHLUETER *et al.*, 2012). Portanto, diferentes abordagens têm sido investigadas para evitar um desequilíbrio no processo desmineralização/remineralização com consequente perda de minerais.

Existem vários métodos disponíveis para o tratamento da hipersensibilidade dentinária (ADDY e WEST 2013; MALEKI *et al.*, 2015; TAHA *et al.*, 2015), todos com o mesmo intuito: vedar os túbulos dentinários. Dentre esses métodos, pode-se citar: uso de vernizes fluoretados, oxalato de potássio, sistema adesivo autocondicionante, gel fluoretado, laser e dentifrícios especiais como: o gel bifásico com silicato de cálcio e sais de fosfato de sódio e pastas com monofluorofosfato de sódio. Outro método também utilizado para tratar a hipersensibilidade dentinária é a iontoforese. Porém, os compostos fluoretados são os mais utilizados para a redução da hipersensibilidade dentinária (VAN DEN BERGHE *et al.*, 1984; CAMILOTTI *et al.*, 2012).

GAFFAR (1999) em sua pesquisa com o verniz fluoretado Duraphat gerou a formação de cristais de fluoreto de cálcio que impediam a abertura dos túbulos dentinários, promovendo a remineralização e consequentemente um alívio da hipersensibilidade dentinária. O oxalato de potássio é um agente dessensibilizante que age na obliteração dos túbulos e despolarização de terminações nervosas; é apresentado tanto na forma de dentifrícios quanto em aplicações tópicas (ASSIS *et al.*, 2011). STEAD *et al.*, (1996) notaram a redução da permeabilidade dentinária devido à obliteração dos túbulos dentinários, porém esse resultado era temporário pois os cristais eram dissolvidos parcialmente na saliva.

Uma nova tecnologia foi desenvolvida com base na combinação de silicato de cálcio, sais de fosfato de sódio e fluoreto, que propõe aumentar os processos de mineralização natural da saliva e a formação de minerais de esmalte dentário. O proposto mecanismo pode ajudar a reparar o esmalte após desafios ácidos (PARKER *et al.*, 2014). O gel de silicato de cálcio e sais de fosfato de sódio se combinam e se integram ao dente, regenerando o esmalte, recuperando assim sua composição mineral. Esse gel dual é aplicado passivamente na moldeira e é composto por duas partes: parte A - composto de

silicato de cálcio, sais de fosfato e monofluorofosfato de sódio e parte B - (ativador gel), composto de fluoreto de sódio (HORNBY *et al.*, 2014).

O composto atua nos estágios iniciais da erosão. O uso do gel aumenta a eficácia do creme dental em 43%, maximizando o poder de remineralização do esmalte, mesmo em locais de difícil acesso como entre os dentes. (JOINER A *et al.*, 2014). Dessa forma, proporciona proteção direcionada para os dentes contra os efeitos da erosão do esmalte e de ataques ácidos. (JOINER A *et al.*, 2014). Entretanto ainda não foram encontrados estudos desses agentes na dentina.

Pesquisas recentes demonstram resultados promissores no que diz respeito ao tratamento da HD com o uso de *laser* na área da odontologia clínica, no qual grandes variedades de procedimentos são realizadas com este dispositivo, como preparo de cavidade, remoção de cárie, remoção de restauração, condicionamento da superfície, tratamento da sensibilidade dentinária, prevenção de cárie e clareamento (HASHIM *et al.*, 2014). Estes procedimentos são realizados através da interação do laser com o tecido, causando diferentes reações teciduais, de acordo com o seu meio ativo, comprimento de onda e densidade de energia para as propriedades ópticas do tecido alvo (HUSEIN, 2006). Desde os experimentos realizados com o *laser* de rubi, outros lasers foram testados e utilizados no tratamento da hipersensibilidade dentinária, tais como: CO₂, diodo (GaAlAs), He-Ne, Nd:YAG, Er:YAG, Er,Cr:YSGG (KUMAR e MEHTA 2005; YILMAZ *et al.*, 2011; ARANHA e EDUARDO 2012).

PALAZON *et al.*, (2013) avaliaram o efeito do laser Nd:YAG e dessensibilizante (pasta Colgate Sensitive Pró- Alívio) na obliteração dos túbulos dentinários. Após o tratamento as amostras foram submetidas a uma sequência de desafios erosivos e abrasivos e observou-se que apenas o tratamento com a irradiação com *laser* Nd:YAG foi capaz de vedar imediatamente os túbulos dentinários, contudo nenhum dos tratamentos realizados mostrou eficácia na manutenção de vedação desses túbulos dentinários após estes serem submetidos aos desafios erosivos e abrasivos. ARANHA e EDUARDO (2012) seguiram a mesma linha de pesquisa e obtiveram resultados semelhantes, avaliando 2 *lasers*: Er,Cr:YSGG com duas potências diferentes (0,25W e 0,50W) e Er:YAG. Baseado nos resultados e dentro dos limites do estudo, concluíram que nenhum dos tratamentos a *laser* foi capaz de eliminar completamente a dor, porém o *laser* Er,Cr:YSGG a uma potência de 0,25W exibiu o melhor desempenho nas avaliações.

O uso do *laser* Er:YAG associado ao flúor tópico (gel de flúor fosfato acidulado 1,23%) na prevenção de lesões erosivas no esmalte também foi estudado em trabalho

recente. Os tratamentos feitos não preveniram o desgaste dental e, de acordo com os autores, é necessária a realização de outros estudos para determinar comprimento de onda, protocolo de aplicação e sua ação com flúor para ser utilizado como um método de prevenção de processos erosivos, visto que existem poucos estudos que abordam o uso do *laser* associado com o flúor na prevenção da erosão dental. (DOS REIS DERCELI *et al.*, 2013).

De acordo com DILSIZ *et al.*, (2009), o Nd:YAG é eficaz no tratamento da HD em relação ao Er:YAG e diodo 685-nm, pois em três meses de estudos obtiveram resultados promissores em relação ao tratamento proposto. A hipersensibilidade dentinária representa um grande problema para pacientes que possuem doença periodontal e constantemente apresentam recessão gengival e superfícies da raiz expostas. O fato mais importante do uso da laserterapia, e que deve ser sempre considerado, é alcançar resultados satisfatórios, sem provocar danos pulparem prejudiciais, fraturas e carbonização (MOHAMMAD e MASOUMEH 2013).

A aplicação do laser Er,Cr:YSGG na superfície dental provoca aumento da temperatura inferior a 5,5°C, o que altera sua estrutura química e deixa a superfície menos solúvel (FREITAS *et al.*, 2010). Um estudo de HOSSAIN *et al.*, (2001) mostrou que o laser Er,Cr:YSGG aumentou a resistência da dentina em meios ácidos.

As características ultra-estruturais da dentina dependem principalmente dos parâmetros do laser como a potência de saída, frequência e modo de aplicação, porque esses parâmetros estão diretamente relacionados com o aumento da temperatura (GERALDO-MARTINS 2005; ALFREDO *et al.*, 2008).

Desta forma, torna-se importante o estudo dos parâmetros adequados para a utilização do laser Er,Cr:YSGG com a finalidade de aumentar a resistência ácida da dentina, pois é uma técnica promissora que pode promover resultados efetivos e de longa duração, gerando maior conforto ao paciente, além de ser um método rápido, podendo atender uma grande parcela da população.

Métodos *in vitro* foram propostos (YU *et al.*, 2010) para avaliação da erosão dentária, incluindo o perfil de desgaste e a rugosidade superficial através do microscópio confocal a laser LEXT OLS4000 (Olympus Japão).

Em 1992, SÖDERHOLM *et al.*, estudaram métodos de medida da rugosidade superficial e atribuíram às técnicas avançadas de perfilometria uma melhor capacidade de resolução e registro de desgastes localizados em relação ao método de microscopia óptica, primeiramente empregado.

A rugosidade é um dos parâmetros mais utilizados para quantificar alterações das superfícies dentárias em estudos *in vitro*. Esta propriedade foi analisada pois a presença de irregularidades superficiais no dente acarreta a retenção de biofilme bacteriano, aumentando o risco de cárie e de inflamação periodontal (LEPRI e PALMA-DIBB 2012). Já foi estudado que existe correlação positiva entre rugosidade e adesão de biofilme (NOGUEIRA *et al.*, 2017). Entretanto, uma limitação do uso desta propriedade é que seu valor não contempla as características de textura da superfície em estudo, apresentando apenas informações sobre sua altura (picos) e profundidade (vales) da superfície em intervalos regulares (FIELD *et al.*, 2010), que é amenizada com o emprego de microscopia confocal a laser (DERCELI *et al.*, 2013) que realiza leituras de um feixe a laser e permite a avaliação tridimensional da superfície.

O uso do microscópio confocal a laser Olympus LEXT OLS4000 leva a maiores resoluções dessas imagens capturadas e permite a observação de mudanças superficiais como prismas de esmalte, túbulos dentinários e áreas com erosão, sendo compatível com a desmineralização pois não causa danos na superfície da amostra porque não há forças de contato entre elas (DERCELI *et al.*, 2013).

É possível quantificar, por meio do microscópio confocal a laser, a perda de substrato dental empregando imagens capturadas. Para esta avaliação, é necessário que a superfície a ser analisada seja plana e que haja uma área de referência (isto é, controle), contra a qual o cálculo da perda de volume da área exposta ao agente erosivo se baseará. A perda de volume é calculada como o volume da relação em μm^3 dividido pela área analisada, o que permite ver quantitativamente o volume perdido na superfície do dente. (DERCELI *et al.*, 2013).

Portanto, parece oportuno analisar a rugosidade superficial de área (um^2) e a perda de volume (um^3), considerando esses dois métodos citados acima para mensurarmos a porcentagem de volume perdido da área tratada seguida de erosão em relação a área não tratada e conseqüentemente o desgaste superficial.

A hipótese nula é que não haveria diferença estatisticamente significativa na rugosidade superficial e na perda de volume nos diferentes grupos, independentemente do tratamento realizado.

2. PROPOSIÇÃO

2. PROPOSIÇÃO

O objetivo do presente estudo foi avaliar a influência do laser Er,Cr:YSGG, associado ou não a agentes dessensibilizantes, na prevenção da erosão ácida em dentina radicular bovina, utilizando os seguintes métodos de análises: rugosidade superficial (parâmetro Ra em μm^2) e avaliação do perfil de desgaste (percentual da perda de volume), realizados por meio da microscopia confocal de varredura a laser.

3. CAPÍTULO 1

Influence of Er,Cr:YSGG laser, associated or not to desensitizing agents, in the prevention of acid erosion in bovine root dentin.

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Concise title: Influence of Er,Cr:YSGG laser on dentin acid erosion prevention

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ABSTRACT

Purpose: This *in vitro* study evaluated the influence of the Er,Cr:YSGG laser, associated or not to desensitizing agents, in the prevention of acid erosion in bovine root dentin.

Methods: Eighty dentin specimens were obtained and divided into 8 groups (n=10). G1:negative control; G2:5% fluoride varnish(positive control); G3:Er,Cr:YSGG laser; G4:5% fluoride varnish+Er,Cr:YSGG; G5:potassium oxalate 3%; G6:3% potassium oxalate+Er,Cr:YSGG; G7:biphasic calcium silicate/phosphate gel; G8:biphasic calcium silicate/phosphate+Er,Cr:YSGG laser. The erosive drink used was a cola soft-drink (pH=2.42 at 4°C), lasting 5 minutes, twice a day, with 6-hour intervals between the challenges, during 14 days. Normality(Kolmogorov-Smirnov) and homogeneity(Levene's) of the data were evaluated. The surface roughness data were submitted to the statistical analysis of Variance Analysis(ANOVA) and Tukey post-hoc. For the wear profile, Kruskal-Wallis and Dunn post-hoc tests were used. Afterwards, the Spearman correlation test was performed. All statistical tests assumed a significance level of 5% ($\alpha=0.05$). **Results:** G1 presented the highest surface roughness value after the erosive challenge ($3.586\mu\text{m}^2\pm 0.205\mu\text{m}^2$) and the G7 presented the lowest surface roughness value after the erosive challenge ($1.071\mu\text{m}^2\pm 0.180\mu\text{m}^2$). For the lost volume, G4 presented the lowest percentage ($9.7\%\pm 0.9\%$), while G1 had the highest percentage ($41.8\%\pm 2.5\%$), both with ($p<0.05$). There was a weak correlation between the response variables ($\rho=0.33$). **Conclusions:** All groups presented lower values of surface roughness and loss of volume when compared to the negative control group. For the surface roughness, the biphasic calcium silicate/phosphate gel presented the best result. For volume loss, the 5% fluoride varnish+Er,Cr:YSGG laser showed the best results compared to the other groups.

Key words: YSGG laser, Dental erosion, Prevention, Dentinal hypersensitivity.

4. INTRODUCTION

Dentin hypersensitivity (DH) or hyperalgesia is understood to be an acute pain, of short duration, manifesting in an uncomfortable way for the patient. This hyperalgesia occurs due to the presence of open dentinal tubules on an exposed dentin surface [1,2,3]. Acid erosion has been pointed out as one of the main triggers of DH, and can act alone or in association with some clinical situations [4,5,6].

There are several methods available for the treatment of DH [7,8,9], with the same intention: to seal the dentinal tubules. Among these methods, we can mention use of fluoride varnishes, potassium oxalate, self-etching adhesive system, fluoride gel, laser and special dentifrices such as biphasic gel with calcium silicate and sodium phosphate salts and slurries with sodium monofluorophosphate.

GAFFAR (1999) in his research with the fluoride varnish Duraphat observed the formation of calcium fluoride crystals, which prevented the opening of the dentinal tubules, and in conjunction promoting the remineralization of a lasting relief of the dentin hypersensitivity. Potassium oxalate is a desensitizing agent that acts on tubule obliteration and depolarization of nerve endings. It is presented both in the form of dentifrices and in topical applications [11]. STEAD *et al.* (1996) noted a decrease in dentin permeability due to obliteration of dentinal tubules, but this result was temporary because the crystals were partially dissolved in the saliva.

The calcium silicate gel and sodium phosphate salts combine and integrate to the tooth, remineralizing the enamel, thus recovering its mineral composition. However, it acts in the early stages of erosion. The use of the potentializer increases the effectiveness of toothpaste by 43%, maximizing the power of the enamel, even in hard-to-reach places such as between the teeth [13]. In this way, it provides a targeted protection for the teeth against the effects of enamel erosion and acid attacks [13]. However, no studies of these agents have been found in dentin.

Recent research has shown promising results regarding the treatment of DH with the use of laser in the area of clinical dentistry, in which large varieties of procedures are performed with this device, such as cavity preparation, caries removal, restoration removal, surface treatment, dentin sensitivity treatment, caries prevention and bleaching [14].

The application of the Er,Cr:YSGG laser on the dental surface causes an increase in temperature and changes its chemical structure, leaving the surface less soluble [15].

Therefore, it is important to study the adequate parameters for the use of Er,Cr:YSGG laser in order to increase the acid resistance of dentin, since it is a promising technique that can promote effective and long-lasting results, generating greater comfort to the patient, besides being a fast method and able to attend a part of the population.

In vitro methods were proposed [16] for evaluation of dental erosion, including wear profile and surface roughness using the LEXT OLS4000 laser confocal microscope (Olympus Japan).

Surface roughness is one of the parameters most used to quantify dental surface changes for *in vitro* studies. This property was analyzed because the presence of superficial irregularities in the tooth entails the retention of bacterial biofilm, increasing the risk of caries and periodontal inflammation [17].

The volume loss is calculated as the volume ratio in μm^3 divided by the analyzed area, which allows to quantitatively seeing the lost volume at the surface of the tooth [18].

Therefore, the present study analyzed surface roughness (μm^2) and volume loss (μm^3), considering these two methods mentioned above to measure the percentage of lost volume of the treated area followed by erosion in relation to the untreated area and consequently the wear superficial.

The null hypothesis is that there would be no statistically significant difference in surface roughness and loss of volume in the different groups, independently of employed treatment.

5. OBJECTIVE

The aim of the present study was to evaluate the influence of Er,Cr:YSGG laser, associated or not to desensitizing agents, in the prevention of acid erosion in bovine root dentin, using the following methods of analysis: surface roughness (parameter Ra in μm^2) and evaluation of the wear profile (percentage of lost volume), performed by laser scanning confocal microscope.

6. MATERIALS AND METHODS

6.1 Experimental design

The factor under study was: treatment of the specimens in eight levels: G1: No treatment (negative control); G2: duraphat varnish (positive control); G3: Er,Cr:YSGG laser; G4: Duraphat varnish + Laser Er,Cr:YSGG; G5: oxagel; G6: oxagel + Laser Er,Cr:YSGG;

G7: Regenerate Boost Serum; G8: Regenerate Boost Serum + Laser Er,Cr:YSGG. All the irradiated groups used the following parameters: 0,1W; 5Hz, without water-cooling and 55% of air. The sample of the experiment was 80 bovine root dentin specimens divided into these 8 groups (n=10). The quantitative response variables were: surface roughness (parameter Ra in μm^2) and evaluation of the wear profile (percentage of lost volume).

6.2 Teeth selection

Forty bovine incisors were selected, with an age of 18-25 years, without the presence of cracks and wear. The teeth were cleaned using a periodontal curette and then immersed in a 10% formalin solution (pH=7) for 7 days for sterilization. These teeth were then washed and stored in distilled and deionized water at a temperature of 4°C, exchanged daily for a period of 14 days.

6.3 Specimens preparation

The incisors were sectioned by separating the coronary portion from the root with the use of a diamond disk under refrigeration in the ISOMET® 1000 cutting machine (Precision Saw Buehler, Illinois - USA). The first cut was performed 1mm below the enamel-cement junction. The second cut was performed in the mesio-distal direction, obtaining two halves (buccal and lingual). Each half was again sectioned to obtain specimens in the initial dimensions of 4.25mm x 4.25mm. The specimens had their sides polished on the Arotec APL-4 polishing machine (Series 41042, Arotec S.A. industry and trade), using sandpaper # 600 with water cooling to standardization at 4mm x 4mm, resulting in a surface area of 16mm². The polishment was not performed on the evaluated surface of the specimen. Changes in dimensions were allowed in 10%, for more or less.

Half of the surface of each specimen was covered with adhesive tape. Two layers of red nail cosmetic enamel and sculpt wax were applied, and their impermeabilization was performed. After this procedure, the insulation tape was removed and each specimen was left with half the free surface of the protection made with cosmetic enamel and wax. The specimens were stored in distilled and deionized water at a temperature of 4°C until the proposed treatment was performed, where they were randomly divided into 8 groups (n=10) and each group received its treatment as described in Table 1.

6.4 Treatment of specimens

The fluoride varnish (5% sodium fluoride) used was Duraphat® (Colgate-Palmolive Ind. And Co., São Paulo, SP, Brazil) with a disposable applicator (microbrush) and after 4 minutes the excess was removed with a sterile gauze. The laser device was the Er,Cr:YSGG (Waterlase Millennium, Biolase Technologies Inc., San Clemente, USA), with fiber containing 600µm in diameter (tip model: ZipTip MZ6 3mm), irradiated for 10 seconds in scan mode, at 1mm irradiance distance (Table 2) and with a wave-length of 2.78µm. The use of the laser without water cooling was based on previous study, where it was verified that the water could ablate the tissue, thus showing less effectiveness in the preventive treatment [15]. For the potassium oxalate, it was used Potassium oxalate monohydrate 3%, with disposable applicator (microbrush); it was maintained with slight excess in contact for 2 minutes, and the excess was removed with a sterile gauze. The dual regenerate gel composed of two parts: part A - calcium silicate, phosphate salts and sodium monofluorophosphate and part B - sodium fluoride (activator gel) was applied passively with microbrush for 3 minutes and the excess was removed with a sterile gauze.

6.5 Erosive challenge

After the treatments, the specimens were submitted to the erosive challenge: Each group was placed separately in a becker and were immersed in Coca-Cola® (Cia. De Bebidas Ipiranga, Ribeirão Preto, SP, Brazil) with a pH of 2.42 at 4°C for 5 minutes on a magnetic stirrer (ABC-LAB, model 221-1).

After this time, the erosive solution was discarded and the specimens were washed with distilled and deionized water for 10 seconds and stored again in that water. This procedure was performed twice a day, with 6-hour intervals between the challenges, for a total period of 14 days. The specimens were stored at 4°C immersed in distilled and deionized water until analysis. The enamel and wax (control area) of each specimen were removed using the lecron instrumentation. There was no contact of the instrument with the central surface of the specimen, only on the sides.

6.6 Analysis of surface roughness and wear profile

For these analyses, the specimens were placed parallel to the table of the confocal laser-scanning microscope LEXT (Olympus, Japan) with the aid of the parallelogram.

After selecting the central region of the 1mm x 1mm specimen, image acquisition was performed with a 20x magnification lens. After obtaining the images, these were analyzed for wear profile and surface roughness (parameter Ra). For the analysis of surface roughness, the central region was measured, encompassing the control region (reference) and the eroded area. The data, in μm^2 , were acquired by means of a specific software (OLS4000®) (Figure 1).

The wear profile was determined by the difference between the volume of the reference area and the eroded area between the midline of the graph. Data were obtained in μm^3 and for the statistical calculations, a comparison was made between the control area and the eroded area, transforming in percentage of lost volume.

6.7 Statistical analysis

The surface roughness data were submitted to the statistical analysis. Firstly, it was observed that the data presented normal distribution (Kolmogorov-Smirnov) and homogeneous (Levene). After this, we applied the analysis of variance (ANOVA) for comparison of the means and Tukey's post-hoc for multiple comparisons. The wear profile data were submitted to Kruskal-Wallis test and the Dunn post-hoc. Afterwards, the Spearman correlation test was performed. All statistical tests assumed a significance level of 5% ($\alpha = 0.05$).

7. RESULTS:

7.1 Surface roughness

The surface roughness values are described in Table 3.

For the reference area, all groups presented surface roughness values with statistical similarity ($p > 0.05$).

In the area submitted to the erosive challenge (treated + eroded), the surface roughness values of the negative control group were higher and with a statistically significant difference in the other groups ($p < 0.05$). There was no statistically significant difference between the groups: G2, G3, G4, G5, G6, G8 ($p > 0.05$).

The G7 group presented a statistically significant difference in relation to the other groups that had treatments ($p < 0.05$), evidencing a lower surface roughness of the analyzed area.

7.2 Wear profile

Regarding the percentages of volume lost, it was observed that G4 (duraphat lacquer + laser Er, Cr: YSGG) had the lowest volume loss ($p < 0.05$), only 9.7%, when compared to the others groups. On the other hand, the greatest loss of volume was observed in G1 (negative control), which presented 41.8% (Figure 2) and a statistically significant difference in relation to the other groups ($p < 0.05$) (Table 4).

7.3 Spearman correlation test:

There was a weak correlation between the response variables (surface roughness X wear profile ($\rho = 0.33$)).

8. DISCUSSION:

This study was conducted to evaluate the possible increase of acid resistance in bovine root dentin after erosive challenge with Coca-Cola®. The results showed that the proposed treatments had a statistically significant difference ($p < 0.05$) for the two analyzed properties. Thus, the null hypothesis that different treatments would have no effect on the dentin acid resistance after erosive challenge was rejected.

The initial standardization of the specimens was confirmed by the surface roughness results of the reference area, as there was no significant difference between the groups ($p > 0.05$). In addition, this similar result indicates that the waterproofing of the specimens was effective, since the surface roughness values of the reference area were smaller than the treated area followed by erosion.

The choice of bovine teeth was made due to the higher standardization of these teeth and was based on previous studies in which tests were made that proved that the human and bovine dentine substrates had similar morphology [19,20]. Furthermore, bovine teeth were used in several *in vitro* tests, such as erosion / abrasion test, shear bond strength test, among others [21].

It was performed the immersion of the specimens in Coca-Cola® because of its erosive potential already studied and discussed in several scientific works [22,23]. In addition to being a widely consumed beverage in the world, its pH of 2.42 (at temperature of consumption) is far below the critical pH of the dentin, unbalancing the process of demineralization-remineralization [24].

In a study of dental erosion induced by different beverages, Coca Cola® was considered the most erosive agent among hot and cold drinks studied (37%). After 10 minutes of

exposure to Coca Cola®, the exposed dental surface showed effects such as clearly visible enamel prisms, fissures, rough surfaces, showing signs of demineralization. In the SEM (scanning electron microscopy), it showed visible debris and exposed prisms and the hardness tends to decrease [25]. This corroborates with the results verified in the present study, in which dentin surface demineralization was observed after erosive challenge with Coca Cola®.

G1 showed the highest value of surface roughness and volume loss in the treated area followed by erosion, demonstrating that the absence of any kind of preventive treatment enhances the action of Coca Cola®, that is, demineralization on the surface of the root dentin.

The obtained data for surface roughness test showed that, in the control region, there was no statistically significant difference between the groups ($p>0.05$). The control region always had a lower roughness when compared to the treated and eroded area, for all groups.

In relation to the surface roughness, the groups G2, G3, G4, G5, G6 and G8 showed that there was no statistically significant difference after the erosive challenge with Coca Cola® among them, but it was more effective than G1 (negative control) that presented the highest surface roughness value ($3,586\mu\text{m}$). This explains the use of preventive treatments to avoid increasing surface roughness.

The G7 (application the calcium silicate/phosphate toothpaste) showed a lower surface roughness ($1,071\mu\text{m}$) after the erosive challenge compared to the other groups. This can be explained because this treatment based on calcium silicate and sodium phosphate can provide protection to the enamel by several mechanisms: releasing calcium ions to the surrounding oral fluids under acidic conditions, thus increasing the local concentration of calcium, the degree saturation with respect to enamel hydroxyapatite and inhibiting dissolution. [26]. Considering that enamel and dentin are constituted mostly by hydroxyapatite, it is expected that this remineralizing agent also protects the root dentin with similar mechanisms described above for the enamel.

As regards wear profile, the Er,Cr:YSGG laser showed to be effective, since it presented low values of lost volume when applied alone (G3) or in association with duraphat (G4), oxagel (G6), regenerate (G8), especially in the association of G4, which presented the lowest value of lost volume among all the studied groups (9.7%). G2, G5 and G7 presented lower lost volume only when compared to G1 that had the highest percentage among all groups (41.8%).

The Er,Cr:YSGG laser can be used in dentistry as a high and low power laser. For preventive purposes, it should be used with sub-ablative parameters to only modify chemically and morphologically the structures [27,28]. Some manuscripts have been published using this enamel laser, and have obtained positive responses for caries prevention [29,30]. For this reason and based on previous studies that showed that the use of the laser emission of 2.78 μ m-wavelength is also effective in dentin, we opted for the choice of this laser in the present study. This wavelength coincides with the water and hydroxyapatite absorption peaks [31,32,33].

Consistent studies such as that of Zach and Cohen (1965) have already shown that healthy pulp tissue is not thermally affected if the temperature rise were less than 5,5°C. Thus, the use of laser to increase the acid resistance of dentin is safe and a new option for preventive treatment [6].

Studies have proven that laser applications combined with fluoride may potentiate its effect. Gao et al. (2006) carried out a study to analyze the association of laser and fluoride application and observed that, when the laser is applied, there is a better absorption of fluoride in the root dentin, resulting in a better inhibition of demineralization.

Studies combining acidulated phosphate fluoride and Er,Cr:YSGG laser irradiation have shown that this association reduced enamel demineralization more than any fluoride treatment or laser treatment isolated [36,37]. The results of this study showed that the association of laser irradiation with fluoride varnish duraphat was more effective, because the ion incorporation on the specimen surface was possibly potentiated with treatment association, leaving the surface more resistant to acids, as demonstrated in G4.

In the literature, many techniques have been used to investigate the effects of erosive challenges on hard dental tissues. Micro-indentation, surface profilometry, microradiography, chemical analysis and SEM were considered the most advanced in this evaluation [16]. The use of confocal laser scanning microscopy allows the qualitative understanding of dentin surface demineralization processes by observing the specific and structural morphology characterizing dentin [38], analyzing the loss of substance and surface characteristics of dentin. The use of the Olympus LEXT OLS4000 laser confocal microscope leads to higher resolutions of these captured images and allows the observation of superficial changes such as enamel prisms, dentinal tubules and erosion areas, being compatible with demineralization, once it does not cause damage to the specimen's surface because there are no contact forces between them [18].

It would be interesting to submit these specimens to longitudinal microhardness analysis in future studies.

The present study demonstrated that these treatments increase the dentin acid resistance after erosive challenge. However, it is important further studies to ensure the use of these treatments over time, as well as periodic monitoring of the patient's oral health. In addition, dentists must recommend and instruct his patients to reduce the frequency of Coca Cola® ingestion.

9. CONCLUSION:

Considering the obtained results and the limitations of an *in vitro* study, it was concluded that all groups had lower values of surface roughness and loss of volume when compared to the negative control group. For the surface roughness, the biphasic Regenerate gel presented the best result. For wear profile, the 5% fluoride varnish + Er,Cr:YSGG laser showed the best results compared to the other groups, suggesting a promising preventive treatment of dentin hypersensitivity.

10. ACKNOWLEDGMENTS:

The authors would like to thank the financial support (scholarship) of the following funding agencies: CAPES (PROSUP-TAXA), CNPq (PIBIC) and FAPEMIG (PIBIC).

11. CONFLICT OF INTEREST

The authors state that there are no conflict of interest.

12. ROLE OF FUNDING SOURCE

CAPES (PROSUP-TAXA), CNPq (PIBIC) and FAPEMIG (PIBIC) provided scholarships and PAPE-UNIBE made possible the financial vialization of this project.

13. ETHICAL APPROVAL

This project followed all the ethical principles for medical research, according to Declaration of Helsinki.

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Legends:

Fig.1 Morphological differences between the control region (left side) and the treated followed by erosion region (right side).

Fig.2 3-D profilometry, showing the control region (left side) and the treated followed by erosion region (right side). The volume lost is represented by the yellow color.

Table 1: Treatment of specimens / experimental groups (n = 10)

Groups	Treatments
G1	No treatment (negative control)
G2	Application of 5% fluoride varnish (positive control)
G3	Application of Er,Cr:YSGG (0.1W; 5Hz, 55% air)
G4	Fluoride varnish + Laser Er,Cr:YSGG application (0.1W; 5Hz; air 55%)
G5	Application of 3% potassium oxalate
G6	Application of 3% potassium oxalate + Laser Er,Cr:YSGG (0.1W; 5Hz; air 55%)
G7	Applying the calcium silicate/phosphate toothpaste
G8	Application the calcium silicate/phosphate toothpaste + Laser Er,Cr:YSGG (0.1W; 5Hz; 55% air)

Table 2: Er,Cr:YSGG laser application parameters

Power	0.5W
Distance	1mm
Time	10 seconds
Mode of application	Surface scanning
Wave-length	2.78 μm
Fiber diameter	600 μm
Energy density	8.92 J/cm ²

Table 3: Mean values and standard deviation of the surface roughness of the groups, considering the control region and the treated + eroded area.

Groups	Reference area	Treated area followed by erosion
G1: No treatment (negative control)	0.844 (0.081) ^a	3.586 (0.205) ^c
G2: Application of 5% fluoride varnish (positive control)	0.821 (0.087) ^a	2.205 (0.084) ^b
G3: Application of Er,Cr:YSGG (0.1W; 5Hz, 55% air)	0.875 (0.069) ^a	2.155 (0.090) ^b
G4: Fluoride varnish + Laser Er,Cr:YSGG application (0.1W; 5Hz; air 55%)	0.893 (0.071) ^a	1.961 (0.104) ^b
G5: Application of 3% potassium oxalate	0.882 (0.091) ^a	2.104 (0.067) ^b
G6: Application of 3% potassium oxalate + Laser Er,Cr:YSGG (0.1W; 5Hz; air 55%)	0.849 (0.080) ^a	2.118 (0.099) ^b
G7: Applying the calcium silicate/phosphate toothpaste	0.852 (0.057) ^a	1.071 (0.180) ^a
G8: Application the calcium silicate/phosphate toothpaste + Laser Er,Cr:YSGG (0.1W; 5Hz; 55% air)	0.889 (0.092) ^a	1.956 (0.055) ^b

* Equal letters represent statistical similarity between groups ($p > 0.05$)

Table 4: Mean values and standard deviations of volume loss (%) in the groups studied:

Groups	Lost volume (%)	Standard Deviation
G1: No treatment (negative control)	4.,8 ^d	2.5
G2: Application of 5% fluoride varnish (positive control)	27.8 ^c	1.9
G3: Application of Er,Cr:YSGG (0.1W; 5Hz, 55% air)	20.5 ^b	1.4
G4: Fluoride varnish + Laser Er,Cr:YSGG application (0.1W; 5Hz; air 55%)	9.7 ^a	0.9
G5: Application of 3% potassium oxalate	29.2 ^c	1.1
G6: Application of 3% potassium oxalate + Laser Er,Cr:YSGG (0.1W; 5Hz; air 55%)	19.4 ^b	1
G7: Applying the calcium silicate/phosphate toothpaste	29.1 ^c	1.2
G8: Application the calcium silicate/phosphate toothpaste + Laser Er,Cr:YSGG (0.1W; 5Hz; 55% air).	19.7 ^b	1.4

* Equal letters represent statistical similarity between groups ($p>0.05$)

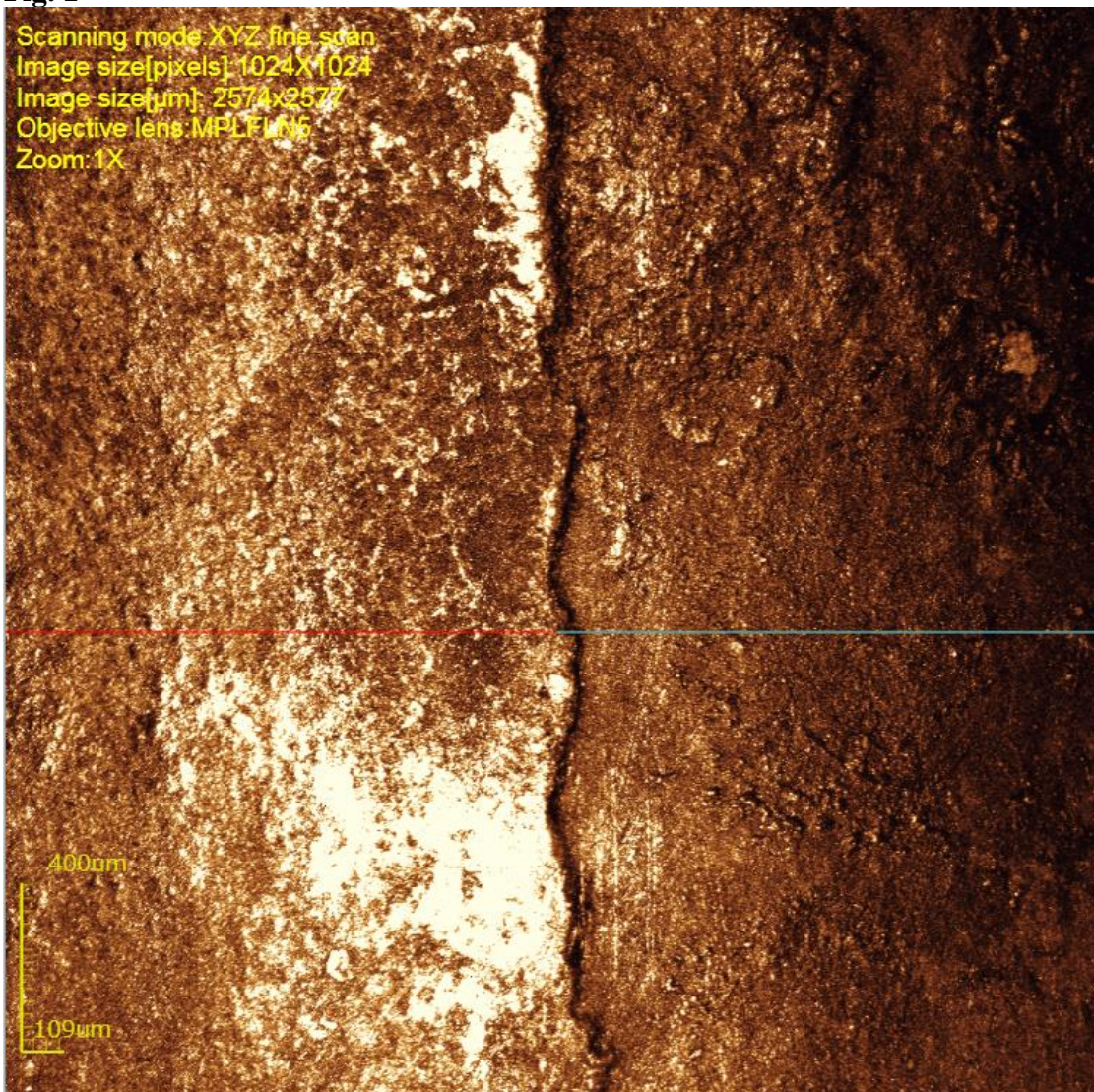
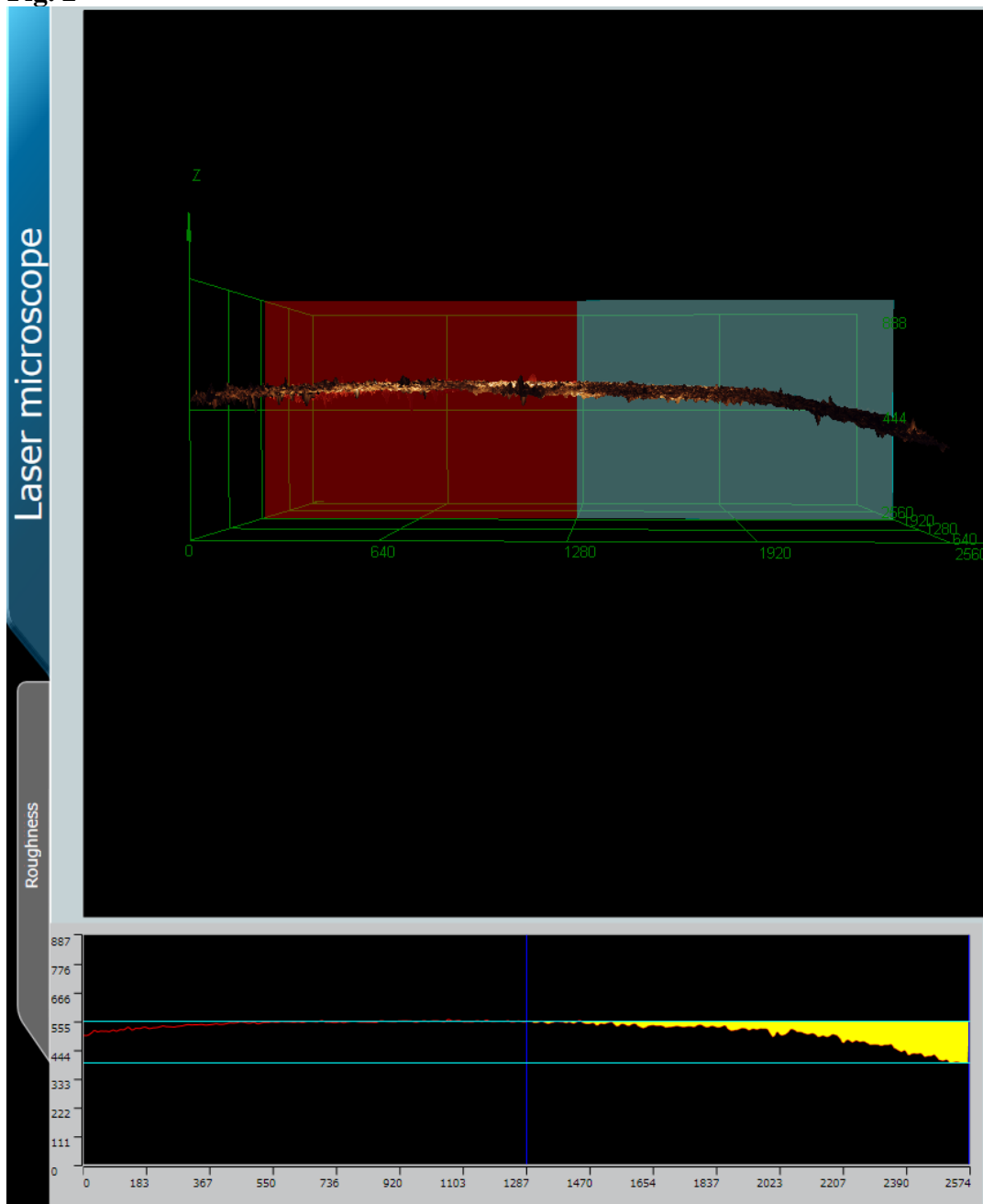
Fig. 1

Fig. 2



15. CONCLUSÃO

15. Conclusão:

Considerando os resultados obtidos e as limitações de um estudo *in vitro*, conclui-se que todos os grupos apresentaram menores valores de rugosidade superficial e perda de volume quando comparados ao grupo controle negativo. Para a rugosidade superficial, a aplicação do gel bifásico regenerate mostrou o melhor resultado, alterando significativamente os valores de rugosidade superficial da dentina radicular bovina. Para a perda de volume, a associação verniz fluoretado + laser Er,Cr:YSGG mostrou o melhor resultado em comparação aos demais grupos, sugerindo efeito promissor na prevenção da erosão ácida.

16. REFERÊNCIAS

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17. APÊNDICE:

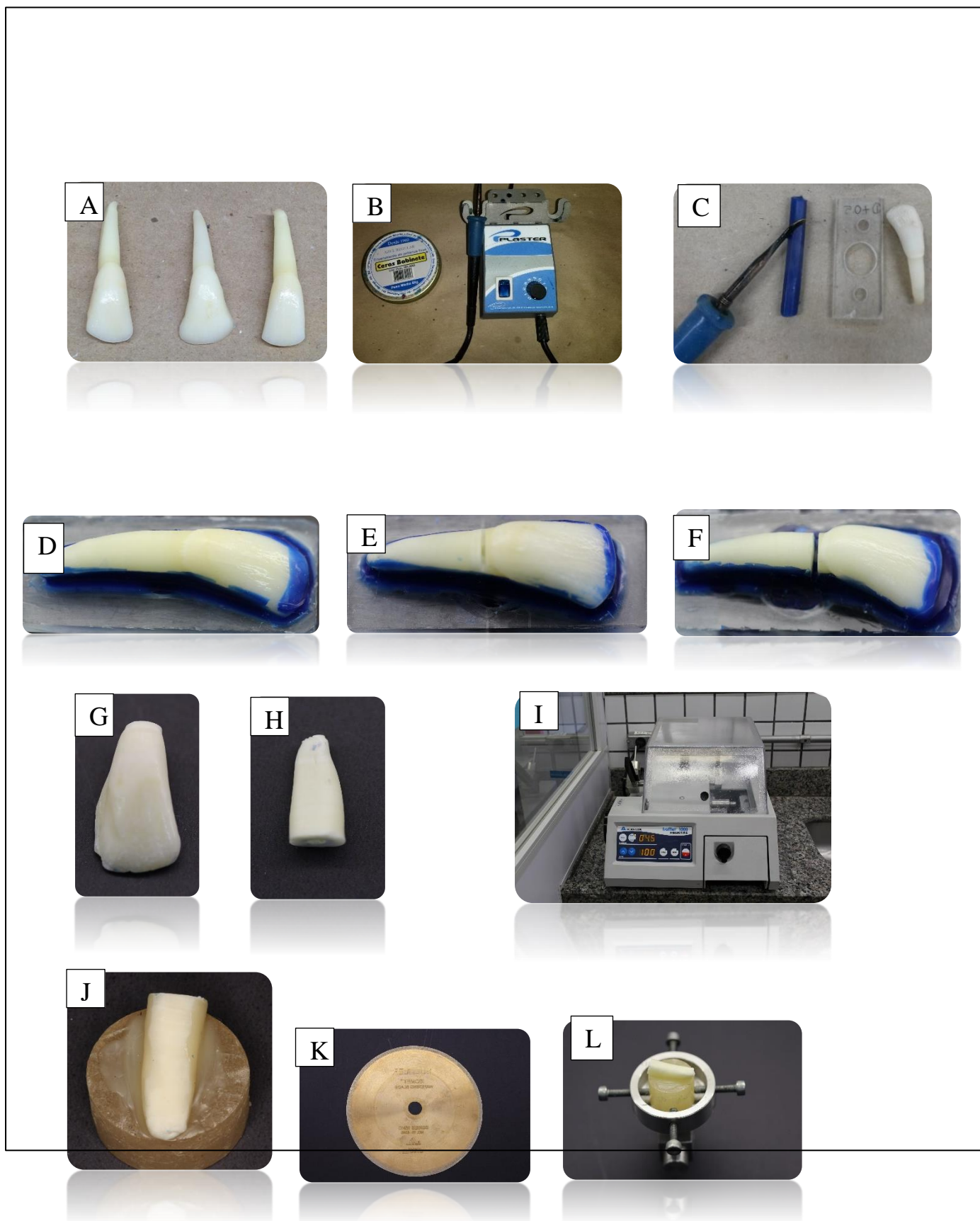


Figura 1: Preparo dos espécimes. A- Dentes hígidos bovinos. B- Gotejador elétrico. C- Gotejador elétrico e cera para fixação do dente na placa acrílica. D- Dente fixado com cera na placa acrílica. E- Início de separação coroa e raiz. F- Separação coroa e raiz. G- Coroa bovina. H- Raiz bovina. I- Máquina de corte -

ISOMET® 1000. J- Cilindro acrílico e fixação de raiz. K- Disco diamantado. L- Dispositivo utilizado para o preparo dos espécimes.

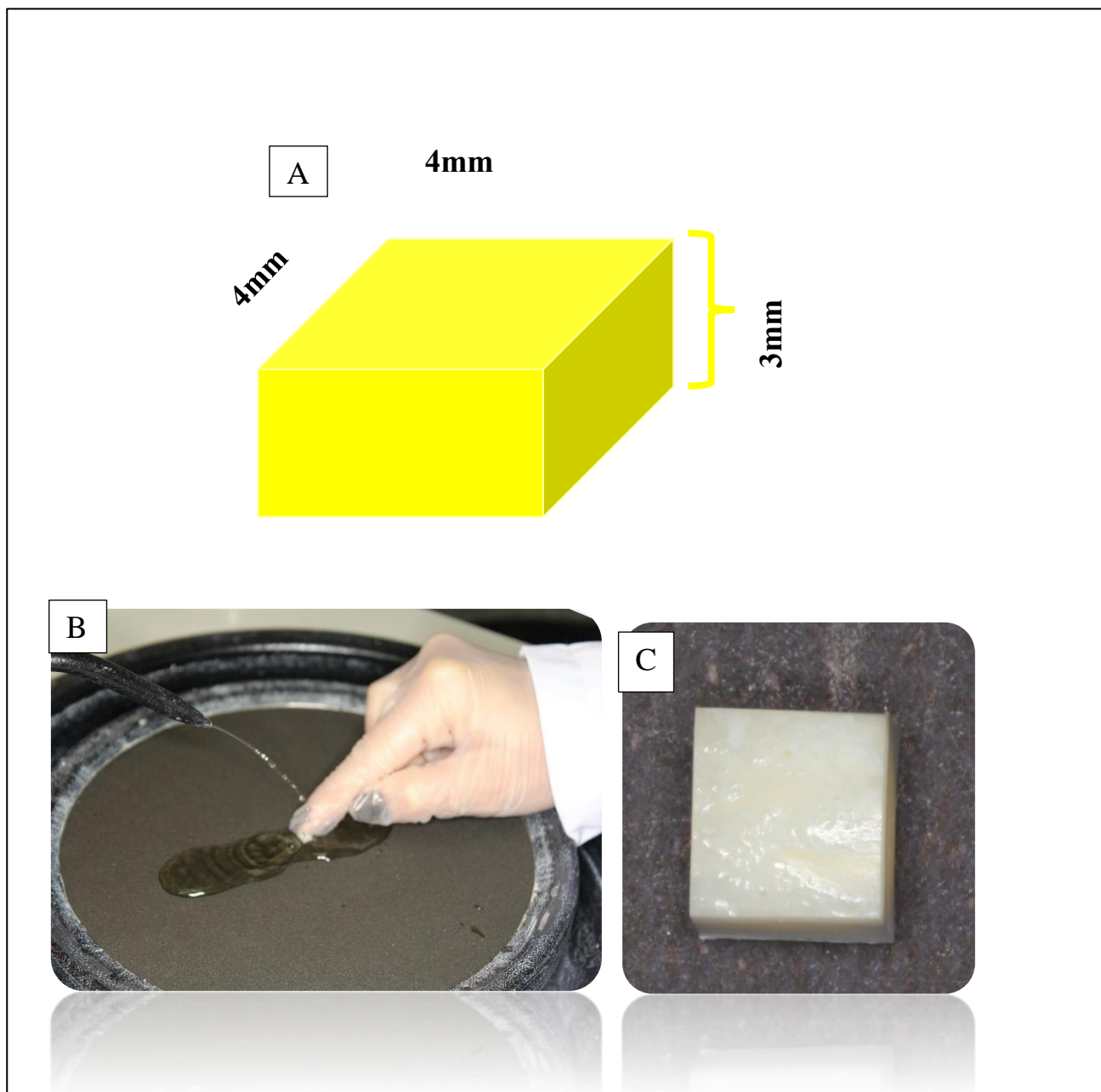


Figura 2: A- Medidas dos espécimes. B- Lixadeira e Politriz Metalográfica APL (Arotec) C- Espécime Padronizado.



Figura 3: A- Espécime com 3 camadas de esmalte. B- Espécime com fita isolante. C- Espécime pronto para o tratamento. D- Laser Er,Cr:YSGG. E- Verniz fluoretado a 5% Duraphat - Colgate. F- Aplicação do Verniz duraphat. G- Oxalato de potássio 3%- oxagel - Kota. H-Gel bifásico Regenerate - Unilever.

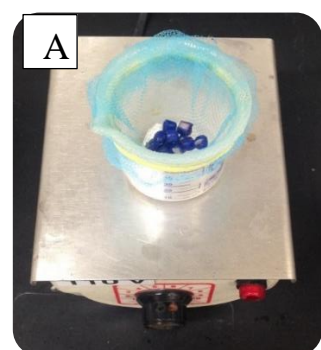


Figura 4: A- Agitador magnético. B- Coca-Cola®. C- Solução de Coca-Cola® no agitador magnético. D- Espécimes lavados em água destilada. E- Microscópio Confocal de varredura a laser 3D.

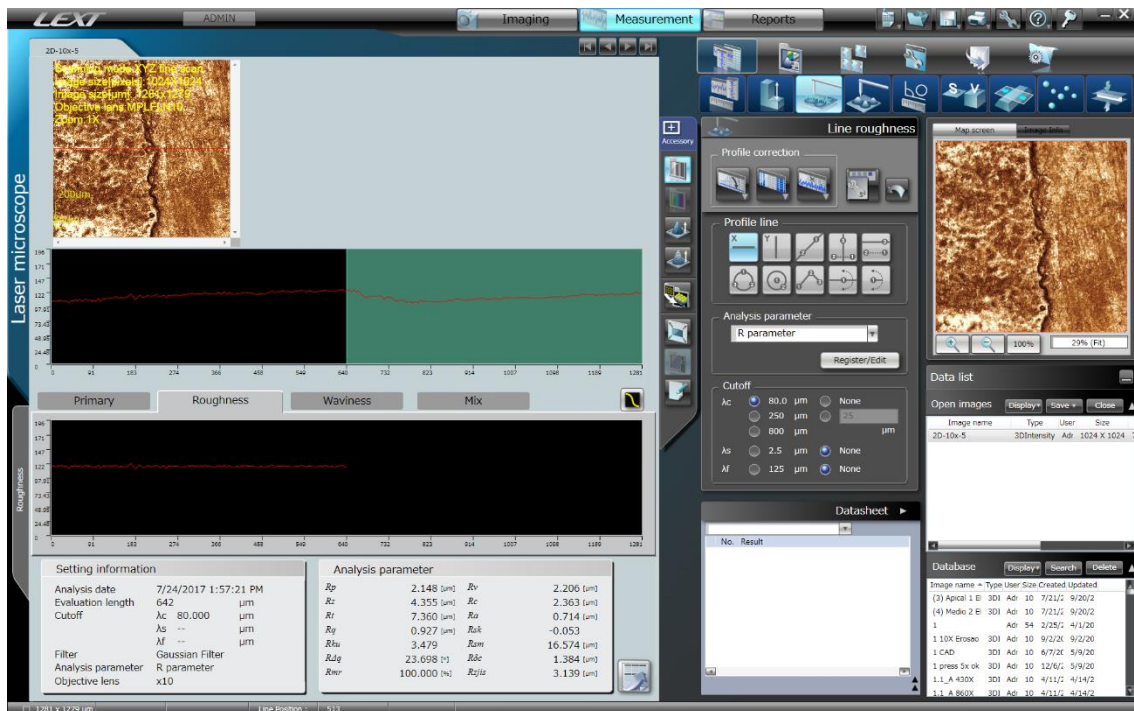


Figura 5: Imagem representativa da rugosidade superficial. A linha de rugosidade R_a está na cor vermelha. Esta linha desenhada dentro da cor preta representa a rugosidade na região controle enquanto dentro da cor verde representa a rugosidade na região tratada + erodida.

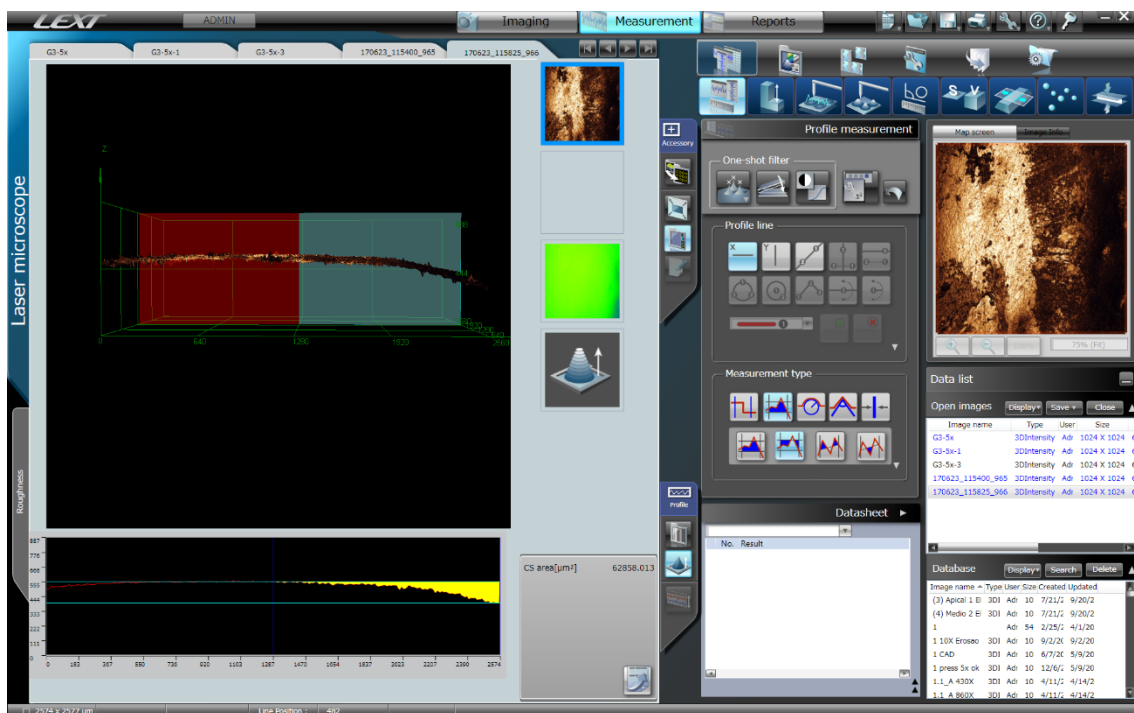
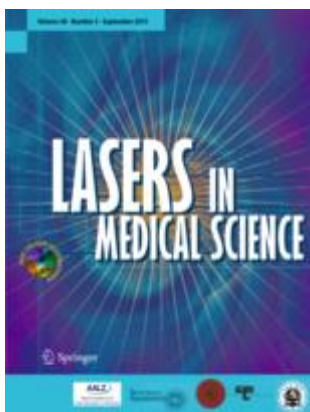


Figura 6: Imagem representativa do perfil de desgaste. A área na cor amarela representa o volume perdido.

18. ANEXOS:

18.1 Anexo 1: Normas para publicação no periódico “Lasers in Medical Science”



Instructions for Authors

TYPES OF PAPERS

- Original Article – limited to 4000 words, 45 references, no more than 5 figures
- Review Article – limited to 5000 words, 50 references, no more than 5 figures
- Brief Report - limited to 2000 words, 25 references, no more than 4 figures - Case Reports will not be accepted!
- Letter to the Editor – up to 600 words

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Ideally, the names of all authors should be provided, but the usage of “et al” in long author lists will also be accepted:
Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. *N Engl J Med* 965:325–329

- Article by DOI
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South J, Blass B (2001) *The future of modern genomics.* Blackwell, London
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 - Online document
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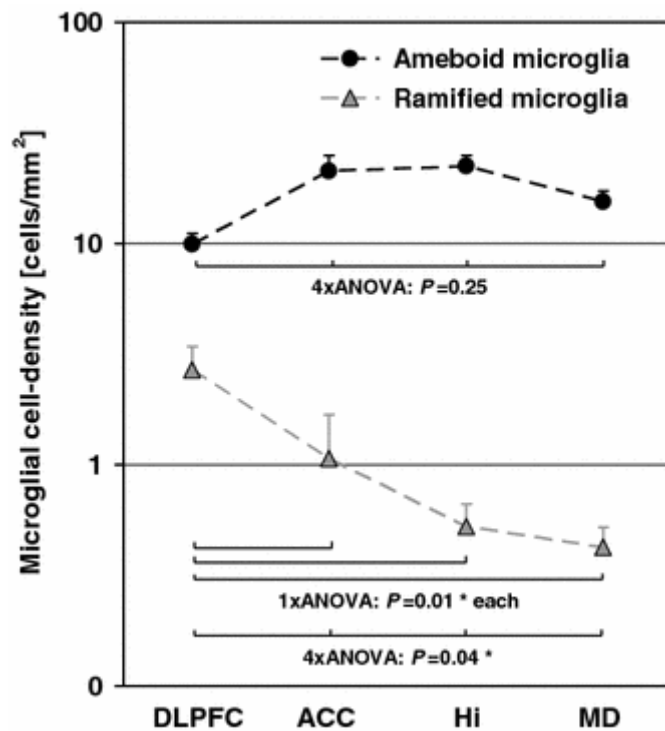
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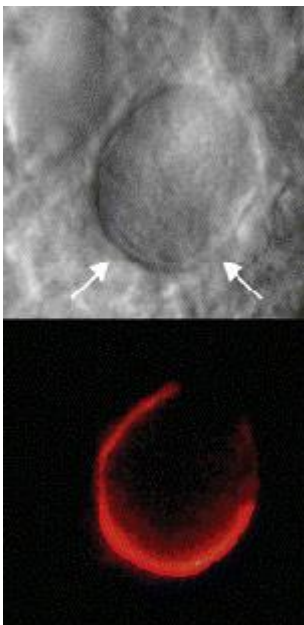
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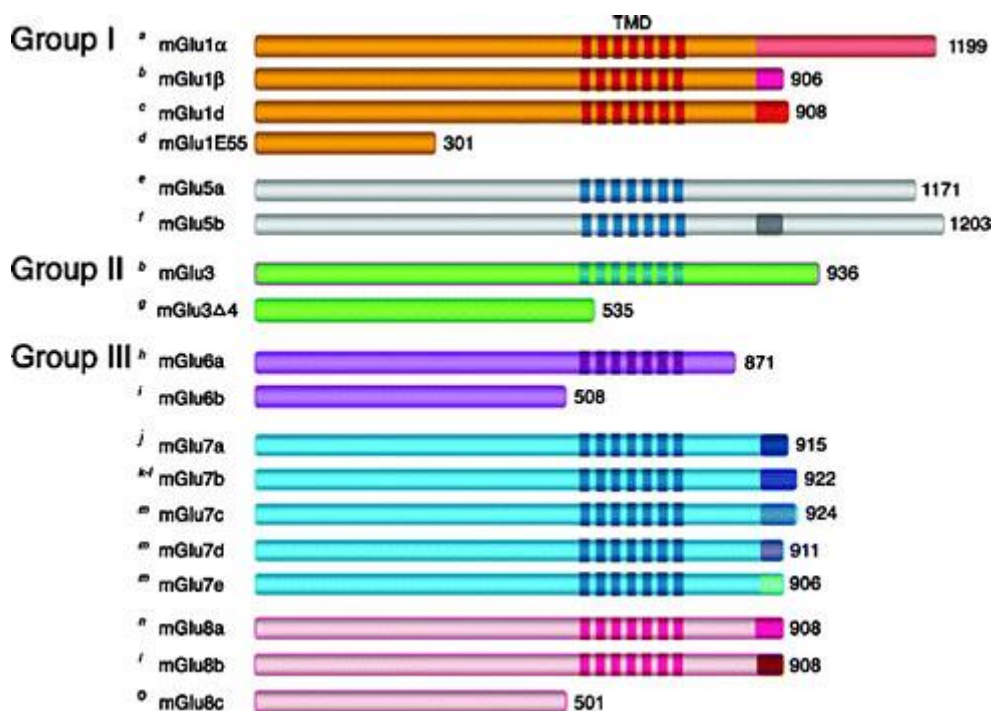
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César Penazzo Lepri <cesar.lepri@uniube.br>

Fwd: Lasers in Medical Science - Submission Notification to co-author

Brenda Ferreira Arantes <brend287@hotmail.com>
Para: "cesar.lepri@uniube.br" <cesar.lepri@uniube.br>

14 de março de 2018 12:20

Enviado do meu iPhone

Início da mensagem encaminhada:

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Assunto: Lasers in Medical Science - Submission Notification to co-author
Responder A: Lasers in Medical Science <josephinedenise.elmedo@springernature.com>

Re: "Influence of Er,Cr:YSGG laser, associated or not to desensitizing agents, in the prevention of acid erosion in bovine root dentin."
Full author list: Brenda Ferreira Arantes, DDS, MSc student; Laura de Oliveira Mendonça, DDS, MSc student; Regina Guenka Palma-Dibb, PhD; Juliana Jendiroba Faraoni, PhD; Denise Tomavoi de Castro, PhD; Vinicius Rangel Geraldo-Martins, PhD; Cesar Penazzo Lepri, PhD

Dear Ms Brenda Arantes,

We have received the submission entitled: "Influence of Er,Cr:YSGG laser, associated or not to desensitizing agents, in the prevention of acid erosion in bovine root dentin." for possible publication in Lasers in Medical Science, and you are listed as one of the co-authors.

The manuscript has been submitted to the journal by Dr. PhD Cesar Penazzo Lepri who will be able to track the status of the paper through his/her login.

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Thank you very much.

With kind regards,

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