**UNIVERSIDADE DE UBERABA**
**MESTRADO EM ODONTOLOGIA**

**NATYELLE FERNANDA SILVA BELLOCCHIO CORRÊA**

# INFLUÊNCIA DOS LASERS Er:YAG E Nd:YAG ASSOCIADOS OU NÃO AO FLUORETO DE SÓDIO NA PREVENÇÃO DA HIPERSENSIBILIDADE DENTINÁRIA

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

Área de concentração: Biomateriais.

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Corrêa, NFSB. Influência dos lasers Er:YAG e Nd:YAG associados ou não ao fluoreto de sódio na prevenção da hipersensibilidade dentinária. [Dissertação de Mestrado]. Uberaba: Universidade de Uberaba- UNIUBE; 2015.

**Resumo**

Hipersensibilidade dentinária (HD) é uma dor aguda, de curta duração, manifestando-se de maneira desconfortável ao paciente. Essa dor ocorre devido a presença de túbulos dentinários abertos em uma superfície dentinária exposta. O objetivo deste estudo foi avaliar a eficácia dos lasers Er:YAG e Nd:YAG na prevenção da hipersensibilidade dentinária associado ou não ao fluoreto de sódio 1,23%, após desafio ácido com Coca-Cola®. Foram obtidos 104 espécimes a partir de dentina radicular bovina (4,25mm x 4,25mm x 3,00mm de altura), os quais foram polidos e divididos aleatoriamente em 8 grupos de acordo com os tratamentos preventivos realizados: G1 irradiação do laser Er:YAG; G2 irradiação laser Er:YAG seguido da aplicação tópica de Flúor Fosfato Acidulado (FFA); G3 aplicação do FFA seguido da irradiação do laser Er:YAG simultaneamente, G4 irradiação laser Nd:YAG; G5 irradiação laser Nd:YAG seguido da aplicação tópica de Flúor Fosfato Acidulado (FFA); G6 aplicação do FFA seguido da irradiação do laser Nd:YAG simultaneamente; G7 aplicação do FFA; G8 sem tratamento. A metade da superfície da dentina de cada espécime foi isolada com esmalte cosmético e cera utilidade (área controle) e a outra metade exposta ao tratamento preventivo. Os parâmetros para irradiação com o laser Er:YAG foram: 10s de irradiação, 4mm de distância (pré-focado), refrigeração com fluxo de água a 2mL/min, taxa de repetição 2Hz e densidade de energia 3,92J/cm$^2$. Para o laser Nd:YAG: 10s de irradiação, 1mm de distância (desfocado), sem refrigeração, taxa de repetição 10Hz e densidade de energia 70,7 J/cm$^2$. Quando utilizado, o fluoreto foi aplicado por um tempo total de 4min. O desafio erosivo foi feito com Coca-Cola, em agitador magnético, à temperatura de 4°C (pH=2,42), durante 1 minuto, 3 vezes ao dia, por 5 dias consecutivos. Após, realizou a análise da rugosidade superficial e do desgaste em microscopia confocal a laser 3-D. Os dados de rugosidade superficial foram submetidos ao teste ANOVA ($\alpha$=5%). Para o desgaste, os dados foram submetidos ao teste estatístico não-paramétrico Kruskal-Wallis seguido do teste de Dunn, ambos com nível de significância de 5%. Em relação à rugosidade superficial, não houve diferença estatisticamente significante entre os grupos ($p>0,05$). Os grupos irradiados com o laser Er:YAG tiveram uma perda de volume significantemente menor quando comparados aos demais grupos ($p<0,05$). O grupo G6 apresentou valores maiores que os grupos irradiados com o laser Er:YAG e valores menores que os demais grupos. Os outros grupos irradiados com o laser Nd:YAG mostraram resultados similares aos grupos controle ($p>0,05$). A rugosidade superficial dos grupos tratados e submetidos ao desafio erosivo foi similar aos grupos controle (tanto positivo quanto negativo) nas mesmas condições experimentais, demonstrando que a irradiação laser em dentina bovina é segura, uma vez que não alterou a propriedade analisada. O laser Er:YAG apresentou os menores valores percentuais de perda de volume na análise do desgaste, sugerindo que este laser aumentou a resistência ácida da dentina. Portanto, a irradiação de dentina radicular bovina com lasers de alta intensidade provou ser um método promissor para aumentar a resistência ácida.

**Palavras-chave:** Hipersensibilidade da dentina; laser Er:YAG; laser Nd:YAG; fluoreto de sódio.

Correa, NFSB. Influence of Er:YAG and Nd:YAG, associated or not with fluoride, on dentin hypersensitivity prevention. [Master's thesis]. Uberaba: University of Uberaba- UNIUBE; 2015.

**Abstract**

Dentin hypersensitivity (DH) is an acute and short-term pain, uncomfortably to the patient. This pain occurs due to the presence of open dentinal tubules in an exposed dentin surface. The objective of this study was to evaluate the effectiveness of Er:YAG and Nd:YAG on dentin hypersensitivity prevention, associated or not to sodium fluoride 1.23%, after erosive challenge with Coca-Cola®. 104 specimens were obtained from bovine root dentine (4mm x 4mm x 3mm height), which were polished and randomly divided 8 groups according to the preventive treatment carried out G1 irradiation of Er:YAG; G2 irradiation laser Er:YAG followed by topical application of acidulated phosphate fluoride (APF); G3 application of APF followed by irradiation of Er:YAG laser simultaneously; G4 laser irradiation Nd:YAG; G5 laser irradiation Nd:YAG followed by topical acidulated phosphate fluoride (APF); G6 application of FFA followed by laser irradiation Nd:YAG simultaneously; G7 application of APF; G8 untreated. Half of the dentin surface of each specimen was isolated and utility wax nail varnish (control area) and the other half exposed to preventive treatment. The parameters for irradiation with the Er:YAG laser were: 10s irradiation, distance of 4mm (pre-focused), water cooling flow of 2mL/min, 2Hz repetition rate and energy density of 3.92J/cm$^2$. For the Nd:YAG laser: 10s irradiation, distance of 1mm (unfocused), without cooling, 10Hz repetition rate and energy density of 70.7J/cm$^2$ . When used, the fluoride was applied for a total time of 4 minutes. The erosive challenge was done in Coca-Cola, magnetic stirrer, at a temperature of 4°C (pH=2.42), 3 times a day for a period of 1 minute for 5 days. Afterwards, surface roughness and wear analysis were evaluated in 3-D confocal laser microscope. Surface roughness data were submitted to ANOVA test ($\alpha$=5%). For wear analysis, data were submitted to non-parametric test of Kruskal-Wallis followed by Dunn test, both with $\alpha$=5%. As regards surface roughness, there was no statistically significant difference among the groups ($p>0.05$). The groups irradiated with Er:YAG laser had a volume loss significantly lower when compared to other groups ($p<0.05$). G6 showed higher values than the groups irradiated with Er:YAG and lower values than the other groups. The other groups irradiated with Nd:YAG laser showed similar wear results to the control groups ($p>0.05$). Surface roughness of the groups, treated and submitted to erosive challenge, was similar to control groups (either positive or negative) in the same experimental conditions, demonstrating that laser irradiation in bovine dentin is safe, because did not alter the analyzed property. The Er:YAG laser showed the lowest percentage values of volume loss from wear analysis, suggesting that this laser has increased the acid resistance of dentin. Therefore, the irradiation of bovine root dentine with high intensity lasers proved to be a promising method for increase the acid resistance.

**Key Words:** Dentinal hypersensitivity; Er:YAG laser; Nd:YAG laser; sodium fluoride.

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## LISTA DE ABREVIATURAS, SIGLAS E SÍMBOLOS

| | |
|---|---|
| **µm** | micrômetro |
| **$CO_2$** | 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 |
| **FFA** | flúor fosfato acidulado |
| **G** | grupo |
| **g/f** | grama força |
| **HD** | hipersensibilidade dentinária |
| **Hz** | hertz |
| **$J/cm^2$** | joule por centímetro quadrado |
| **KHN** | Knoop Hardness Number |
| **kV** | quilovolt(s) |
| **mL** | mililitro(s) |
| **mm** | milímetro(s) |
| **NaF** | fluoreto de sódio |
| **$^oC$** | grau Celsius |
| **pH** | logaritmo negativo de concentração hidrogeniônica ($-\log[H^+]$) |
| **W** | watts |

# SUMÁRIO

# 1 Introdução

# 1 Introdução

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 & ADDY 2002; TORWANE *et al.* 2013). A exposição da dentina ao meio bucal surge em decorrência da perda do esmalte e do cemento (RIMONDINI *et al*. 1995). 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 e 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 (GANDARA & TRUELOVE 1999; EHLEN *et al*. 2009; MAGALHÃES *et al.* 2009; NAIDU *et al.* 2014).

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

A HD é definida como uma dor derivada da dentina exposta em resposta a estímulos químicos, térmicos, tácteis, ou osmóticos que não pode ser explicada como surgimento a partir de qualquer outro defeito dental ou doença (KO *et al*. 2014). Diversas teorias foram propostas para explicar a etiologia da hipersensibilidade dentinária, mas a teoria mais comumente aceita para explicar o mecanismo da transmissão da dor é a “Teoria Hidrodinâmica”, proposta por Brännström. Conforme essa teoria, a exposição dos túbulos dentinários ao meio bucal permitiria a movimentação dos fluidos dentinários, estimulando assim as fibras nervosas, ocasionando desta forma a sensação de dor (BRANNSTROM 1966; BRANNSTROM *et al.* 1979).

A exposição da dentina cervical é mais trivial na face vestibular de caninos e pré-molares devido ao posicionamento destes dentes na arcada dentária. A prevalência aumenta com a idade (ADDY & WEST 1994; SOBRAL 1995; Y ZHANG *et al.* 2014). Além disso, acomete mais mulheres do que homens de acordo com FLYNN *et al*. 1985; OYAMA & MATSUMOTO, 1991; FISCHER *et al.* 1992; WALTERS 2005. Em contrapartida, em pesquisa recente, RANE *et al.* 2013 avalariam 960 pacientes, 528 homens e 432 mulheres.

Estes foram classificados de acordo com a faixa etária e sexo, onde 288 pessoas tinham entre 20 e 29 anos, outros 432 indivíduos entre 30 e 39 e os demais variavam de 40 a 50 anos de idade. Os resultados mostraram que a hipersensibilidade dentinária foi mais comum nos indivíduos do sexo masculino (60,8%) quando comparado ao sexo feminino (39,2%), acometendo indivíduos da faixa etária dos 30-39 anos (39,2%), seguido de 40-50 (37,3%) e por último o grupo de 20-29 anos (23,5%).

Esta prevalência pode variar de um país para o outro e em territórios diferentes dentro do mesmo país, devido à diversidade de hábitos alimentares, sociais e culturais (PEREIRA, 1995). Na América do Norte, segundo GAFFAR 1998, calcula-se que quarenta milhões de adultos relataram ter apresentado hipersensibilidade dentinária e a cada seis pacientes que procuram atendimento clínico, um apresenta algum grau de hipersensibilidade dentinária em pelo menos um dente (SOBRAL *et al*. 1995).

A literatura (LEE & EAKLE 1996; BURKE *et al.* 2000; PRADEEP & SHARMA 2010) afirma que uma extensa variedade de agentes dessensibilizantes são eficazes para a cura da hipersensibilidade dentinária, entretanto outras pesquisas (ARANHA *et al.* 2009; DOS REIS DERCELI *et al*. 2013) mostram que o uso de agentes dessensibilizantes produz uma resposta de curta duração, ou seja, o efeito do tratamento não é duradouro.

Existem vários métodos disponíveis (ADDY & WEST 2013; MALEKI *et al*. 2015; TAHA *et al.* 2015) para o tratamento da hipersensibilidade dentinária, 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, dentifrícios especiais. Outro método também utilizado para tratar a hipersensibilidade dentinária é a iontoforese. Os compostos fluoretados são os mais utilizados para a redução da hipersensibilidade dentinária (VAN DEN BERGHE *et al.*1984; CAMILOTTI *et al*. 2012).

GAFFAR (1998) em sua pesquisa com o verniz fluoretado Duraphat observou a formação de cristais de fluoreto de cálcio que impediam a abertura dos túbulos dentinários, promovendo a remineralização e consequentemente um alívio duradouro da hipersensibilidade dentinária. O oxalato de potássio é um agente dessensibilizante que age na obliteração dos túbulos e despolarização de termininações nervosas; é apresentado tanto na forma de dentifrícios quanto em aplicações tópicas (ASSIS *et al*. 2011). STEAD *et al.* (1996) notaram redução da permeabilidade dentinária devido à obliteração dos túbulos dentinários, porém esse resultado era temporário pois os cristais eram dissolvidos parcialmente na saliva.

SANTIAGO *et al.* (2006) observaram que várias formulações de oxalato de potássio diminuíram a permeabilidade dentinária em cerca de 75%, atestando a eficácia destes produtos. OSMARI *et al.* (2013) verificaram a ação do verniz fluoretado Duraphat Colgate-Palmolive Company (New York, EUA), oxalato de potássio monohidratado (Oxa-gel Kota

Indústria e Comércio LTda (São Paulo, Brasil), sistema adesivo autocondicionante de 2 passos (SA) Clearfil TM SE Bond Kuraray (Osaka, Japão) e laser diodo (Thera Lase Surgery DMC Equipamentos Ltda São Carlos SP, Brasil), para uma maior compreensão dos mecanismos de ação quando da sua aplicação clínica.

Avaliando as modificações morfológicas da dentina após a aplicação desses quatro agentes dessensibilizantes usados no tratamento da hipersensibilidade dentinária, os autores concluíram que os quatro agentes dessensibilizantes mostraram ser eficazes na oclusão dos túbulos dentinários, com os diferentes mecanismos de ação, sendo que quando aplicado o sistema adesivo autocondiconante, visualizou-se uma película contínua e uniforme sobre a superfície dentinária, não sendo possível visualizar os túbulos. Dessa forma, os autores sugerem a realização de estudos clínicos para verificar a efetividade dos achados (OSMARI *et al*. 2013)

A utilização de dentifrícios especiais tem sido uma das primeiras opções no tratamento da hipersensibilidade dentinária devido ao fácil acesso, entretanto possui um baixo custo-benefício (PRATI *et al.* 2002; WANG *et al.* 2010). PINTO *et al.* (2012) compararam os efeitos de diferentes marcas comerciais de dentifrícios dessensibilizantes em combinação com a escovação dental e concluíram que estes foram capazes de diminuir a permeabilidade da dentina, embora tenham causado a obliteração parcial dos túbulos dentinários. O dentifrício à base de nitrato de potássio reestabelece o fluxo de potássio no interior do odontoblasto, onde esse fluxo é perdido devido a estímulos externos. Dessa forma, estabiliza-se a polaridade das terminações nervosas (PURRA et al. 2014). Já os dentifrícios à base de cloreto de estrôncio atuam na obliteração dos túbulos dentinários, criando uma barreira impermeável, estimulando a formação de dentina reparativa, diminuindo a hipersensibilidade dentinária (RICO, 1992). A iontoforese usa um potencial elétrico que é capaz de transferir íons dentro do corpo humano. Na hipersensibilidade dentinária o objetivo é levar íons flúor mais profundamente aos túbulos dentinários (BRAHMBHATT et al. 2012).

De acordo com PETERSSON (2013) o flúor e diferentes combinações de agentes apresentam propriedades que são capazes de ocluir os túbulos dentinários, tais como íons, sílica, nitrato e oxalatos, podendo amenizar os efeitos adversos. Entretanto, a pasta dental com fluoreto de estanho apresenta um resultado mais satisfatório em relação aos outros componentes, porém com uma desvantagem: seu uso acarreta na descoloração dos dentes. Para PETERSSON (2013) os tratamentos dessensibilizantes devem ser empregados sistematicamente, a começar com a prevenção e tratamentos realizados em casa com o uso de creme dental com flúor e complementado com as modalidades realizadas em consultório pelo cirurgião-dentista, com a sua supervisão conforme necessário.

Outra forma de tratamento da hipersensibilidade dentinária pode ser obtida através da utilização de *lasers.* A utilização de terapias com *laser,* associado ou não ao flúor, nos casos de hipersensibilidade dentinária, têm promovido resultados satisfatórios (LOPES & ARANHA. 2013). O primeiro laser foi descoberto por MAIMAN (1960) criando o primeiro *laser* sólido, utilizando o rubi como meio. Este *laser* é localizado na faixa visível do espectro eletromagnético. Em 1961 houve a primeira intervenção cirúrgica com *laser* em um tumor de retina (BRUGNERA *et al.* 1991). PATEL em 1964 criou o *laser* cirúrgico de dióxido de carbono ($CO_2$), e na mesma época Sinclair & Knoll desenvolveram outro tipo de *laser*, conhecido como *soft laser* (BRUGNERA 2003*).* Em 1968 destacava-se o *laser* argônio, por permitir maior controle do operador. TAYLOR *et al.* (1965) observaram o efeito do laser de rubi nos dentes e mucosa de hamster sírio. No ano de 1971 o pesquisador Hall comparou a ação do laser de $CO_2$, eletrocautério e bisturi em cirurgia de tecido mole e constatou que as incisões realizadas com este *laser* curavam mais lentamente do que as realizadas com bisturi. BRUGNERA & PINHEIRO (1998) demonstraram que se obtém um grande sucesso nas cirurgias realizadas com o *laser* de $CO_2$, motivo pelo qual é largamente utilizado na Odontologia.

O primeiro trabalho publicado com a utilização de *laser* na Odontologia foi em 1964 (STERN & SOGNNAES). Eles utilizaram o *laser* de rubi para irradiar esmalte e dentina e observaram redução da permeabilidade dentinária e consequentemente redução da desmineralização do esmalte dental. ADRIAN *et al* (1971) demonstraram por meio de pesquisas que o *laser* de rubi é nocivo no que se diz respeito à vitalidade pulpar, devido a grande quantidade de energia que é gerada, resultando em um calor excessivo e causando danos pulpares irreversíveis.

De acordo com HE *et al*. (2011) uma revisão sistemática da literatura indicou que a terapia a laser tem uma leve vantagem clínica em relação aos medicamentos tópicos utilizados no tratamento da hipersensibilidade dentinária (CUNHA-CRUZ, 2011). Muitos estudos avaliaram apenas a aplicação isolada dos lasers, sem a associação do flúor tópico, porém poucos estudos elucidam a combinação do laser juntamente com a aplicação tópica de fluoreto, além de não apresentarem um resultado duradouro (BELA & YASSIN, 2014; MALEKI *et al*. 2015).

Tratamentos da HD nem sempre produzem os efeitos esperados pelos pacientes, pois seus efeitos muitas vezes não são permanentes, levando o paciente a sofrer novamente com as dores incômodas devido a estímulos externos (*YAZICI et al.* 2010). Pesquisas recentes estão demonstrando resultados promissores no que diz respeito ao tratamento da HD com o uso de *laser.* Desde os experimentos realizados com o *laser* de rubi, outros lasers foram testados e utilizados no tratamento da hipersensibilidade dentinária, tais como: $CO_2$, diodo (GaAlAs), He-Ne, Nd:YAG, Er:YAG, Er,Cr:YSGG (KUMAR & MEHTA 2005; YILMAZ et al. 2011; ARANHA & EDUARDO 2012).

PALAZON *et al.* (2013) avaliaram o efeito do laser Nd:YAG e dessensibilizante (pasta Colgate Sensitive Pró- Alívio) na vedação dos túbulos dentinários. Após o tratamento as amostras foram submetidas a uma sequência de desafios erosivos e abrasivos. 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 & 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 0,25 W 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 necessário 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).Portanto, tanto o *laser* Er:YAG quanto o Nd: YAG podem ser usados para reduzir a hipersensibilidade dentinária.

De acordo com DILSIZ *et al.* 2009, o Nd:YAG é mais eficaz no tratamento da HD em relação ao Er:YAG e diodo, 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 que constantemente apresentam recessão gengival e superfícies da raiz exposta. O fato mais importante do uso da laserterapia, e que deve ser sempre considerado, é alcançar resultados satisfatórios, sem provocar danos pulpares prejudiciais, fraturas e carbonização (MOHAMMAD & MASOUMEH 2013).

Devido a uma grande variedade nos métodos e tipos de *lasers*, ainda não foi possível propor um método definitivo para tratar a HD. Desta forma, seria interessante a obtenção de parâmetros seguros e ideais, utilizando *lasers* de alta potência, no intuito de se obter alterações morfológicas nos tecidos dentais, como selamento e oclusão dos túbulos dentinários pelo derretimento e recristalização da dentina.

# 2 Proposição

# 2 Proposição

O objetivo desse estudo *in vitro* foi avaliar a efetividade da irradiação de *lasers* na prevenção da hipersensibilidade dentinária, após desafio erosivo (imersão em Coca-Cola®), analisando a influência do tipo de *laser* (Er:YAG, Nd:YAG) associado ou não ao flúor, por meio das análises de:

-rugosidade superficial dos espécimes, através da *microscopia confocaa laser;*
-avaliação do desgaste, através de *microscopia confocal a laser*

# 3 Capítulo 1

**Influence of Er:YAG and Nd:YAG laser irradiation, associated or not with fluoride, on dentin hypersensitivity prevention**

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*Concise title:* Influence of lasers on dentin hypersensitivity prevention

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**Abstract**

Dentin hypersensitivity (DH) is an acute and short-term pain, uncomfortably to the patient. This pain occurs due to the presence of open dentinal tubules in an exposed dentin surface. The objective of this study was to evaluate the effectiveness of Er:YAG and Nd:YAG on dentin hypersensitivity prevention, associated or not to sodium fluoride 1.23%, after erosive challenge with Coca-Cola®. 104 specimens were obtained from bovine root dentine (4mm x 4mm x 3mm height), which were polished and randomly divided into 8 groups according to the preventive treatment carried out G1 irradiation of Er:YAG; G2 irradiation laser Er:YAG followed by topical application of acidulated phosphate fluoride (APF); G3 application of APF followed by irradiation Er:YAG laser simultaneously; G4 laser irradiation Nd:YAG; G5 laser irradiation Nd:YAG followed by topical acidulated phosphate fluoride (APF); G6 application of APF followed by laser irradiation Nd:YAG simultaneously; G7 application of APF; G8 untreated). Half of the dentin surface of each specimen was isolated and utility wax nail varnish (control area) and the other half exposed to preventive treatment. The parameters for irradiation with the Er:YAG laser were: 10s irradiation, distance of 4mm (pre-focused), water cooling flow of 2mL/min, 2Hz repetition rate and energy density 3.92J/cm$^2$. For the Nd:YAG laser: 10s irradiation, distance of 1mm (unfocused), without cooling, 10Hz repetition rate and energy density 70.7J/cm$^2$. When used, the fluoride was applied for a total time of 4 minutes. The erosive challenge was done in Coca-Cola, magnetic stirrer, at a temperature of 4°C, 3 times a day for a period of 1 minute for 5 days. Afterwards, surface roughness and wear analysis were done in 3-D confocal laser microscope. Surface roughness data were submitted to ANOVA test ($\alpha$=5%). For wear analysis, data were submitted to non-parametric test of Kruskal-Wallis followed by Dunn test, both with $\alpha$=5%. As regards surface roughness, there was no statistically significant difference among the groups ($p>0.05$). The groups irradiated with Er:YAG laser had a volume loss significantly lower when compared to other groups ($p<0.05$). G6 showed higher values than the groups irradiated with Er:YAG and lower values than the other groups. The other groups irradiated with Nd:YAG laser showed similar wear results to the control groups ($p>0.05$). Surface roughness of the groups, treated and submitted to erosive challenge, was similar to control groups (either positive or negative) in the same experimental conditions, demonstrating that laser irradiation in bovine dentin is safe, because did not alter the analyzed property. The Er:YAG laser showed the lowest percentage values of volume loss from wear analysis, suggesting that this laser has increased the acid resistance of dentin.


**Key Words:** Dentinal hypersensitivity; Er:YAG laser; Nd:YAG laser; sodium fluoride.

# 4. Introduction

The dentin hypersensitivity (DH) or hyperalgesia is understood to be a sharp pain, short, manifesting itself uncomfortably to the patient. This pain occurs as a result of exposed dentine in response to chemical, thermal, tactile or osmotic stimulus, which can not be explained as arising from any other dental defect or disease [1]. It occurs due to the presence of open dentinal tubules on an exposed dentin surface [2-4]. Enamel and cementum loss causes dentine exposure to the oral environment. [2].

This loss is derived from several factors, such as sub-gingival scaling, dental crowding, or the combination of two or more factors. The combination of these factors, such as abrasion, abfraction and acid erosion also cause DH and acid erosion can arise due to extrinsic factors (acidic foods and drinks such as citrus fruits, coffee, soft drinks, wine and other alcoholic drinks) and intrinsic, caused by eating disorders and gastroesophageal disorders (anorexia, xerostomia, bulimia and acid reflux), and even the force applied during dental hygiene can be an aggravating factor of erosion [5-8].

The most commonly accepted theory to explain the pain transmission mechanism is the hydrodynamic theory, proposed by Brännström. Under this theory, exposure of dentinal tubules to the oral environment would allow the movement of dentinal fluid, thereby stimulating the nerve fibers, thus causing the pain sensation [9, 10].

Several methods [11-13] are available for the treatment of dentin hypersensitivity, all with the same purpose: seal the dentinal tubules. Among these methods, it can be cited the use of fluoride varnishes, potassium oxalate, self-etching adhesive system, special toothpastes. Another method also used to treat tooth sensitivity is iontophoresis [14]. Fluoride compounds are the most commonly used for the reduction of dentin hypersensitivity [15, 16]. These desensitizing treatments should be used systematically, beginning with prevention and treatments performed at home with the use of fluoride dental toothpaste and complemented by dentists, with their supervision with the procedures performed at dental office [17].

The fluoride topical application prevents the dissolution of the dental substrate [18, 19], consequently increasing the acid resistance of enamel, but its mechanism will depend on its ability to interfere with the demineralization and remineralization process.

Another way to treat dentinal hypersensitivity may be obtained by using lasers. Currently, the laser therapy is used, with or without fluoride, with satisfactory results [20]. The first laser was discovered in 1960 by Maiman [21], creating the first solid-state laser and using ruby as the medium. This laser is situated in the visible range of the electromagnetic spectrum. From the experiments carried out with the ruby laser, other lasers have been developed and used in the treatment of dentinal hypersensitivity, such as $CO_2$, diode (GaAlAs), He-Ne, Nd:YAG, Er:YAG and Er,Cr:YSGG [22-24].

Due to the variety methods and types of lasers, it was not possible to propose a definite method for treating DH. This way, it would be interesting to obtain safe and ideal parameters using high power lasers, in order to get morphological changes in dental tissues, such as sealing and occlusion of dentinal tubules by melting and recrystallization of dentin.

## 5. Objective

The aim of the present study was to analyse the effects of Er:YAG and Nd:YAG laser irradiation, associated or not with 1,23% sodium fluoride (NaF) application on dentin hypersensitivity prevention, after erosive challenge, assessed by surface roughness and wear analysis (confocal laser microscopy).

## 6. Materials and Methods

### *6.1. Preparation of the Samples*

Fifty two bovine incisive teeth were collected and immediately stored in distilled water. The teeth that had microcracks, stains due hypoplasia or wear were discarded. After cleansing and root planning using a curette until the dentin exposition, the teeth were stored in distilled water under refrigeration at 4°C. The crowns were separated from the roots at the cement-enamel junction using a section machine (Iso Met® 1000, BUEHLER-Lake Bluff, IL 60044/USA) with a water-cooled diamond disk (Isomet; 10.2cm×0.3mm, arbour size 1/2 in., series 15HC diamond; Buehler Ltd., Lake Bluff, IL, the USA) in low speed.

Then, the roots were sectioned and divided in half to obtain 104 fragments of 4.25×4.25×3.00mm. The specimens were delineated and polished under water cooling and sandpaper (granulation #600 and #1200).

After polishing, all fragments were coated with two layers of nail varnish and wax (reference area), leaving half of the dentin surface without protection ($9mm^2$) to apply the preventive treatments and induce erosive challenge. Afterwards, the specimens were randomly divided eight groups according to the treatments performed.

### *6.2. Experimental Groups*

One hundred and four root dentin samples were randomly divided into 8 groups (n=13). In each sample, the delimitated area was treated according to Table 1.

Group 1 was only irradiated with Er:YAG laser; G4 received only Nd:YAG laser. In groups 2 and 5, the NaF (1,23% fluoride gel - DFL Industria e Comercio SA - RJ/Brazil) was applied after irradiation during 4 minutes. The samples of the groups 3 and 6 received NaF during 1 minute, simultaneously irradiated (10 seconds) and NaF was left in the specimen until completing 4 minutes. In group 7, a NaF gel was applied on the samples for 4 minutes (positive control group). For all groups that received NaF, the excess gel was removed with gauze immediately after completing the fourth minute and then the specimens were stored in distilled water at 37°C until the next step of the experiment. Finally, group 8 received no treatment (negative control group).

To ensure consistent spot size with the hand irradiation, an endodontic file was fixed on the handpiece, and kept a determined distance from the surface during the irradiation procedures. The laser parameters used for laser irradiation in each group are shown in Table 2. The handpiece was positioned perpendicularly to the root dentin surface, and the samples were irradiated once in each direction, moving the handpiece slowly horizontally and vertically, in order to promote homogeneous irradiation and to cover the entire sample area. The irradiation was performed by hand (simulating a clinical situation) and scanning the dentin surface during 10 seconds. The output power was measured with a power meter (TM-744D,Tenmars Electronics Co. Ltd., Taipei, Taiwan). At the end of these treatments, all samples were kept in distilled water at 37°C until the next step. Afterwards, the samples of all groups were submitted to an erosive challenge.

*6.3. Erosive Challenge*

For the erosive challenge, samples were submitted to daily immersion in 50mL of Coca-Cola at 4°C (pH=2.42), under stirring, during one minute, three times a day. This cycle was carried out for 5 days. The specimens were storage in distilled water between the cycles. At the end of each day, these also remained in distilled water, which was daily changed.

*6.4. Surface roughness measurement and Wear analysis*

The specimens were washed with distilled water and dried with paper tissue. The wax and nail varnish were carefully removed, exposing the control area. The surface roughness and dentin wear were evaluated with a laser confocal microscope (LEXT-Olympus) connected to a computer with specific software (OLS4000).

As regards surface roughness, each specimen was measured seven times in each area (reference or treated). This variable was evaluated in Ra parameter, measured in micrometers (ISO 25178).

The wear measurements of the treated/eroded surface were performed in relation to the untreated area (reference area). After profile determination, the wear measurement was calculated in volume ($\mu m^3$), considering the medium line of the graphic (referring to the protected area = reference area) and the erosion line (treated/eroded area). Each specimen was measured in a central area of $1mm^2$. Finally, we considered the percentage of lost volume, comparing the treated area to the reference area.

*6.5. Statistical Analysis*

For the surface roughness analysis, firstly, the assumptions of equality of variances (modified Levene equal-variance test) and the normality of the error distributions (Shapiro-Wilk test) were checked for the response variables tested. Since the assumptions were satisfied, the ANOVA test ($\alpha$=5%) was applied using SPSS Statistics Version 17.0 software (Chicago: SPSS Inc.). For wear analysis, data were submitted to non-parametric test of Kruskal-Wallis followed by Dunn test, both with $\alpha$=5%.

## 7. Results

There results, expressed in Ra (μm), are described in Table 3. There was no statistically significant difference among all groups ($p>0.05$).

The groups irradiated with Er:YAG laser had a volume loss significantly lower when compared to other groups ($p<0.05$). G6 group (NaF application followed by Nd:YAG laser irradiation, simultaneously) showed higher values than the groups irradiated with Er:YAG and lower values than the other groups. The other groups irradiated with Nd:YAG laser showed similar wear results to the control groups ($p>0.05$). The percentages of lost volume are shown in Table 4.

## 8. Discussion

The use of laser therapy for dentin hypersensitivity prevention has been shown to be a promising method. Our study confirmed this hypothesis.

Although exists evidences on the effects of fluoride on dental tissue, it is also known that such methods have limited actions in an acid environment [25, 26]. Fluoride application leads to the formation of a calcium fluoride-like compound that is more instable and easily dissolved by most acidic beverages and acids from the cariogenic challenge.

Thus, new technologies, including laser therapy, have been developed to allow the enamel to obtain greater resistance to acid attack [27, 28].

The parameters of the Er:YAG laser used to treat HD, according to Mohammad & Masoumeh [29] are 1W and 10-12 Hz, with irradiation duration of less than 60 seconds, in order to prevent damage to dental surface and soft tissues. According to Aranha *et al.* the Er:YAG laser is highly effective in reducing the diameter of dentinal tubules under specific conditions, with partial obliteration of the tubules [30].

In the present study, Er:YAG and Nd:YAG lasers with sub-ablative parameters were used to obtain an adequate energy density for the prevention of dental demineralization, without damaging the surface through the ablative process. We proposed to study surface roughness because the presence of irregularities can lead bacterial biofilm retention and gingival irritation, increasing the risk of caries and periodontal inflammation [31].

Dilber *et al*. used three types of lasers: Er:YAG, Nd:YAG and KTP. They concluded that irradiation with these lasers did not affect the structure and the composition of the dentin surface. The average percentage of minerals weight, such as Ca, K, Mg, Na and P were not affected [32]. Previously, in other research with Er:YAG and Nd:YAG lasers, Rohanizadeh *et al,* they noted that the proportion of minerals Ca and P was decreased in Er:YAG irradiated tissue, and increased in the Nd:YAG irradiated tissue [33]. This might be explained by the Nd:YAG action mechanism: the hydroxyapatite crystals melt in the presence of energy, immediately occluding the tubules [34].

The Nd:YAG laser was effective only when it was previously performed the application of fluoride. This finding is different to that found by Raucci-Neto *et al.* [35], probably because the substrate evaluated in that study was the enamel, witch has significant differences from the dentin studied in this study.

The findings in the present study suggest that the laser irradiation with both devices are effective when the roughness parameter was analyzed, however, more studies are needed to assess whether there is change in the percentage of dentin minerals.

Lastly, the Er:YAG laser has been shown to be safe in dental irradiation, since it promoted acceptable temperature increases [36, 37]. Furthermore, it also presented in the present study the advantage of significantly reduce the mineral volume loss after erosive challenge. Therefore, further studies are needed in human teeth to validate these findings and determine the optimal parameters of irradiation.

## 9. Conclusion

Surface roughness of the groups, treated and submitted to erosive challenge, was similar to control group (either positive or negative) in the same experimental conditions, demonstrating that laser irradiation in dentin is safe, because did not alter the analyzed property.

The Er:YAG laser showed the lowest percentage values of volume loss from wear analysis, suggesting that this laser has increased the acid resistance of dentin.

Therefore, the irradiation of bovine root dentine with high intensity lasers proved to be a promising method for dentin hypersensitivity prevention.

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**Legends**

**Table 1.** Treatment employed in the different groups

**Table 2.** Lasers parameters of the experimental groups

**Table 3.** Means (μm) ± standard deviations of the surface roughness of the dentin surface after different preventive pretreatments followed by erosive challenge

**Table 4.** Lost volume (%) and standard deviations of the wear of the dentin surface after different preventive pretreatments followed by erosive challenge, comparing the treated area to the reference area.

Table 1. Treatment used in the different groups

| Group | Treatment |
|---|---|
| G1 | Er:YAG laser irradiation |
| G2 | Er:YAG laser irradiation followed by NaF application |
| G3 | NaF application followed by Er:YAG laser irradiation, simultaneously |
| G4 | Nd:YAG laser irradiation |
| G5 | Nd:YAG laser irradiation followed by NaF application |
| G6 | NaF application followed by Nd:YAG laser irradiation, simultaneously |
| G7 | NaF application (positive control group) |
| G8 | No treatment (negative control group) |

Table 2. Lasers parameters of the experimental group

| Parameters | *Lasers* | |
|---|---|---|
| | Er:YAG | Nd:YAG |
| Manufacturer | Kavo Co., Germany | Deka, Italy |
| Equipament Template | Kavo Key Laser II | Smartfile |
| Wavelength (nm) | 2,940 | 1,064 |
| Repetition Rate (Hz) | 2 | 10 |
| Pulse Length (µs) | 250 (short-pulsed) | 350 (short-pulsed) |
| Beam Diameter (mm) | 0.63 | 0.30 |
| Irradiation distance (mm) | 4 (prefocused) | 1 (unfocused) |
| Output Power (W) | 0.6 | 0.5 |
| Energy Density ($J/cm^2$) | 3.92 | 70.7 |
| Water Flow | 2.0mL/min | No cooling |
| Irradiation time (s) | 10 | 10 |

Table 3. Means (µm) ± standard deviations of the surface roughness of the dentin surface after different preventive pretreatments followed by erosive challenge

| Group | Reference Area (1) | Pretreated + Eroded Area (2) | Surface Roughness Difference (2-1) |
|---|---|---|---|
| G1 – Er:YAG | 1.845 ± 0.278 | 2.258 ± 0.537 | 0.413[a] |
| G2 – Er:YAG followed by NaF | 1.901 ± 0.198 | 2.145 ± 0.449 | 0.244[a] |
| G3 – NaF followed by Er:YAG | 1.881 ± 0.097 | 2.189 ± 0.522 | 0.308 [a] |
| G4 – Nd:YAG | 1.756 ± 0.277 | 2.204 ± 0.477 | 0.448 [a] |
| G5 – Nd:YAG followed by NaF | 1.823 ± 0.117 | 2.263 ± 0.501 | 0.440 [a] |
| G6 – NaF followed by Nd:YAG | 1.940 ± 0,273 | 2.208 ± 0.560 | 0.268 [a] |
| G7 – NaF (positive control) | 1.934 ± 0.129 | 2.155 ± 0.432 | 0.221 [a] |
| G8 – no treatment (negative control) | 1.850 ± 0.207 | 2.205 ± 0.382 | 0.355 [a] |

*Same letter represents statistical similarity.

Table 4. Lost volume (%) and standard deviations of the wear of the dentin surface after different preventive pretreatments followed by erosive challenge, comparing the treated area to the reference area.

| Group | Lost Volume (%) | Standard Deviation | |
|---|---|---|---|
| G1 – Er:YAG | 17.9 | 1.8 | a |
| G2 – Er:YAG followed by NaF | 18.2 | 1.1 | a |
| G3 – NaF followed by Er:YAG | 15.5 | 1.9 | a |
| G4 – Nd:YAG | 30.8 | 2.7 | c |
| G5 – Nd:YAG followed by NaF | 29.5 | 3.9 | c |
| G6 – NaF followed by Nd:YAG | 22.7 | 2.3 | b |
| G7 – NaF (positive control) | 32.1 | 4.1 | c |
| G8 – no treatment (negative control) | 35.7 | 3.3 | c |

*Same letter represents statistical similarity.

# 12 Conclusão

# Conclusão

A rugosidade superficial dos grupos, tratados e submetidos a desafio erosivo, foi similar aos grupos controle (tanto positivo quanto negativo) nas mesmas condições experimentais, demonstrando que a irradiação laser em dentina bovina é segura, uma vez que não alterou a propriedade analisada.

O laser Er:YAG mostrou os menores valores percentuais de perda de volume mineral na análise de desgaste, sugerindo que este laser aumentou a resistência ácida de dentina.

Portanto, a irradiação de dentina radicular bovina com lasers de alta intensidade provou ser um método promissor na prevenção da hipersensibilidade dentinária.

# 13 Agradecimentos

# *Agradecimentos*

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- 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.

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*De acordo com o estilo Vancouver. Disponível em http://www.nlm.nih.gov/bsd/uniform_requiriments.html. Abreviaturas dos periódicos em conformidade com *Baseline.*

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# 15 Anexos

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



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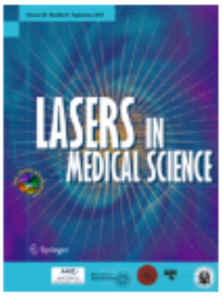


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If you are unsure, please use the full journal title.

For authors using EndNote, Springer provides an output style that supports the formatting of in-text citations and reference list.

EndNote style (zip, 2 kB)

Authors preparing their manuscript in LaTeX can use the bibtex file spbasic.bst which is included in Springer’s LaTeX macro package.

TABLES

- All tables are to be numbered using Arabic numerals.
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- For each table, please supply a table caption (title) explaining the components of the table.
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- Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

ARTWORK AND ILLUSTRATIONS GUIDELINES

## Electronic Figure Submission

- Supply all figures electronically.
- Indicate what graphics program was used to create the artwork.
- For vector graphics, the preferred format is EPS; for halftones, please use TIFF format. MSOffice files are also acceptable.
- Vector graphics containing fonts must have the fonts embedded in the files.
- Name your figure files with "Fig" and the figure number, e.g., Fig1.eps.

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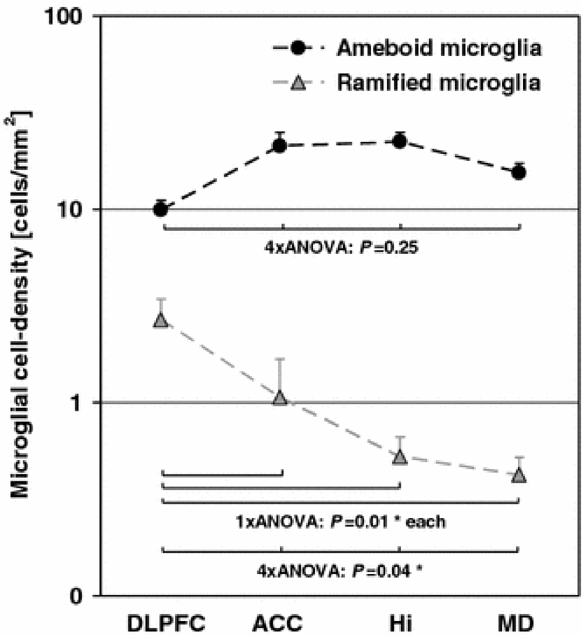


- Definition: Black and white graphic with no shading.
- Do not use faint lines and/or lettering and check that all lines and lettering within the figures are legible at final size.
- All lines should be at least 0.1 mm (0.3 pt) wide.
- Scanned line drawings and line drawings in bitmap format should have a minimum resolution of 1200 dpi.
- Vector graphics containing fonts must have the fonts embedded in the files.

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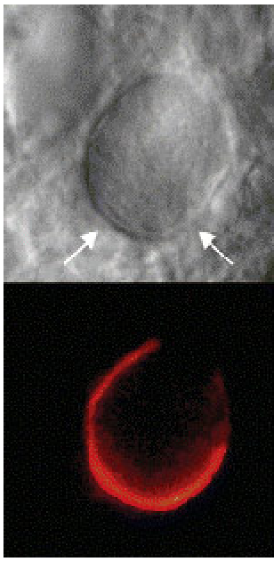
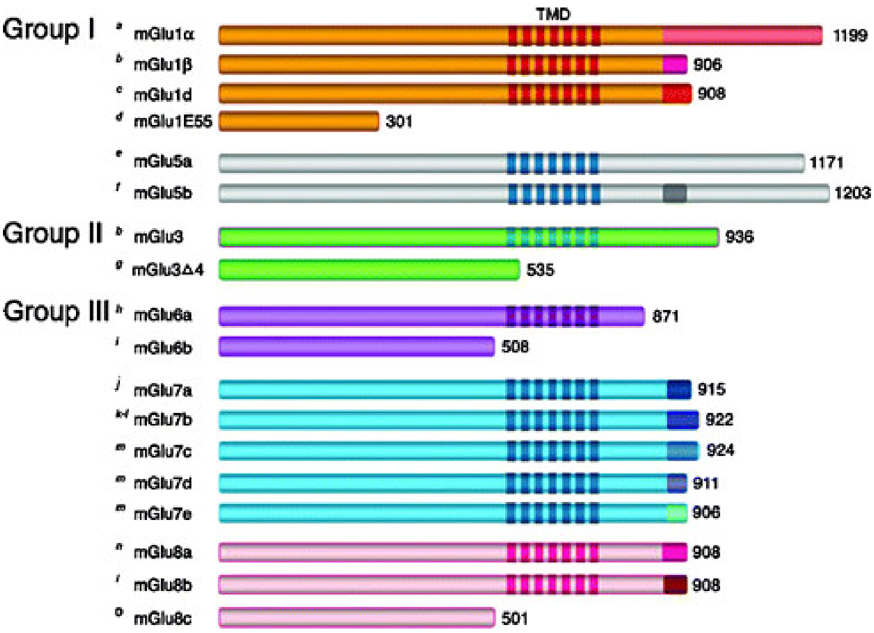
Definition: Photographs, drawings, or paintings with fine shading, etc.
If any magnification is used in the photographs, indicate this by using scale bars within the figures themselves.
Halftones should have a minimum resolution of 300 dpi.



Combination Art

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Combination artwork should have a minimum resolution of 600 dpi.

Color Art

Color art is free of charge for online publication.
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Color illustrations should be submitted as RGB (8 bits per channel).

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- Keep lettering consistently sized throughout your final-sized artwork, usually about 2–3 mm (8–12 pt).
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- Do not include titles or captions within your illustrations.

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Figures should always be cited in text in consecutive numerical order.
Figure parts should be denoted by lowercase letters (a, b, c, etc.).
If an appendix appears in your article and it contains one or more figures, continue the consecutive numbering of the main text. Do not number the appendix figures, "A1, A2, A3, etc." Figures in online appendices (Electronic Supplementary Material) should, however, be numbered separately.

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- Each figure should have a concise caption describing accurately what the figure depicts. Include the captions in the text file of the manuscript, not in the figure file.
- Figure captions begin with the term Fig. in bold type, followed by the figure number, also in bold type.
- No punctuation is to be included after the number, nor is any punctuation to be placed at the end of the caption.
- Identify all elements found in the figure in the figure caption; and use boxes, circles, etc., as coordinate points in graphs.
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- If the readers should be encouraged to make their own calculations, spreadsheets should be submitted as .xls files (MS Excel).

### Specialized Formats

- Specialized format such as .pdb (chemical), .wrl (VRML), .nb (Mathematica notebook), and .tex can also be supplied.

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- It is possible to collect multiple files in a .zip or .gz file.

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- Refer to the supplementary files as “Online Resource”, e.g., "... as shown in the animation (Online Resource 3)", “... additional data are given in Online Resource 4”.
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**Apêndice I:** Figuras referentes aos Materiais e Métodos

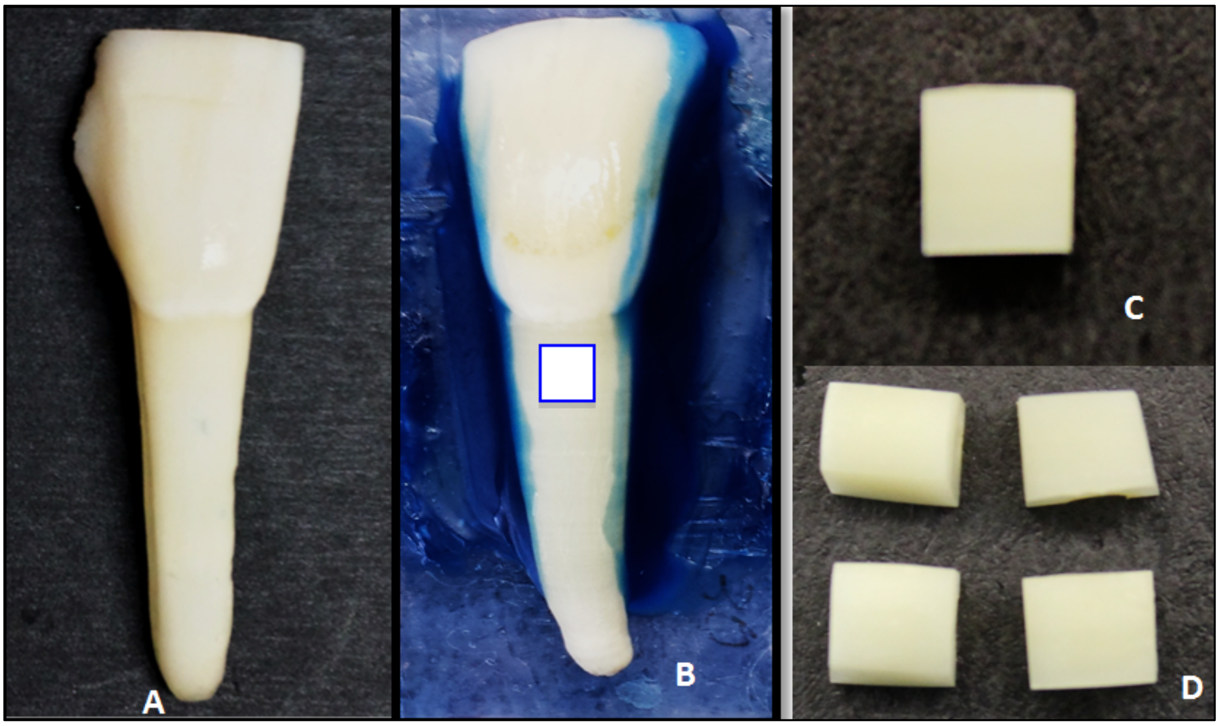


**Figura 1:** Obtenção dos espécimes – A) Incisivo bovino. B) Ilustração dos cortes que foram realizados. C) e D) Espécimes obtidos após os cortes.

**Figura 2:** Máquina de corte.

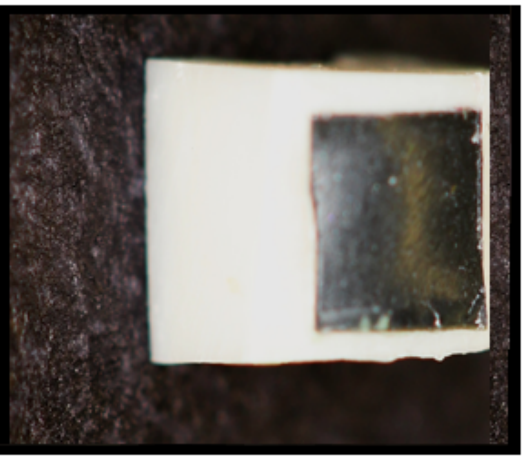

**Figura 3:** Fita isolante fixada no espécime.

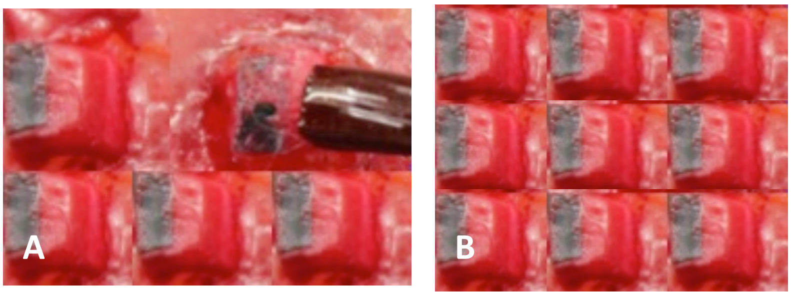


**Figura 4:** A) Proteção da área controle com esmalte cosmético. B) Espécimes protegidos com esmalte cosmético.

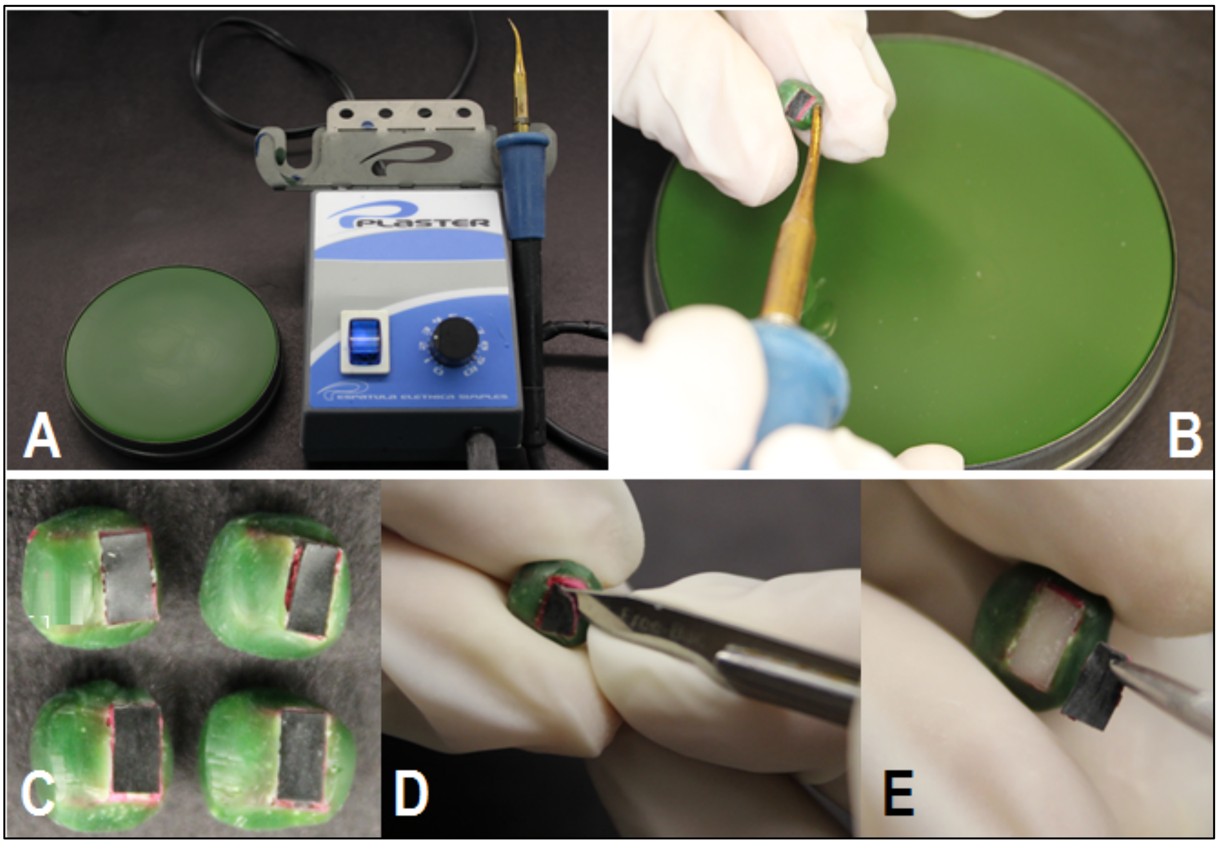


**Figura 5:** A) Cera de escultura e gotejador elétrico. B) Impermeabilização dos espécimes. C) Espécimes impermeabilizados. D) Remoção da fita isolante com lâmina de bisturi. E) Exposição da área que receberá os tratamentos preventivos e erosivos.

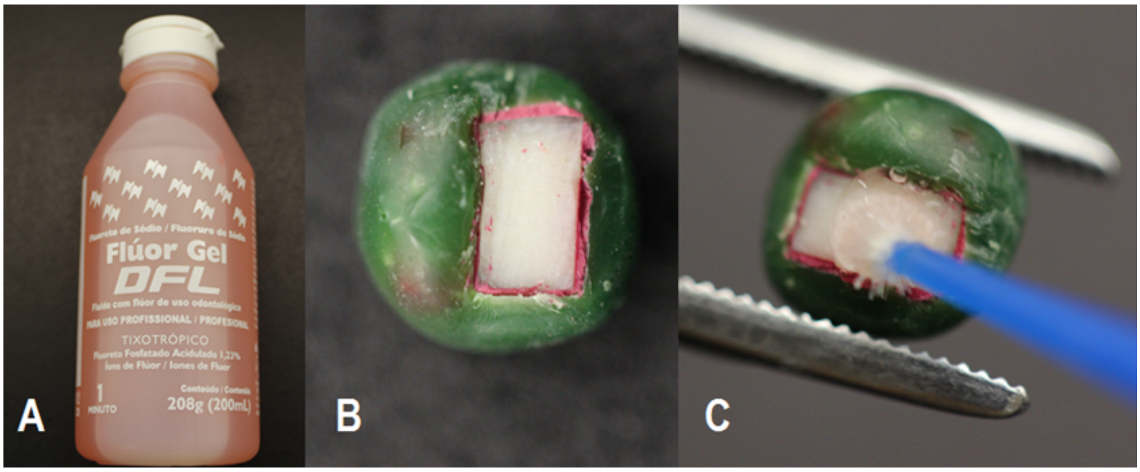


**Figura 6:** A) Fluoreto de sódio 1,23%. B) Espécime que receberá os tratamentos preventivos. C) Aplicação do fluoreto de sódio com auxílio do microbrush.

**Figura 7:** Laser Er:YAG

**Figura 8:** Laser Nd:YAG


**Figura 9:** Refrigerante à base de cola.


**Figura 10:** Máquina de agitação

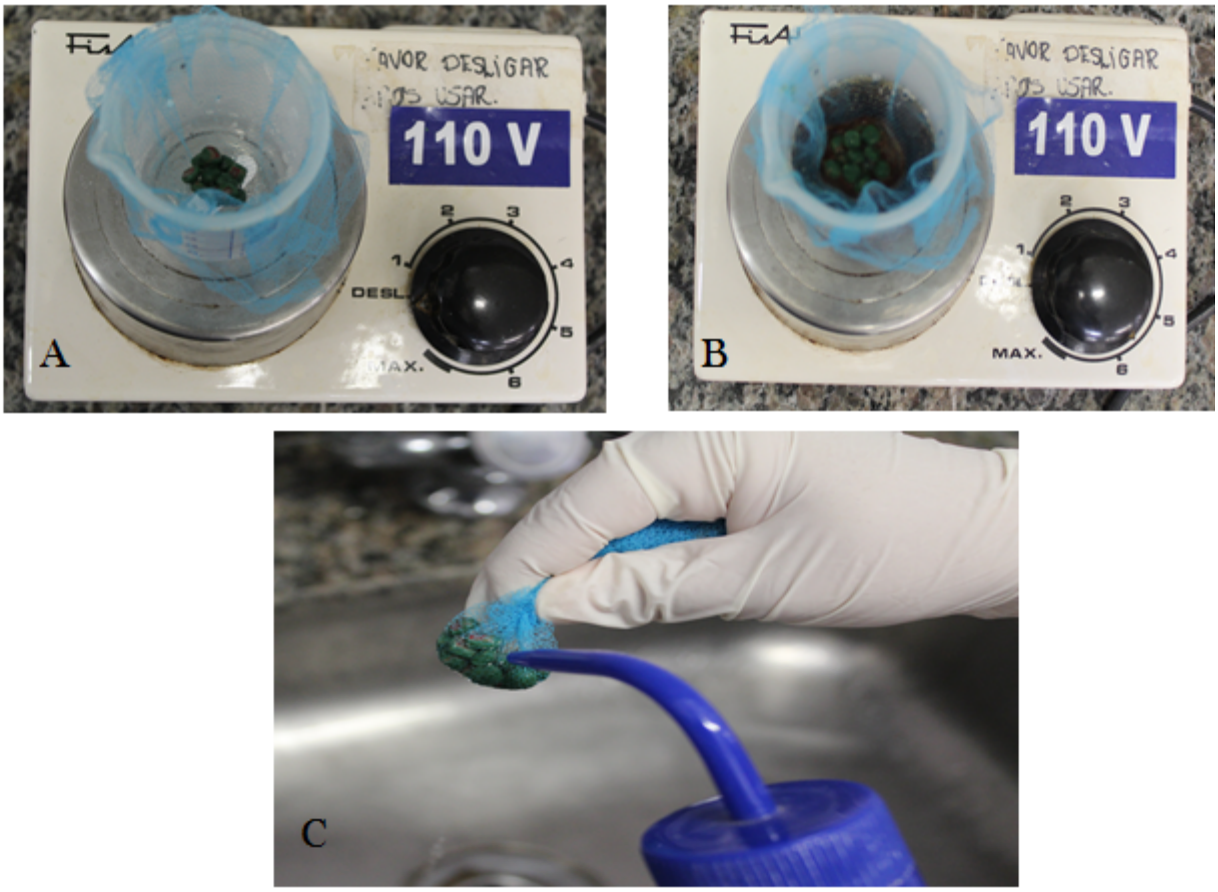


**Figura 11:** A) Espécimes inseridos em um Becker de 50 mL. B) Desafio erosivo em Coca-Cola. C) Espécimes sendo lavados com água destilada.

**Figura 12:** Remoção da cera e esmalte, para as análises de rugosidade superficial e desgaste.


**Figura 13:** Microscópio Confocal a laser 3D.

**Apêndice II:** Figuras referentes aos Resultados

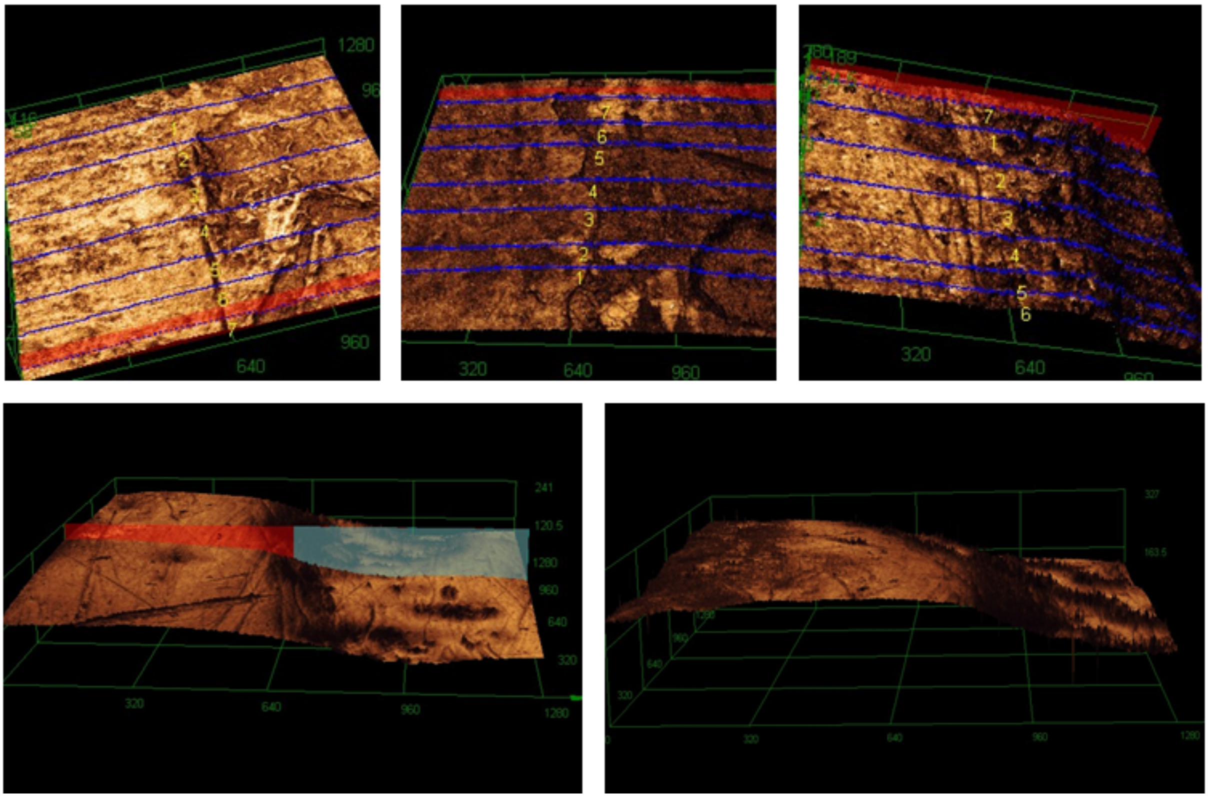


**Figura 14:** Fotos referentes à análise de rugosidade superficial

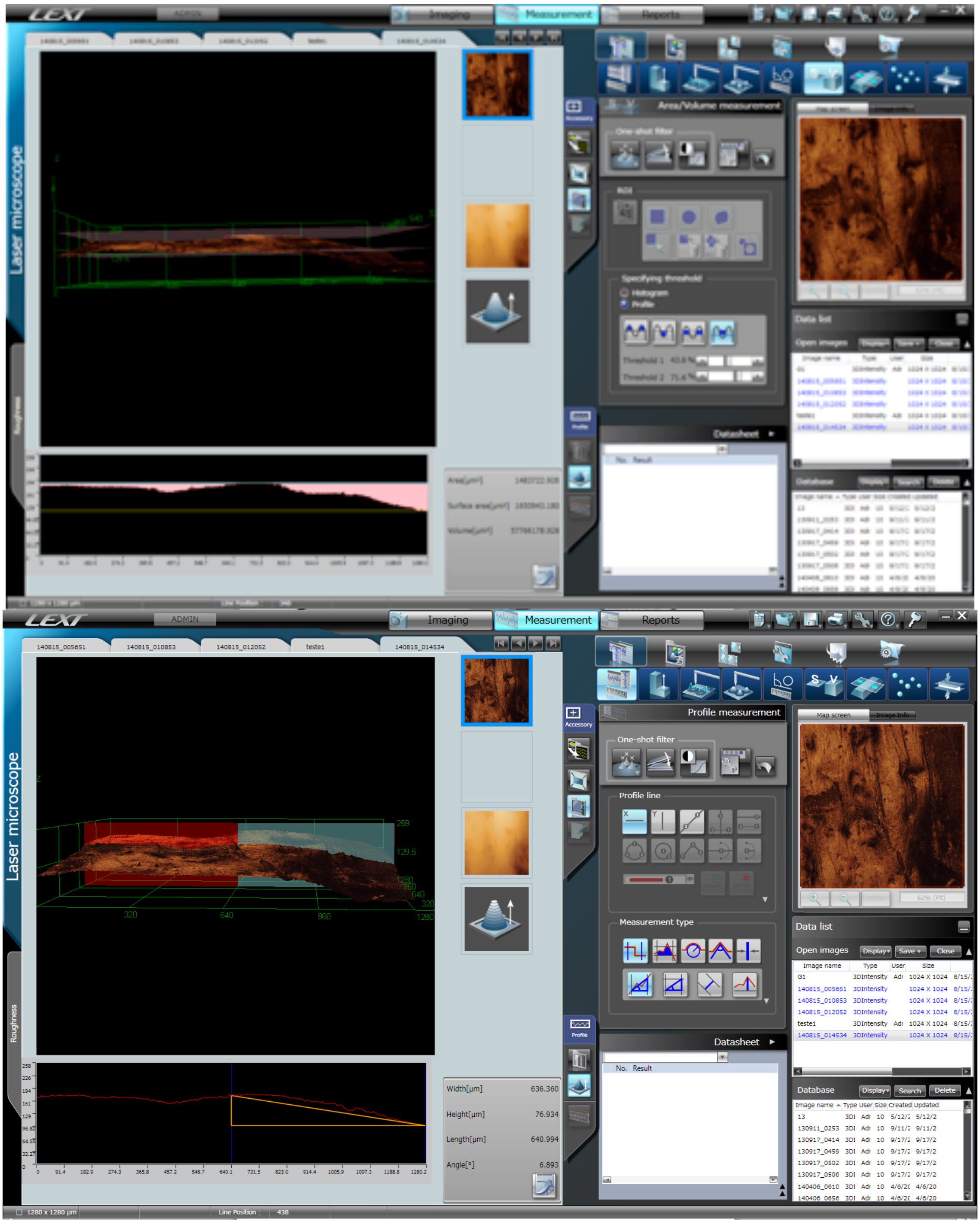


**Figura 15:** Imagens demonstrativas da avaliação do desgaste no Software OLS4000