UNIVERSIDADE DE UBERABA
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INFLUÊNCIA DO *LASER* Er, Cr: YSGG ASSOCIADO OU NÃO A AGENTES DESSENSIBILIZANTES NA MICRODUREZA LONGITUDINAL DA DENTINA RADICULAR BOVINA APÓS DESAFIO EROSIVO

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

Orientador: Prof. Dr. Cesar Penazzo Lepri

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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

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EDICATÓRIA

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RESUMO

O objetivo desse estudo *in vitro* foi avaliar a influência do laser Er,Cr:YSGG, associado ou não a agentes dessensibilizantes, na microdureza longitudinal da dentina radicular bovina após erosão ácida. Foram selecionados 40 incisivos bovinos; as dimensões dos espécimes foram: 4mm x 4mm e 3mm de espessura, divididos aleatoriamente em 8 grupos (n=10). CO: grupo controle; FV: verniz fluoretado (NaF 5%); L: Er,Cr:YSGG; FV + L: verniz fluoretado seguido de irradiação com laser; OXA: oxalato de potássio a 3%; OXA + L: oxalato de potássio a 3% seguido de irradiação com laser; GEL: silicato de cálcio bifásico / gel de fosfato; GEL + L: silicato de cálcio bifásico / gel de fosfato seguido de irradiação com laser. Metade do espécime foi devidamente isolada (região controle) e a outra metade recebeu um dos tratamentos propostos. Os parâmetros do laser foram: potência 0,5W; distância de irradiação=1,0mm; densidade de energia= 8,92J/cm². Após, os espécimes foram imersos em Coca-Cola a 4ºC, com pH de 2,42 durante 5 minutos. Este procedimento foi realizado 2 vezes ao dia, com intervalos de 6 horas entre os desafios, por um período total de 14 dias. Após esta etapa, os espécimes foram incluídos em resina epóxi e seccionados no sentido transversal para a análise de microdureza longitudinal (25gf por 40 segundos), nas seguintes profundidades: 20µm, 50µm, 100µm, 200µm e 500µm, tanto na região controle quanto na experimental. Os dados foram analisados percentualmente em relação aos valores do grupo controle e submetidos ao teste de Kruskall-Wallis, seguido do pós-teste de Dunn. Para comparar as profundidades, os dados foram submetidos ao teste de ANOVA. Todos os testes estatísticos adotaram o nível de significância de 5% (α=0,05). Observou-se que o grupo FV+L apresentou uma maior porcentagem de microdureza dentinária (345%) em relação ao grupo CO que não recebeu nenhum tratamento. O grupo L apresentou 221%, seguido pelos grupos OXA+L = GEL+L com 212% e 209% respectivamente. Os demais grupos sem irradiação (GEL, OXA, F) apresentaram menores valores de dureza ($p<0,05$). Não houve diferença estatisticamente significante entre as profundidades ($p>0,05$). Conclui-se que todos os grupos apresentaram maiores valores de microdureza quando comparados ao grupo controle negativo. O grupo FV + L, seguido do grupo L, mostraram os maiores valores de microdureza Knoop, seguidos dos grupos OXA + L = GEL + L, em relação ao grupo controle, que não recebeu nenhum tratamento preventivo. Portanto, a irradiação com laser é uma alternativa promissora na prevenção da erosão dentária especialmente quando associada à agentes dessensibilizantes.

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

ABSTRACT

The objective of this in vitro study was to evaluate the influence of Er, Cr: YSGG laser, associated or not to desensitizing agents, on the longitudinal microhardness of bovine root dentin after acid erosion. Forty bovine incisors were selected; the dimensions of the specimens were: 4mm x 4mm and 3mm thickness, randomly divided into 8 groups (n = 10). CO: control group; FV: fluoride varnish (5% NaF); L: Er,Cr:YSGG; FV + L: fluoride varnish followed by laser irradiation; OXA: 3% potassium oxalate; OXA + L: 3% potassium oxalate followed by laser irradiation; GEL: biphasic calcium silicate/phosphate gel; GEL + L: biphasic calcium silicate/phosphate gel followed by laser irradiation. Half of the specimen was properly isolated (control region) and the other half received one of the treatments proposed. The parameters of the laser were: power 0.5W; irradiation distance = 1.0mm; energy density = 8.92J/cm². Afterwards, specimens were immersed in Coca-Cola at 4ºC, pH 2.42 for 5 minutes. This procedure was performed twice a day, with 6-hour intervals between the challenges, for a total period of 14 days. After this step, the specimens were included in epoxy resin and cross-sectioned for the analysis of longitudinal microhardness (25gf - 40 seconds) at the following depths: 20μm, 50μm, 100μm, 200μm and 500μm, in both the control and experimental regions. The data were analyzed in relation to the values of the control group and submitted to the Kruskall-Wallis test, followed by the Dunn post-hoc. To compare the depths, the data were submitted to one-way-ANOVA. All the statistical tests adopted the level of significance of 5% ($\alpha = 0.05$). It was observed that the FV + L group had a higher percentage of dentin microhardness increase (345%) than the CO group that received no treatment. Group L presented 221%, followed by groups OXA + L = GEL + L with 212% and 209% respectively. The other groups without irradiation (GEL, OXA, F) had lower hardness values ($p<0.05$). There was no statistically significant difference between the depths ($p>0,05$). It was concluded that all groups had higher hardness values when compared to the negative control group. The group FV + L: fluoride varnish followed by laser irradiation, showed the higher values of Knoop microhardness, followed by OXA + L = GEL + L, in relation to the CO group, which received no preventive treatment. Therefore, laser irradiation is a promising alternative in the prevention of dental erosion, especially when associated with desensitizing agents.

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

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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

G grupo

HD hipersensibilidade dentinária

Hz hertz

J/cm^2 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

KHN Knoop Hardness Number (valor de dureza Knoop)

SUMÁRIO

1. *INTRODUÇÃO*

Induzida por episódios recorrentes de exposição ácida, a erosão dentária é uma condição que tem recebido crescente atenção de profissionais e pesquisadores nos últimos anos (ZHANG *et al*., 1998).

Estudos foram conduzidos na tentativa de alcançar uma melhor compreensão desse processo, juntamente com suas terapias preventivas e terapêuticas. Uma perda crônica patológica de tecidos duros dentais devido à influência química de ácidos extrínsecos e intrínsecos sem o envolvimento de microrganismos foi dedicado à modelagem desta lesão, tanto em condições de laboratório como *in situ* (BEZERRA *et al*., 2018; TURSSI *et al*., 2010).

Desse modo, a formação de depósitos semelhantes a fluoreto de cálcio (CaF_2) nas superfícies dos dentes é considerada o principal mecanismo de proteção contra a erosão dentária proporcionada por compostos fluoretados convencionais, como o fluoreto de sódio (NaF) e o fluoreto de amina (AmF). Esses depósitos podem atuar como uma barreira física contra os ácidos erosivos ou como um reservatório mineral para a remineralização (SCARAMUCCI *et al*., 2015).

Quando o desgaste erosivo está em um nível avançado com o envolvimento da dentina e da integridade estrutural do dente, comprometendo até a estética, é necessário realizar um tratamento restaurador para reduzir a sensibilidade térmica, como também prevenir o envolvimento pulpar, causado por abrasão de dentifrícios, erosão ácida adicional, impactação da comida, desconforto da língua e bochechas. Esse tratamento é feito principalmente para restabelecer a estética e aumentar a resistência dentária posteriormente (MIRKARIMI; TOOMARIAN, 2012; RAMOS *et al.*, 2013).

A desmineralização erosiva da coroa dentária é caracterizada pelo amolecimento inicial da superfície do esmalte, que varia dependendo do tempo de imersão e dos ácidos na cavidade bucal. (LUSSI *et al*., 2011).

A dentina é frequentemente exposta mesmo em estágios relativamente iniciais da formação de lesões erosivas, conhecido como o principal e mais importante local para estratégias de inibição da erosão. *In vitro*, a formação de lesões erosivas da dentina resulta no desenvolvimento de uma camada superficial de matriz de colágeno totalmente desmineralizada e solúvel em ácido, no entanto, clinicamente, ainda não está claro se e até que ponto esta estrutura é retida na cavidade bucal (DIAMANTI; KOLETSI-KOUNARI; MAMAI-HOMATA, 2016).

Produtos foram desenvolvidos para aliviar esta condição clínica, como oxalato de potássio, flúor, laser e outros agentes com efeitos oclusivos da dentina. A ação do oxalato de potássio na diminuição da HD (hipersensibilidade dentinária) é bem conhecida. Este agente dessensibilizante oblitera os túbulos dentinários pela precipitação de cristais de oxalato de cálcio. No entanto, parece que os cristais formados pela reação entre o oxalato de potássio e a hidroxiapatita, são dissolvidos ao longo do tempo. Além disso, o oxalato de potássio não tem efeito sobre o desgaste erosivo contínuo da dentina (MIYOSHI, 2015; PEREIRA; SEGALA; GILLAM, 2005).

Um novo dentifrício chamado Regenerate, de silicato de cálcio e fosfato de sódio (fosfato monossódico e fosfato trissódico), contendo 1450ppm de fluoreto (adicionado como monofluorofosfato de sódio), foi desenvolvido para proporcionar benefícios aprimorados à saúde do esmalte em relação aos dentifrícios fluoretados padrão (HORNBY *et al*.,2014). Além disso, um gel de dupla fase também foi desenvolvido para fornecer benefícios adicionais à remineralização do esmalte. O gel Regenerate de dupla fase contém parte A: uma fase fluoretada contendo sais de silicato de cálcio e fosfato de sódio e parte B: uma fase contendo fluoreto de sódio. Este gel de fase dupla é proposto como um complemento ao uso diário do creme dental. Demonstrou-se que o creme dental deposita silicato de cálcio na superfície do esmalte e forma a hidroxiapatita (HAP) em protocolos *in vitro* e *in situ*. Além disso, foi demonstrado usando modelos *in vitro*, que esta nova formulação de dentifrício pode reduzir a desmineralização do esmalte por ácido e promover a remineralização do mesmo, que o novo gel de dupla fase pode auxiliar no processo de remineralização do esmalte (HORNBY *et al*., 2014; JOINER *et al*., 2014; SUN *et al*., 2014, ARANTES *et al*., 2018).

Nos últimos anos, os lasers têm sido amplamente utilizados no tratamento de doenças bucais. A terapia com laser foi aplicada pela primeira vez para o tratamento da hipersensibilidade dentinária por Matsumoto e colaboradores em 1985. Até o momento, alguns tipos de lasers de alta potência (Nd:YAG, Er:YAG, CO_2 e GaAlAs) são comumente usados no tratamento da hipersensibilidade dentinária, e sua eficácia varia dependendo do tipo de laser e parâmetros utilizados. A terapia a laser tem sido um tema muito estudado no tratamento da hipersensibilidade dentinária, especialmente no que se refere ao seu efeito analgésico, imediato e reprodutível (JOINER *et al*., 2014). Por outro lado, a relação custo-benefício e a modalidade de tratamento complexo, bem como seu potencial dano à polpa dentária, devem ser levados em consideração (MIYOSHI, 2015). Assim, até o momento, o uso da terapia a laser para tratar a hipersensibilidade dentinária é incerto.

Para a prevenção da desmineralização dentária, a luz laser deve ser fortemente absorvida e convertida eficientemente em calor sem danificar os tecidos subjacentes ou circundantes, com o objetivo de alterar a composição ou diminuir a solubilidade dos tecidos duros dentais (RAMALHO *et al*., 2015; MOLAASADOLLAH *et al*., 2017;).

Foi demonstrado que a irradiação da superfície dentária com laser Er,Cr:YSGG, de 2.780nm de comprimento de onda, usando uma densidade de energia de 8J/cm^2, promove um aumento de temperatura suficiente para alterar a estrutura química do esmalte, transformando-o em uma estrutura menos solúvel (AL-OMARI; PALAMARA, 2013; GERALDO-MARTINS *et al*., 2014; RAMOS *et al*., 2013).

A dureza é uma propriedade amplamente utilizada para comparar materiais restauradores e tecidos biológicos. É definida pela resistência do substrato à deformação plástica local e medida pela relação entre a força aplicada e a área de indentação (CASTANHO et al., 1984). A medida dessa propriedade pode ser realizada de três maneiras diferentes: testes de penetração, risco ou choque. O teste mais utilizado na área odontológica é o teste de penetração. Para realizar este teste, é aplicada uma carga particular a um equipamento tendo uma pirâmide ou extremidade penetradora que penetre ou marque a superfície do material a ser testado (Vickers, Knoop, Rockwell e Brinell). A medida da marca deixada pelo penetrador é convertida em índices de dureza e expressa a propriedade do material em resistir à deformação plástica (FLAMINI 2012; DONASSOLLO *et al*., 2007; SUGAWARA *et al*., 2015).

Apesar de existirem vários tratamentos, até o momento não se sabe qual deles é o mais efetivo à longo prazo. Portanto, este trabalho comparou a microdureza longitudinal da dentina bovina erodida, submetida a diferentes tratamentos preventivos, utilizando o teste de microdureza Knoop.

A hipótese nula do presente estudo é de que não existiria uma diferença estatisticamente significante na microdureza, independentemente do tratamento preventivo.

2. *PROPOSIÇÃO*

O objetivo do presente estudo foi avaliar a influência do laser Er,Cr:YSGG, associado ou não a agentes dessensibilizantes, na microdureza longitudinal da dentina radicular bovina após erosão ácida.

3. *CAPÍTULO 1*

Influence of Er,Cr:YSGG laser, associated or not to desensitizing agents, on longitudinal microhardness of bovine dentin after erosive challenge

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ABSTRACT

Purpose: Evaluate the influence of Er,Cr:YSGG laser, associated or not to desensitizing agents, on the longitudinal microhardness of bovine root dentin after acid erosion. **Methods**: Forty-bovine incisors were selected (4mmX4mmX3mm-thickness) and divided into 8 groups (n=10). CO:control group; FV:fluoride varnish(5%NaF); L:Er,Cr:YSGG; FV+L; OXA:3% potassium oxalate; OXA+L; GEL:biphasic calcium silicate/phosphate gel; GEL+L. Half of each specimen was isolated (control area) and the other half received one of the treatments proposed before immersion in Coca-Cola at 4ºC, pH=2.42 for 5-minutes; twice a day, with 6-hour intervals between the challenges, during 14 days. Afterwards, the specimens were included in epoxy resin and cross-sectioned for the analysis of longitudinal microhardness (25gf-40seconds) at the following depths: 20µm, 50µm, 100µm, 200µm and 500µm, in both the control and experimental regions. The data were analyzed in relation to the values of the control group and submitted to Kruskall-Wallis followed by Dunn post-hoc tests. To compare the depths, the data were submitted to the one-way-ANOVA. **Results:** The FV+L group presented greater hardness increase of dentin microhardness (345%) than the CO group that received no treatment. Group L presented 221%, followed by groups OXA+L and GEL+L with 212% and 209% respectively. The other groups without irradiation (GEL, OXA, F) had lower hardness values ($p<0.05$). There was no statistically significant difference between the depths ($p>0.05$). **Conclusion:** FV+L group presented statistically significant hardness increase in relation to the other groups. Therefore, laser irradiation is a promising alternative in the prevention of dental erosion, especially when associated with desensitizing agents.

Key words: YSGG laser, Dental erosion, Hardness Tests, Dentinal hypersensitivity

4. INTRODUCTION

Induced by recurrent episodes of acid exposure, dental erosion is a condition that has received increasing attention from both, professionals and researchers, in the last few years. Many studies have been conducted in an attempt to reach better understanding of this process, along with its preventive/treatment therapies (1). Due to the pathological chronic loss of dental hard tissues as a result of chemical influence of extrinsic and intrinsic acids without the involvement of microorganisms, a substantial effort has been devoted to simulate this lesion, both under laboratory and *in situ* conditions (2,3).

This way, the formation of calcium fluoride (CaF_2)-like deposits on the tooth surfaces is assumed to be the main mechanism of protection against dental erosion provided by conventional fluoride compounds, such as sodium fluoride (NaF) and amine fluoride (AmF). These deposits can act either as a physical barrier against erosive acids or as a mineral reservoir for remineralization (4). When erosive wear is at an advanced level with involvement of the dentin of the tooth's structural integrity and compromising the aesthetics, it is necessary to perform a restorative treatment to reduce thermal sensitivity, prevent pulpal involvement, abrasion by toothpastes, further acid erosion, food impaction, discomfort of the tongue and cheeks, and especially to restore the aesthetics and increase the tooth resistance (5,6).

Dental erosion, however, consists on the tooth demineralization that occurs because of intrinsic and extrinsic acids, without the involvement of bacteria. Extrinsic factors include acidic foods, carbonated beverages, sports drinks, red and white wines, citrus fruits, and, to a lesser degree, occupational exposure to acidic environments. The most common intrinsic factors include hydrochloride acid, caused by chronic gastrointestinal disorders such as gastroesophageal disease, as well as health issues such as eating disorders (anorexia and bulimia), in which regurgitation and frequent vomiting are common (7). Erosive demineralization of the tooth crown is characterised by initial softening of the enamel surface, which varies depending on the immersion time and the acids under study (8). Dentine is often exposed even at relatively early stages of tooth erosion lesion formation, which is an important tissue target for erosion-inhibiting strategies. *In vitro*, dentine erosive lesion formation results in the development of a surface layer of fully demineralized, acid-insoluble collagen matrix, however, clinically, it is not yet clear whether and to what extent this structure is retained in the oral cavity (9).

Many products have been developed to relieve this clinical condition, such as potassium oxalate, fluoride, laser, and other agents with dentin occlusive effects. The action of potassium oxalate in diminishing DH (dentin hypersensitivity) is well known (30). This desensitizing agent obliterates the dentinal tubules by the precipitation of calcium oxalate crystals. However, it seems that the crystals formed by the reaction between potassium oxalate and hydroxyapatite are dissolved over time (10,11).

A new calcium silicate and sodium phosphate, Regenerate salts (mono sodium phosphate and trisodium phosphate) toothpaste containing 1450ppm fluoride (added as sodium mono- fluorophosphate) has been developed to provide enhanced enamel health benefits versus standard fluoride toothpastes. Furthermore, a new dual phase gel has also been developed to provide additional enamel remineralisation benefits. The new dual phase gel contains part A: a fluoridated phase containing calcium silicate and sodium phosphate salts and part B: a phase containing sodium fluoride. This dual phase gel is proposed as an adjunct to daily use of the toothpaste. The toothpaste has been shown to deposit calcium silicate onto the enamel surface and form hydroxyapatite (HAP) in both *in vitro* and *in situ* protocols. In addition, it has been shown using *in vitro* models that this new toothpaste formulation can reduce enamel demineralization by acid and promote remineralization of acid softened enamel and that the new dual phase gel can aid in the enamel remineralization process (12–14).

It was demonstrated that the irradiation of the enamel surface with an Er,Cr:YSGG laser, of 2,780nm wavelength, using a pulsed-beam system, fiber delivery, and a sapphire tip bathed in a mixture of air and water vapor laser device, using an energy density of 8 J/cm^2, promotes a suficient temperature increase to change the chemical structure of enamel, turning it into a less soluble structure (5,16,17).

For the prevention of tooth demineralization, laser light must be strongly absorbed and converted efficiently to heat without damage to the underlying or surrounding tissues, with the advantage of changing the composition or decreased solubility of dental hard tissues (7,15).

Hardness is a property widely used to compare both restorative materials and biological tissues. It is defined by the resistance of the substrate to the local plastic deformation and measured by the relation between the applied force and the indentation area (18). The measure of this property can be done in three different ways: through penetration, risk or shock tests. The most used test in the dental area is the penetration test. To perform this test, a particular load is applied to an apparatus which will penetrate or mark the surface of the material to be tested (Vickers, Knoop, Rockwell and Brinell). The measurement of the mark left by the penetrator is converted to hardness indexes and expresses the property of the material to resist plastic deformation (19–21).

In a previous study, the morphological similarities between bovine and human sclerotic dentine were examined at the microscopic level. These similarities were confirmed by the density of open tubules in both species. Likewise, bond strength tests comparing human and bovine sclerotic dentine have been carried out to assess whether the morphological similarities would result in similar behavior of these substrates with regard to dental adhesives(22,23).

The null hypothesis of the present study is that there would be no statistical significance difference on microhardness, regardless of the preventive treatment.

5. OBJECTIVE

The aim of the present study was to evaluate the influence of Er,Cr:YSGG, associated or not to desensitizing agents, on the longitudinal microhardness of bovine root dentin after erosive challenge.

6. MATERIALS AND METHODS

6.1 Experimental design

The factor under study was: treatment of the specimens in eight levels: CO: control group; FV: fluoride varnish; L: Er,Cr:YSGG laser; FV + L: fluoride varnish followed by laser irradiation; OXA: 3% potassium oxalate; OXA + L: 3% potassium oxalate followed by laser irradiation; GEL: biphasic calcium silicate/phosphate gel; GEL + L: biphasic calcium silicate/phosphate gel followed by laser irradiation. All the irradiated groups used the following parameters: 0,1W; 5Hz, energy density = 8.92J / cm² 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).

6.2 Teeth selection

Forty bovine incisors were selected, 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 water cooling 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 polishiment 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 a 600μm diameter sapphire fiber (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 (16). For the potassium oxalate, it was used Potassium oxalate monohydrate 3% (OXA-GEL. Kota Indústria e Comércio Ltda), with disposable applicator (microbrush); it was maintained with slight excess in contact for 2 minutes, and the excess was removed with a sterile gauze, according to the manufacturer's recommendations. The dual Regenerate Enamel Science™ (Unilever) 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

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 longitudinal Knoop microhardness test

The specimens were included in polyester resin and sectioned crosswise. After the polishing of these surfaces with silicon carbide (#600 and #1200) and felt disk with alumina, control and experimental area were evaluated, using aload of 25gf by 40s at the following depths: 20µm, 50µm, 100µm, 200µm and 500µm (Shimadzu HMV 2000, Shimadzu Corporation Kyoto, Japan). The KHN (Knoop Hardness Number) values were used for data analysis.

6.7 Statistical analysis

The data on the percentage of microhardness increased in relation to the control group were submitted to the Kruskall-Wallis test. Once a statistically significant difference was observed, the Dunn post-test was performed to differentiate the groups. To compare the depths, the data were submitted to the ANOVA test. All statistical tests assumed a significance level of 5% (α=0.05).

7. RESULTS:

7.1 Knoop Microhardness

The microhardness values are showed in Tables 3 and 4.
It was observed that the FV + L group had a higher percentage of dentin microhardness (345%) than the CO group that received no treatment. Group L presented 221%, followed by groups OXA + L = GEL + L with 212% and 209% respectively. The other groups without irradiation, GEL, OXA, F had lower hardness values, both with ($p<0.05$). There was no statistically significant difference between the depths ($p>0,05$).

8. DISCUSSION:

The present 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 microhardness. Thus, the null hypothesis that different treatments would have no effect on the dentin acid resistance after erosive challenge was rejected.

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 (21,25).

For the study of microhardness of dental tissue, basically two methodologies are used: Vickers and Knoop microhardness, being the Knoop method widely used in the literature (38).

There is a great difficulty in comparing microhardness values between different studiesdue to the use of several types of tests to determine this property (24). Human teeth would be the first choice for laboratory studies. However, a more preventive approach to dentistry, reducing the number of extractions, and increased awareness of the precepts of bioethics, has led to a reduction in the availability of these teeth for use in laboratory tests (19,25). Studies using different animal teeth have been performed and bovine teeth have shown results comparable to humans in various laboratory tests (24, 26, 30). Bovine teeth have similar hardness to human teeth, enamel and superficial dentin. In addition, the proportion of organic and inorganic components is similar in both enamel and dentin (18,19,21).

It was performed the immersion of the specimens in Coca-Cola® because of its erosive potential already studied and discussed in several scientific works (27). In addition to being a widely consumed beverage in the world, its pH of 2.42 (at temperature of consumption=4ºC) is far below the critical pH of the dentin (ph 6,5), unbalancing the process of demineralization-remineralization (27,28).

In a study of dental erosion induced by different beverages, Coca Cola® was considered the most erosive agent among hot and cold drinks studied (1,29).

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 (29). This corroborates with the results verified in the present study, in which dentin surface demineralization was observed after erosive challenge with Coca Cola®.

The present study aimed to evaluate the microhardness of the bovine dentin after the specimens were treated followed by erosion by Coca Cola®, where all the groups that received treatment were compared in relation to the CO group, which received no treatment and was only eroded.

Hardness is the resistance of the material to indentation, and it is a property directly related to the susceptibility of a material to deformation and fracture. The micromechanical properties such as microhardness of irradiated human teeth should be thoroughly investigated for a better understanding that would possibly improve clinical treatment of tooth fracture and predict susceptibility of teeth to fracture (17,20,30,31).

The FV + L group presented the highest increase in dentin hardness value in relation to the CO group and the other treated and eroded groups, demonstrating that the absence of any type of preventive treatment enhances the action of Coca Cola®, that is, surface demineralization dentin hardness, decreasing the value of dentin hardness.

The data obtained for the longitudinal microhardness test showed that all groups receiving treatment presented more than 100% hardness in relation to the group without CO preventive treatment ($p<0.05$), that means a microhardness increase.

Following the FV + L group, the other groups presented this sequence: L> OXA + L = GEL + L> GEL> OXA> FV in order from highest to lowest hardness increase.
Thus, it is demonstrated the need to perform preventive treatment for erosion, rather than performing any type of treatment.

The FV + L group (Fluoride varnish + Laser Er, Cr: YSGG application) presenting a higher hardness, after the erosive challenge compared to the other groups, followed by the L group. This can be explained because the interaction of fluoride and laser, increases the acid resistance, leaving the dentin less soluble (16,17,32). This explains the fact that the irradiated groups presented better results.

The OXA + L and GEL + L groups also presented a statistically similar hardness and higher than the OXA, GEL and FV groups, which were not irradiated and to the CO, after the erosive challenge. This can be explained because the action of potassium oxalate is desensitizing agent obliterates the dentinal tubules by the precipitation of calcium oxalate crystals (10). However, it seems that the crystals formed by the reaction between potassium oxalate and hydroxyapatite are dissolved over time. Additionally, the potassium oxalate does not have any effect against continual dentin erosive wear (10), however it proved to be efficient when used in combination with the laser.

The GEL + L group (application of calcium silicate / phosphate + laser) also presented a higher value of Knoop hardness in dentin after erosive challenge. This treatment based on calcium silicate and sodium phosphate can provide protection to the enamel by several mechanisms: release of calcium ions into adjacent oral fluids under acidic conditions, thereby increasing local calcium concentration, degree of saturation relative to hydroxyapatite enamel and inhibition of dissolution (14,33). 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 (5,7,15,34).

Simultaneous application of fluoride and laser as a caries prevention method has been extensively evaluated. A studie have reported that application of both methods is beneficial and have shown that fluoride uptake increases after laser irradiation (15). For this reason and based on previous studies that showed that the use of the laser emission of 2.78μm-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 (5,16).

The use of laser to increase the acid resistance of dentin is safe and a new option for preventive treatment, since the literature have already shown that healthy pulp tissue is not thermally affected if the temperature rise were less than 5,5°C (1,35).

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 FV+L showing higher values of dentin microhardness. Kaur et al. 2017 observed that the Er,Cr:YSGG laser was successful in increasing the enamel surface microhardness whereas surface treatment with fluoride varnish did not significantly affect the microhardness of enamel. It could also be stated that surface treatment with Er,Cr:YSGG laser causes a positive alteration of the enamel surface increasing its ability to resist demineralization with optimum microhardness (36). Kumar et al. 2016 verified that fluoride gel treatment followed by laser irradiation leads to maximum increase in the microhardness of the enamel surface with highest increase in case of APF gel (37).

In spite of the favorable properties of erbium lasers, several other evaluations and comparisons need to be carried out, especially evaluations related to the compositional changes and microhardness of tooth hard tissues irradiated with these lasers before they can be routinely used in dental clinics (39).

In the present study, the microhardness test was used and one of the advantages of this test is that it provides the opportunity to observe the dentin at various depths of indentations (20µm, 50µm, 100µm, 200µm and 500µm) to observe the extent to which the material enters contact with the substrate, with reliable results. Although microhardness determination does not provide specific information on mechanical properties and dentin structures, it provides indirect evidence of mineral loss or dentin hard tissue gain (40,41).

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, especially in relation to prevention, so that there are no future problems.

9. CONCLUSION:

Considering the results obtained and the limitations of an in *vitro* study, it was concluded that all groups had better hardness values when compared to the negative control group. The group FV + L: fluoride varnish followed by laser irradiation, showed the higher values Knoop microhardness, followed by L, OXA + L, GEL + L, GEL, OXA and FV, in relation to the CO group, which received no preventive treatment. Therefore, laser irradiation is a promising alternative in the prevention of dental erosion, especially when associated with desensitizing agents.

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11. CONFLICT OF INTEREST

The authors state that there are no conflict of interest.

12. ROLE OF FUNDING SOURCE

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13. ETHICAL APPROVAL

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

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

Groups	Treatments
CO	No treatment (negative control)
FV	Application of 5% fluoride varnish
L	Application of Er,Cr:YSGG (0.1W; 5Hz, 55% air)
FV + L	Fluoride varnish + Laser Er,Cr:YSGG application (0.1W; 5Hz; air 55%)
OXA	Application of 3% potassium oxalate
OXA + L	Application of 3% potassium oxalate + Laser Er,Cr:YSGG (0.1W; 5Hz; air 55%)
GEL	Applying the calcium silicate/phosphate toothpaste
GEL + L	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
Irradiation Distance	1,0mm
Time	10 seconds
Mode of application	Surface scanning
Wave-length	2.78 µm
Fiber diameter	600µm
Energy density	8.92 J/cm²

Table 3: Mean values and percentage (%) in relation to CO: control group of the longitudinal microhardness of the groups, considering the control region and the treated + eroded area.

Groups	Mean difference		Percentage in relation to CO
CO: control group	20µm 50µm 100µm 200µm 500µm	1,843 0,684 1,798 1,775 2,060	/
FV : Fluoride varnish	20µm 50µm 100µm 200µm 500µm	2,163 2,024 0,918 2,422 3,128	151% [f]
L: Er,Cr:YSGG (0.1W; 5Hz, 55% air)	20µm 50µm 100µm 200µm 500µm	2,341 3,629 3,051 3,856 1,294	221% [b]
FV + L: Fluoride varnish followed by Laser Er,Cr:YSGG application (0.1W; 5Hz; air 55%)	20µm 50µm 100µm 200µm 500µm	5,740 4,220 5,500 5,150 4,180	345% [a]
OXA: 3% potassium oxalate	20µm 50µm 100µm 200µm 500µm	0,380 2,710 2,479 3,575 1,797	169% [e]
OXA + L: 3% potassium oxalate followed by Laser Er,Cr:YSGG (0.1W; 5Hz; air 55%)	20µm 50µm 100µm 200µm 500µm	5,596 2,830 3,080 1,045 2,280	212% [c]
GEL: biphasic calcium silicate/phosphate gel	20µm 50µm 100µm 200µm 500µm	4,069 3,336 2,762 0,780 1,094	192% [d]
GEL + L: biphasic calcium silicate/phosphate gel followed by Laser Er,Cr:YSGG (0.1W; 5Hz; 55% air)	20µm 50µm 100µm 200µm 500µm	3,209 2,869 3,222 3,023 2,148	209% [c]

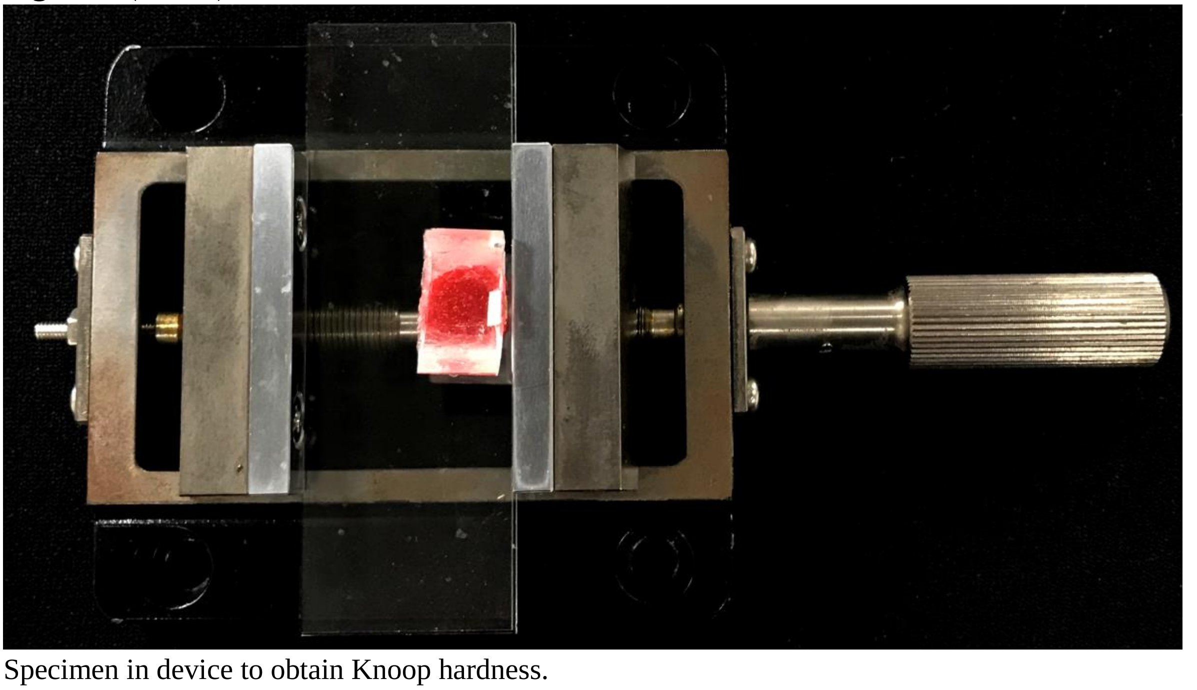
* Equal letters represent statistical similarity between groups (p>0.05)

Table 4: Mean (standard deviation) of the mean microhardness difference at the different depths analyzed.

20µm	**3,1678 (1,8725)[a]**
50µm	**2,7879 (1,0747)[a]**
100µm	**2,8514 (1,3222)[a]**
200µm	**2,7032 (1,4912)[a]**
500µm	**2,2478 (0,9998)[a]**

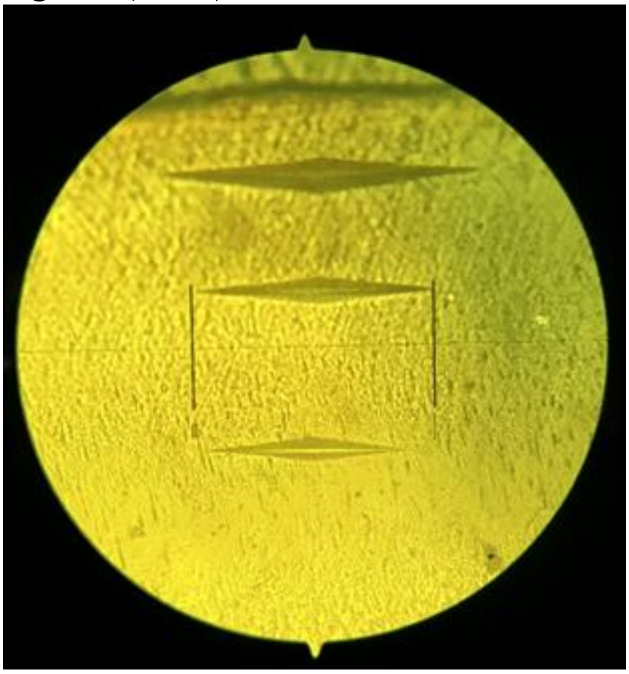
*Equal letters represent statistical similarity between depths (p>0.05)

Figura 1 (article):



Specimen in device to obtain Knoop hardness.

Figura 2 (article):



Hardness measurements at depths of 20 μm, 50 μm and 100 μm.

15 Conclusão:

Considerando os resultados obtidos e as limitações de um estudo *in vitro*, conclui-se que todos os grupos apresentaram maiores valores de microdureza quando comparados ao grupo controle negativo. O grupo FV + L mostrou o melhor resultado de dureza Knoop da dentina radicular bovina, seguido dos grupos L, OXA + L = GEL + L, GEL, OXA e FV, todos em relação ao grupo CO, que não recebeu nenhum tratamento preventivo. Portanto, a irradiação com laser é uma alternativa promissora na prevenção da erosão dentária especialmente quando associada à agentes dessensibilizantes.

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17. APÊNDICE :

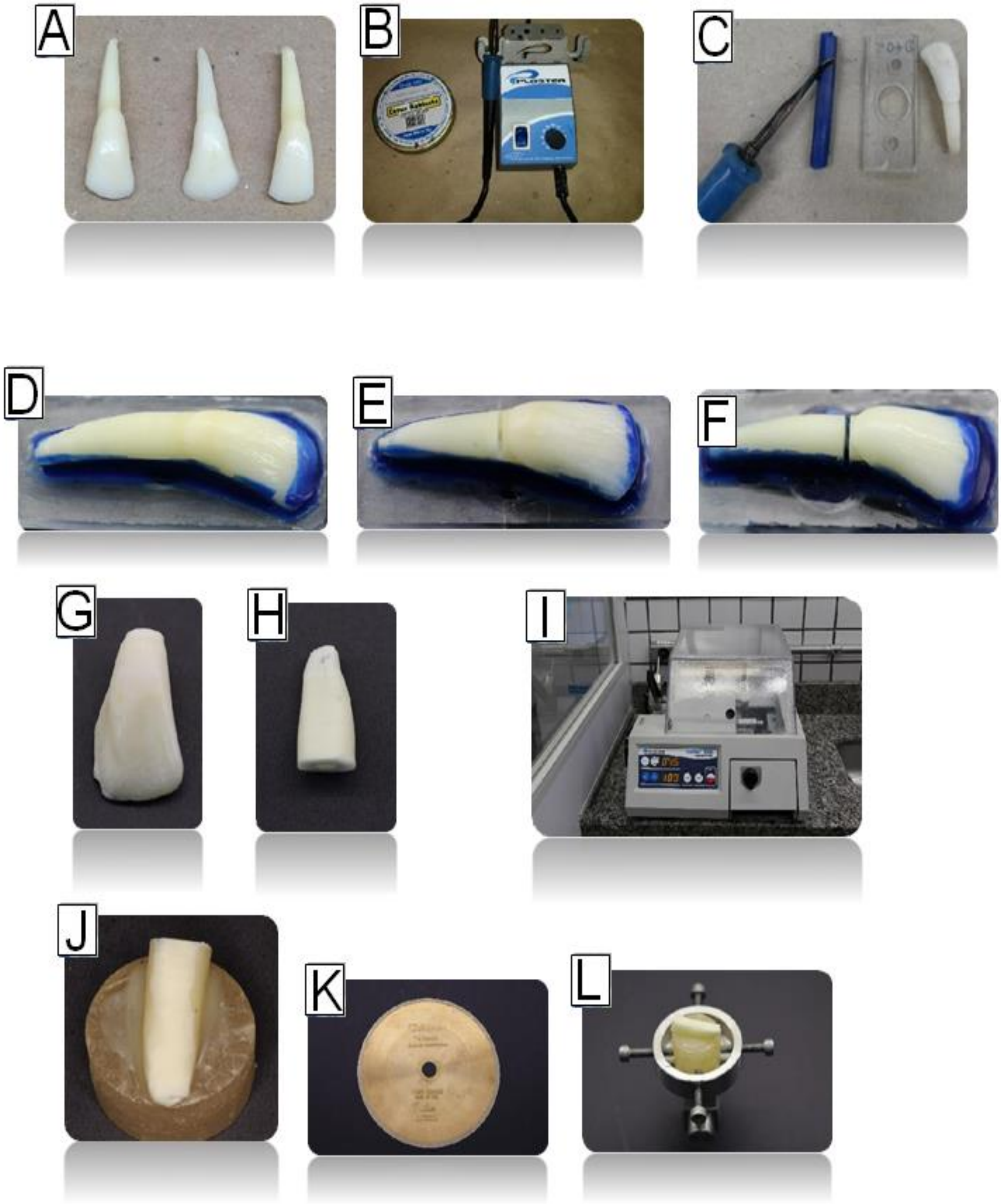


Figura1: Preparo dos espécimes. A- Dentes hígidos bovinos. B- Gotejador elétrico. C- Gotejador elétrico e cera para fixação do dente na placa acrílica. D- Dente fixado com cera na placa acrílica. E- Início de separação coroa e raiz. F- Separação coroa e raiz. G- Coroa bovina. H- Raiz bovina. I- Máquina de corte ISOMET® 1000. J- Cilindro acrílico e fixação de raiz. K- Disco diamantado. L- Dispositivo utilizado para o preparo dos espécimes.

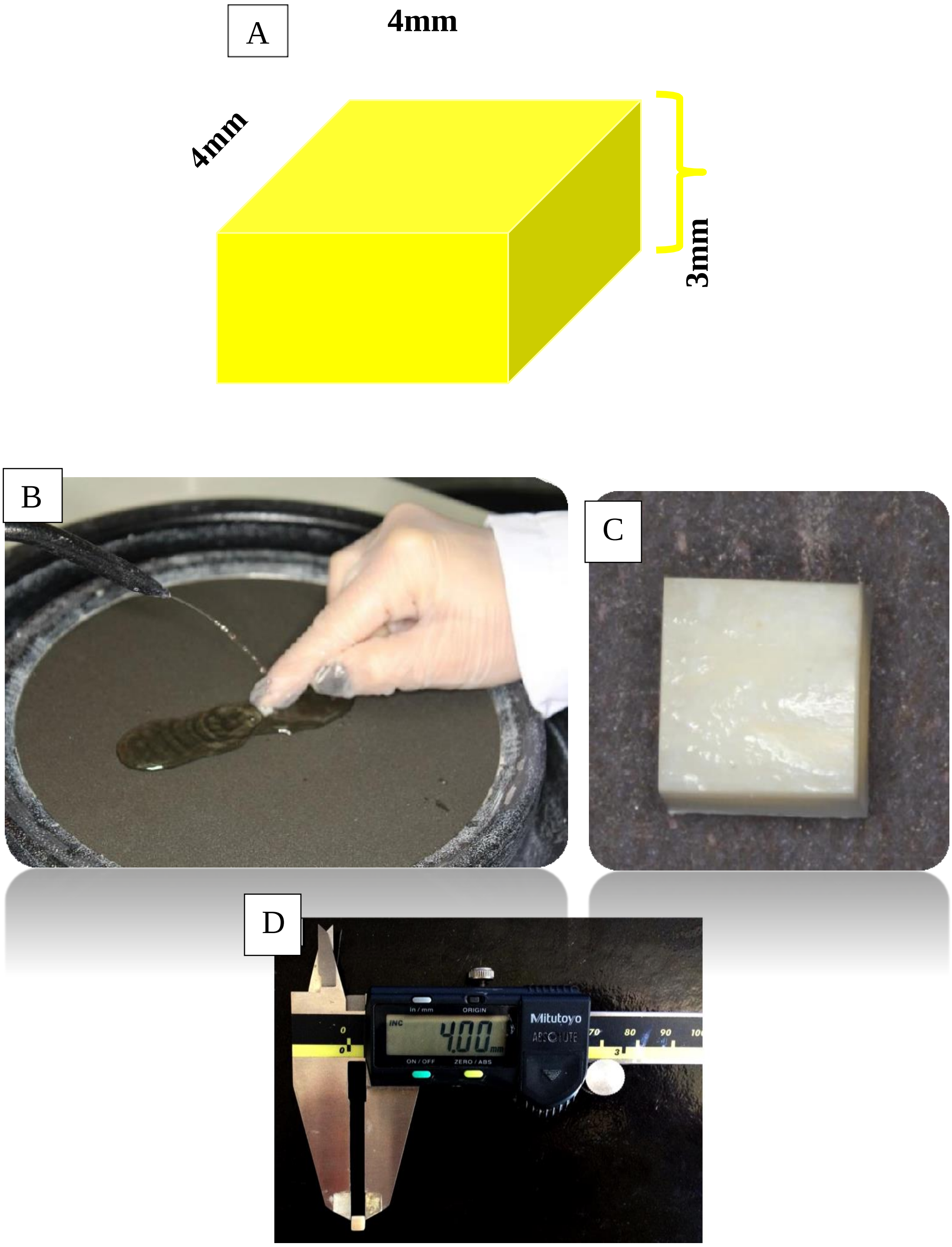


Figura 2: A- Medidas dos espécimes. B- Lixadeira e Politriz Metalográfica APL (Arotec) C- Espécime Padronizado. D: Paquímetro digital.

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.

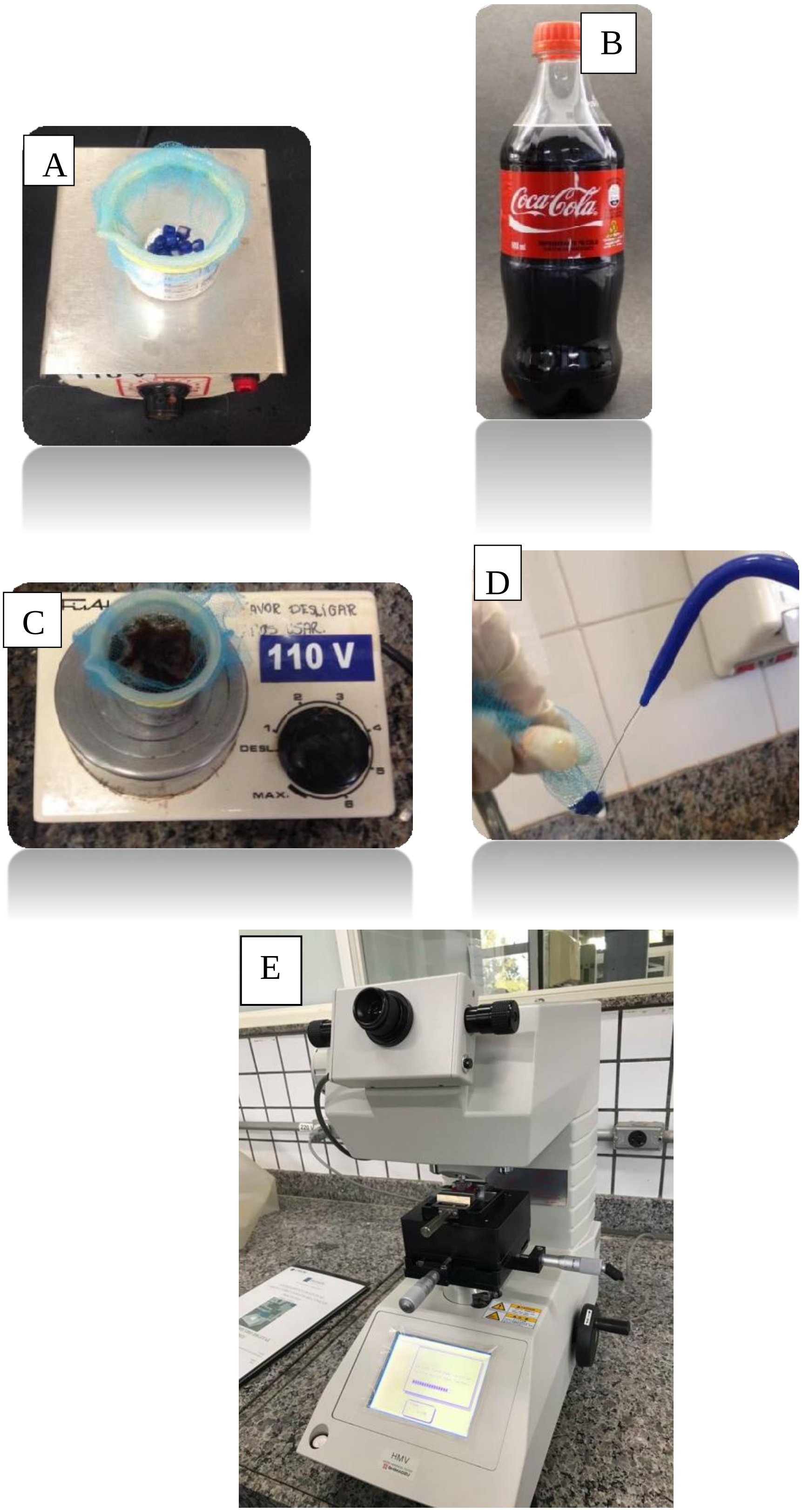


Figura 4: A- Agitador magnético. B- Coca-Cola®. C- Solução de Coca-Cola® no aitador magnético. D- Espécimes lavados em água destilada. E- Microdurômetro digital (Shimadzu HMV 2000, Kyoto, Japan).

Figura 5: F: Tubos de plástico em cera, com espécime acoplados lado a lado. G: Resina Epóxi com endurecedor. H: Espécime no interior da esfera de resina. I: Corte dividindo área experimental e área controle.

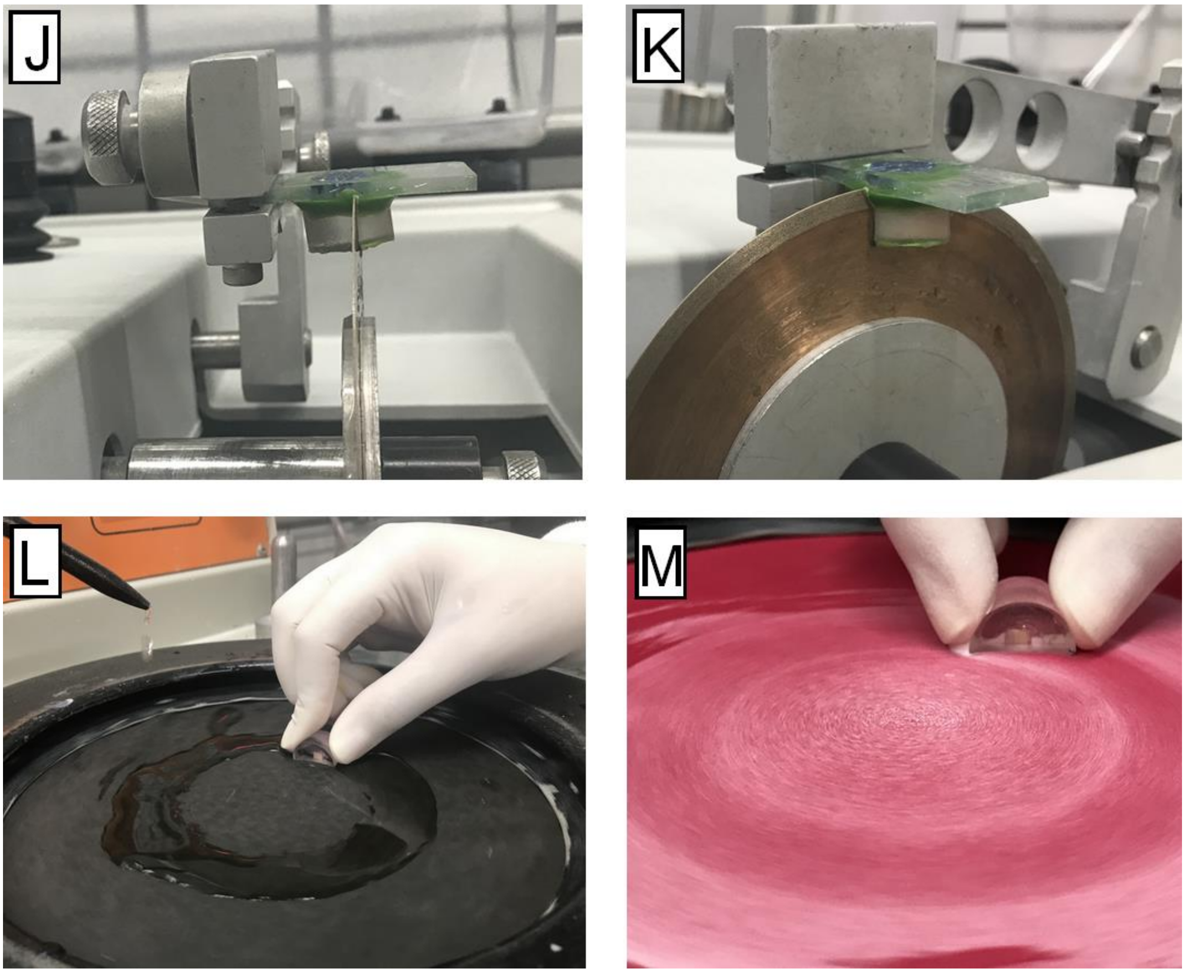


Figura 6: J: Espécime sendo dividido na Máquina de corte ISOMET® 1000. K: Espécime dividido pelo disco diamantado. L: Metade do espécime sendo lixado em lixa d´´agua na Lixadeira e Politriz Metalográfica APL (Arotec). M: Polimento final em disco de feltro com alumina.

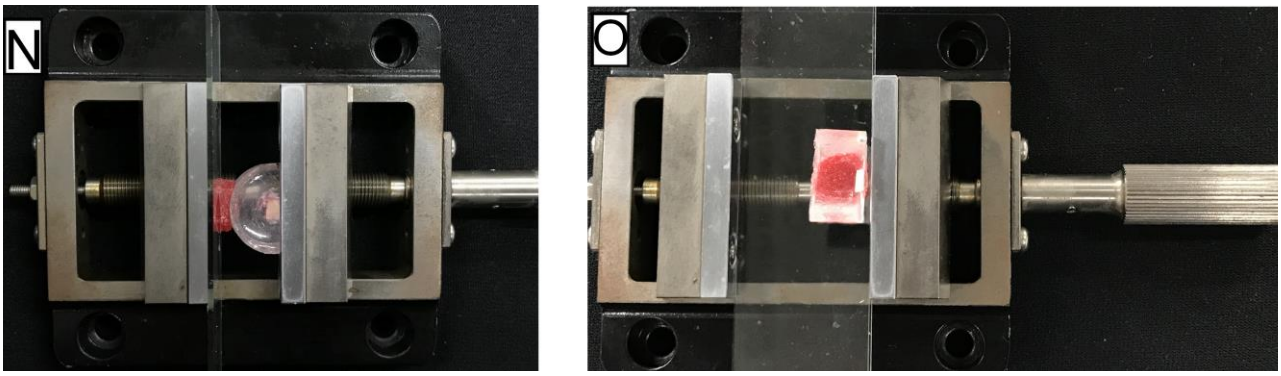


Figura 7: N: Espécime acoplado em lâmina de vidro, no dispositivo morsa “paralelômetro”, para obtenção do paralelismo. O: Espécime preparado para leitura em microdurômetro.

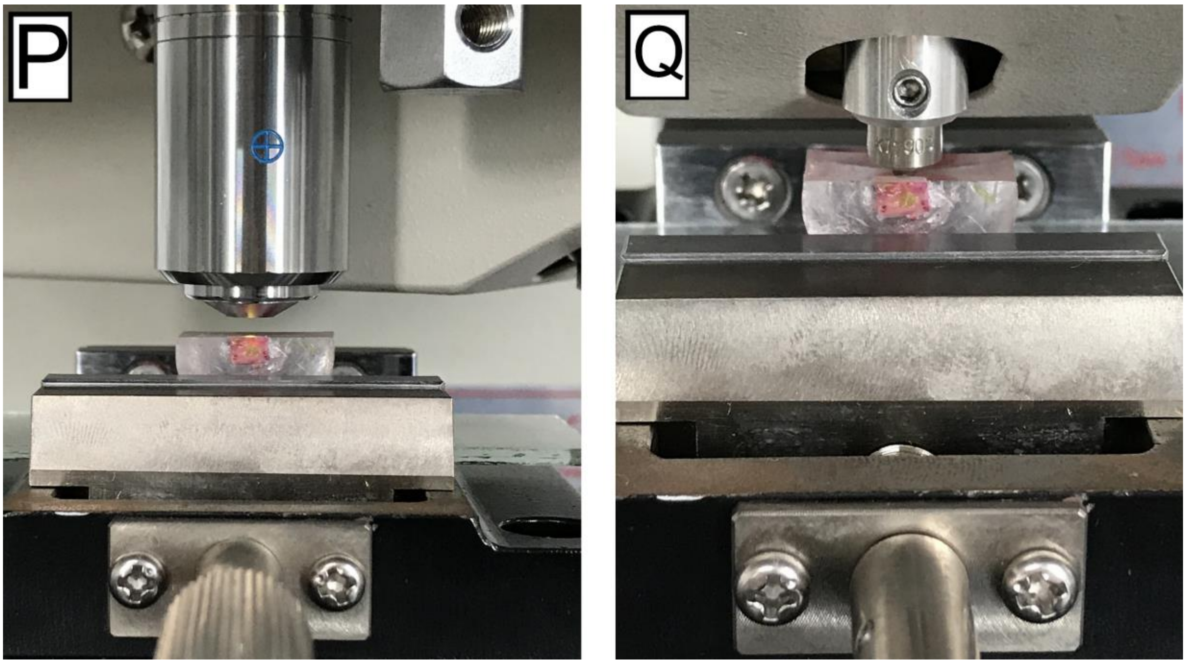


Figura 8: P: Lente indicando ótica de 40X em área para endentação. Q: Endentador Knoop com carga de 25gf em ação para obtenção da dureza.

Figura 9: Display do microdurômetro digital (Shimadzu HMV 2000, Kyoto, Japan) demarcando carga de 25gf

(0,025) em 40 sec em ótica de 40X com endentaodr Knoop. Valor HK demonstrando um valor de dureza obtido.

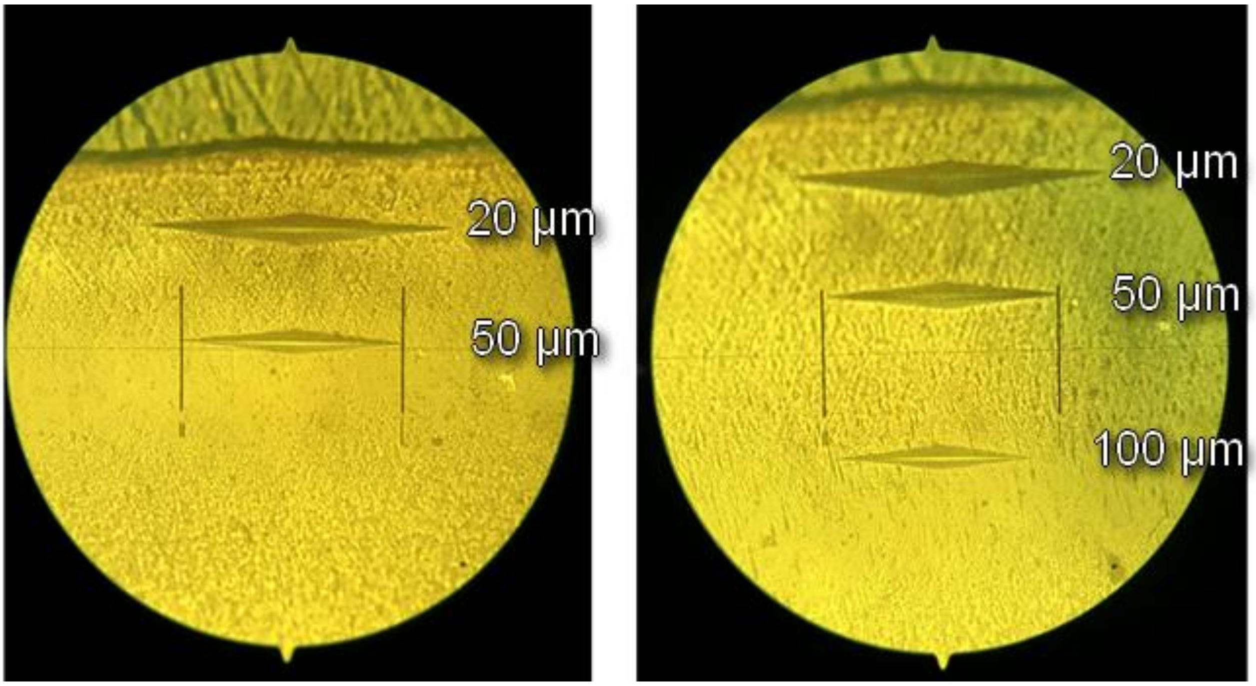
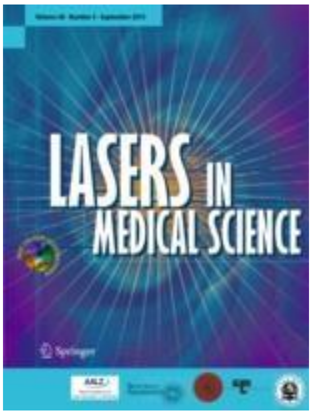


Figura 10: Imagem vista pela lente de 40x no microdurômetro, demarcando endentações em Knoop, em profundidades de 20µm, 50µm na foto à esquerda e 20µm, 50µm e 100µm, na imagem à direita.

18. ANEXO :

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



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SCIENTIFIC STYLE

Generic names of drugs and pesticides are preferred; if trade names are used, the generic name should be given at first mention.

Units and abbreviations

- Please adhere to internationally agreed standards such as those adopted by the commission of the International Union of Pure and Applied Physics (IUPAP) or defined by the International Organization of Standardization (ISO). Metric SI units should be used throughout except where non-SI units are more common [e.g. litre (l) for volume].
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1. Negotiation research spans many disciplines [3].

2. This result was later contradicted by Becker and Seligman [5].

3. This effect has been widely studied [1-3, 7].

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  Smith J, Jones M Jr, Houghton L et al (1999) Future of health insurance. N Engl J Med 965:325–329

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- Book

  South J, Blass B (2001) The future of modern genomics. Blackwell, London
- Book chapter

  Brown B, Aaron M (2001) The politics of nature. In: Smith J (ed) The rise of modern genomics, 3rd edn. Wiley, New York, pp 230-257
- Online document

  Cartwright J (2007) Big stars have weather too. IOP Publishing PhysicsWeb. http://physicsweb.org/articles/news/11/6/16/1. Accessed 26 June 2007
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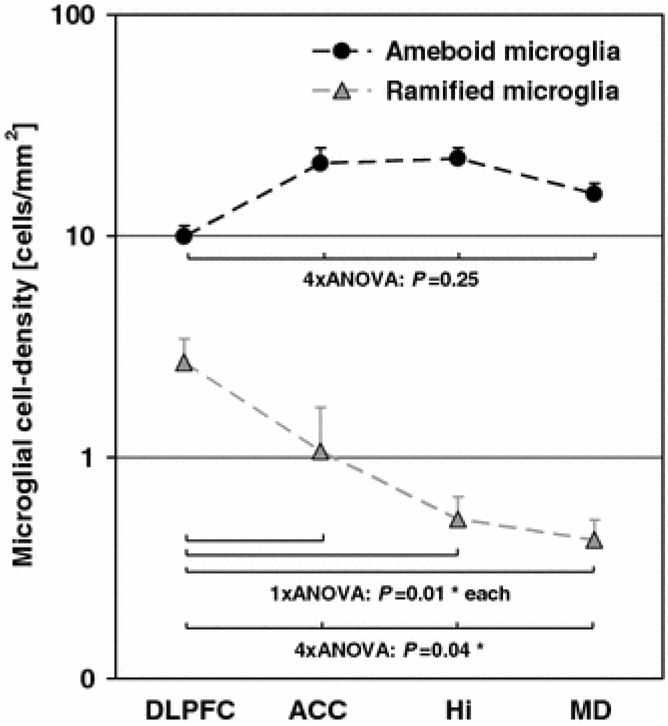

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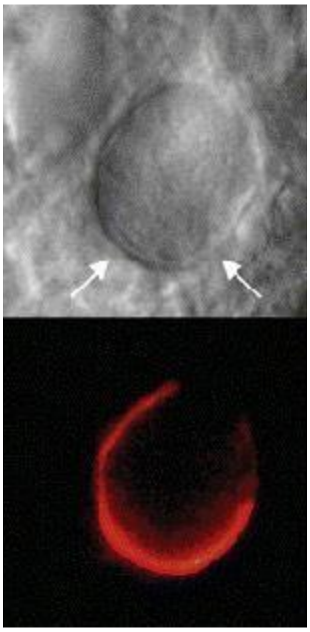

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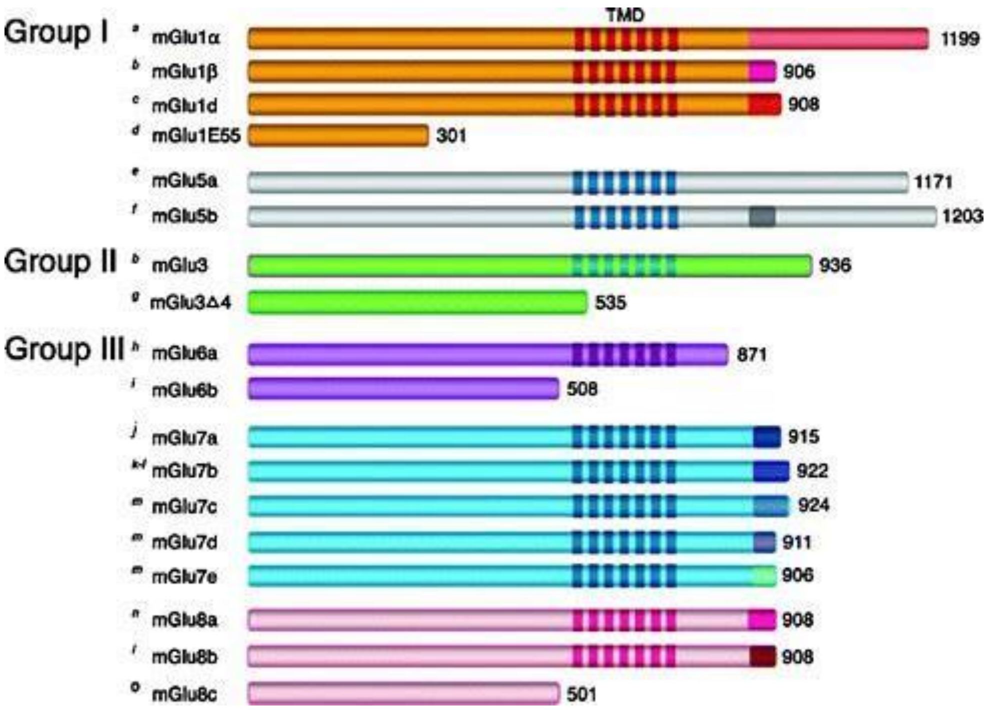

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