Universidade Estadual de Campinas

UNICAMP

Faculdade de Odontologia de Piracicaba

Carolina Steiner Oliveira

Cirurgiã-dentista

“DETERMINAÇÃO DE PARÂMETROS DE SEGURANÇA E DA ASSOCIAÇÃO DO LASER DE CO_2 (λ=10,6 μm) A COMPOSTOS FLUORETADOS NA PROGRESSÃO DA DESMINERALIZAÇÃO DO ESMALTE DENTÁRIO – ESTUDOS *IN VITRO*”

Dissertação apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como requisito para obtenção do título de Mestre em Odontologia, Área de Odontopediatria.

Piracicaba

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Orientadora: Profa. Dra. Marinês Nobre dos Santos Uchôa

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Profa. Dra. Lidiany Karla Azevedo Rodrigues
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PROFa. DRa. LIDIANY KARLA AZEVEDO RODRIGUES

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À Deus,

Pela iluminação e força durante os dois anos...

Aos meus pais, Haydée, Wilson e Fernando

Pelo amor, apoio incondicional, dedicação e compreensão...

Ao meu irmão Gabriel,

Por estar sempre torcendo por mim, mesmo estando longe...

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"É importante esforçar-se por um objetivo

que não seja visível de imediato. Um

objetivo que não esteja relacionado

à inteligência, mas ao espírito."

[Antoine de Saint-Exupéry]

SUMÁRIO

RESUMO

A irradiação do esmalte dentário com laser de CO_2, especialmente se associada ao flúor, pode aumentar a resistência deste substrato ao desafio ácido. Esta tese, constituída por 2 artigos, teve por objetivos: estabelecer a menor densidade de energia obtida com um laser de CO_2 (λ=10,6 µm) pulsado, que quando aplicada sobre o esmalte dentário humano, seja capaz de promover mudanças químicas e morfológicas e de reduzir sua suscetibilidade a ácidos, sem causar danos pulpares; avaliar, *in vitro,* os efeitos combinados de um laser de CO_2 pulsado (λ=10,6 µm) e do dentifrício e enxaguatório fluoretados na redução da progressão de lesão de cárie artificial em esmalte dentário humano. No estudo 1, durante a irradiação de terceiros molares inclusos com densidades de energia de 1,5 a 11,5 J/cm^2, foram avaliadas as alterações na temperatura pulpar através de um termopar. As modificações químicas e morfológicas da superfície do esmalte induzidas pelo laser foram determinadas, respectivamente, através de espectroscopia FT-Raman e microscopia eletrônica de varredura. Os dentes foram submetidos à ciclagem de pH e a perda mineral foi determinada através de microdureza em corte longitudinal. Os espectros Raman obtidos foram analisados pelo teste t pareado e os resultados das variáveis alteração de temperatura e microdureza, foram analisados estatisticamente pelos testes ANOVA e Tukey, todos com nível de significância fixado em 5%. No segundo estudo, espécimes de esmalte obtidos de terceiros molares inclusos foram previamente desmineralizados e em seguida aleatoriamente divididos em nove grupos tratados ou não com laser de CO_2, com ou sem dentifrício fluoretado e com ou sem enxaguatório fluoretado, fazendo todas as associações possíveis entre os tratamentos. Após a ciclagem de pH, as concentrações de flúor das soluções des e remineralizadora foram determinadas e os espécimes foram analisados, qualitativamente, por microscopia de luz polarizada e teste de microdureza em corte longitudinal para quantificar mudanças no conteúdo mineral. Os resultados foram analisados estatisticamente pelos testes ANOVA e Tukey, com nível de significância fixado em 5%. No estudo 1, as mudanças de temperatura intrapulpares não excederam 3°C para todos os grupos irradiados. A espectroscopia FT-Raman e a microscopia eletrônica de varredura indicaram que as densidades de energia iguais ou superiores a 6,0 J/cm^2 foram

suficientes para promover modificações químicas e morfológicas na superfície do esmalte. A redução da desmineralização do esmalte promovida pelo laser foi observada com as densidades de energia a partir de 10,0 J/cm^2. No estudo 2, todos os tratamentos foram capazes de reduzir a perda mineral do esmalte, quando comparados ao grupo controle ($p<0,05$). Todas as terapias combinadas, exceto a associação de laser e enxaguatório, causaram remineralização do esmalte dentário. Concluindo, os resultados desses estudos indicaram que a densidade de energia do laser de CO_2, que pode ser aplicada sobre o esmalte dentário capaz de produzir efeito na prevenção da desmineralização sem oferecer danos para à polpa dentária, é 10,0 J/cm^2 e que o laser de CO_2, combinado ou não com flúor, é capaz de reduzir a progressão da desmineralização do esmalte dentário em situações de alto desafio cariogênico *in vitro*.

ABSTRACT

The irradiation of dental enamel by CO_2 laser, especially if combined with fluoride, can increase the enamel acid resistance. Thus, this thesis, comprised by 2 manuscripts, aimed: to establish the lowest energy fluency of a pulsed 10.6 μm CO_2 laser that, when applied on enamel surface, is able to cause chemical and morphological changes and reduces its acid resistance reactivity, without causing pulpal harm; to assess, *in vitro*, the combined effects of a 10.6 μm CO_2 laser, fluoridated dentifrice and fluoridated mouthrinse in the reduction of lesion progression in human carious dental enamel. In study 1, during the irradiation of human teeth with 1.5-11.5J/cm^2, intrapulpal thermal effects were evaluated by a thermocouple. Moreover, chemical and morphological modifications were assessed on enamel surface, through FT-Raman and MEV analysis, respectively. The teeth were submitted to a pH-cycling model and the enamel mineral loss was determined by cross-sectional microhardness. The Raman spectra obtained were assessed by the paired t test and thermal and microhardness variables were analyzed by ANOVA and Tukey tests, all with 5% of statistical significance. In study 2, slabs of previously demineralized dental enamel were randomly assigned to nine groups (n = 10) and treated with/without CO_2 laser and with/without fluoridated dentifrice and with/without fluoridated mouthrinse, making all possible associations between these treatments. After pH-cycling, fluoride concentrations were determined in the de and remineralizing solutions and qualitative polarized light analysis was performed. In addition, cross-sectional microhardness test was done to quantify changes in mineral content. In the first study, for all irradiated groups, intrapulpal temperature changes were below 3°C. FT-Raman spectroscopy and scanning electron microscopy indicated that fluencies as low as 6.0 J/cm^2 were sufficient to induce chemical and morphological changes in enamel. Laser-induced inhibitory effects on enamel demineralization were observed in fluencies of 10.0 and 11.5 J/cm^2. In the second study, all treatments were able to decrease mineral loss when compared to control group ($p < 0.05$). Additionally, except for the association of laser with fluoridated mouthrinse, all combined treatments have caused enamel remineralization. In conclusion, the results of these studies suggest that the lowest laser fluency capable of producing chemical and morphological changes to reduce acid reactivity of enamel without exposing pulp vitality to danger is 10.0

J/cm^2 and that pulsed CO_2 laser irradiation alone or combined with fluoridated products produced an effective protection against demineralization progression in dental enamel *in vitro*.

I- INTRODUÇÃO GERAL

Um forte declínio da doença cárie tem sito observado no mundo nas últimas décadas em conseqüência do amplo uso de compostos fluoretados (Clarkson *et al.*, 2000). Entretanto, a cárie dentária ainda se manifesta em certos indivíduos ou grupos de indivíduos e tem se mostrado como uma das mais prevalentes doenças em adolescentes, com apenas 15% dos indivíduos de 17 anos livres de cárie (Narvai, *et al.*,1999; Clarkson & Mc Loughlin, 2000; Marthaler, 2004, Gushi *et al.*, 2005). Isto enfatiza a necessidade do aperfeiçoamento de métodos preventivos para que possam agir como coadjuvantes na prevenção e controle da cárie dentária neste segmento da população.

Dentre os métodos tópicos de uso de flúor de alta freqüência e baixa concentração, o dentifrício e o enxaguatório fluoretados são os mais comumente empregados. A estratégia da associação de métodos como enxaguatórios, dentifrícios e aplicações tópicas profissionais visa a manutenção de níveis constantes de flúor no meio bucal a fim de prevenir a desmineralização e aumentar a remineralização do esmalte dentário (Cruz *et al.*, 1994, Paes Leme *et al.*, 2003).

O dentifrício fluoretado é considerado o método de emprego de flúor mais relevante na acentuada redução da prevalência de cárie, observada tanto em países desenvolvidos como naqueles em desenvolvimento (Bratthall, 1996, Marthaler, 2003). Já foi demonstrado que o flúor do dentifrício é capaz de reduzir a perda de mineral do esmalte de dente íntegro, ou ativar a reposição de mineral do dente com lesão de cárie (Lynch *et al.*, 2004), aumentando em 2 vezes a capacidade da saliva de repor mineral na superfície do esmalte desmineralizado (Cury, 2002). Outro agente fluoretado efetivo em reduzir a desmineralizacão do esmalte dentário é o enxaguatório diário, que combina alta freqüência de uso com baixa concentração, o que segundo Wefel (1990) e Mellberg (1990), é a recomendação para o uso do flúor. Nos trabalhos de Øgaard, *et al.*, (1986) e Sonju Clasen, *et al.*, (1997) usando como tratamento apenas os enxaguatórios fluoretados, foi relatada uma redução de até 80% da desmineralizacão do esmalte dentário.

Assim, há um consenso que o principal efeito do flúor dá-se pela interferência na dinâmica da cárie dentária, reduzindo a desmineralização e aumentando a remineralização dos tecidos duros dentários. No entanto, o efeito do flúor é parcial já que o mesmo não consegue impedir completamente o desenvolvimento de lesões de cárie. Em função disto, o emprego combinado de compostos fluoretados a outros métodos preventivos poderia resultar em procedimentos mais efetivos na prevenção e controle da cárie.

Estudos foram realizados, visando demonstrar o potencial do laser de CO_2, em inibir a progressão da cárie dentária quando aplicado sobre a estrutura do esmalte (Lobene *et al.*, 1968; Stern, 1970; Stern & Sognnaes, 1972; Kantola *et al.*, 1973; Borggraven *et al.*, 1980; Brune, 1980; Liberman *et al.*, 1984; Nelson *et al.*, 1986, 1987; Ferreira *et al.*; 1989; Featherstone et al., 1998; Tange et al., 2000, Hsu et al., 2000). A este respeito, Kantorowitz *et al.* (1998), comprovaram um efeito de inibição de cárie do esmalte de até 87% utilizando o comprimento de onda 10,6 µm. Outros estudos relataram que a irradiação do esmalte dentário com laser de CO_2, utilizando densidades de energia de 0,3 a 12,5 J/cm^2 tem promovido redução significativa na perda mineral (Featherstone, *et al.*, 1998; Hsu, *et al*, 2000; Klein, 2005). Ainda, pesquisadores como Featherstone *et al.* (1991), Nobre dos Santos *et al.*(2001), Hsu *et al.*(2001), Nobre dos Santos *et al.*(2002) e Tepper *et al* (2004), investigaram a ação combinada do laser com o flúor. Esses autores observaram que a associação da aplicação tópica de flúor e a posterior irradiação com laser diminuiu a solubilidade do esmalte dentário, mais do que o tratamento isolado com flúor ou laser. No entanto, não existem na literatura estudos que tenham avaliado os efeitos da associação do laser de CO_2 e da terapia com flúor de alta freqüência e baixa concentração sobre a desmineralização do esmalte dentário.

Os lasers de CO_2 possuem comprimentos de onda que variam de λ= 9,3 até 10,6 µm e são considerados os mais apropriados para a utilização em esmalte dentário, pois produzem radiação na região do infravermelho que coincide com as bandas de absorção da hidroxiapatita, principalmente os grupamentos fosfato e carbonato (Nelson & Featherstone, 1982, Featherstone & Nelson, 1987). Assim, uma maior efetividade na diminuição da solubilidade do esmalte dentário pode ser obtida com menor risco de efeitos deletérios aos tecidos dentários (Zuerlein *et al.*, 1999), uma vez que ocorre uma menor dissipação de raios incidentes, com maior rapidez e eficácia do laser.

O laser de CO_2 produz seus efeitos principalmente devido à geração de calor, que produzido a partir de qualquer fonte operatória (utilização de brocas e pontas diamantadas, lasers, aparelhos fotopolimerizadores, entre outros), pode se tornar o principal agente estressante para a polpa dentária. Zach & Cohen, em 1965, determinaram que um aumento da temperatura pulpar superior a 5°C pode resultar em dano permanente à polpa. Portanto, as condições de irradiação devem ser ideais para evitar o espalhamento do calor e dano térmico aos tecidos circunvizinhos.

O emprego bem sucedido do laser de CO_2 com comprimento de 10,6 μm para procedimentos odontológicos preventivos depende da seleção de densidades de energia específicas do laser de CO_2, que não causem danos à vitalidade pulpar e ainda reduzam a reatividade ácida do esmalte dentário. Entretanto, não encontramos relatos na literatura de estudos que tenham relacionado os aspectos químicos, morfológicos e térmicos do laser de CO_2 pulsado à redução da desmineralizacão do esmalte dentário.

Assim, a utilização da tecnologia laser associada ao flúor em indivíduos com alto risco de cárie ou lesões incipientes poderia ser um recurso preventivo efetivo, com as vantagens de uma única aplicação mostrar-se ser efetiva no controle do desenvolvimento e progressão da doença, além de ser um método indolor e não invasivo. Conseqüentemente, para que esta tecnologia possa ser empregada clinicamente com segurança, torna-se necessária a realização de estudos que comprovem sua eficácia em situações de alto desafio cariogênico.

II – PROPOSIÇÃO

Os objetivos desse estudo foram:

1. Estabelecer a menor densidade de energia obtida com um laser de CO_2 (λ = 10,6 μm) pulsado que, quando aplicada sobre o esmalte dentário humano, seja capaz de promover mudanças químicas e morfológicas e de reduzir sua reatividade a ácidos, sem causar danos pulpares.

2. Avaliar, *in vitro,* os efeitos combinados de um laser de CO_2 (λ = 10,6 μm) pulsado e de dentifrício e enxaguatório fluoretados na progressão da lesão em esmalte dentário humano.

III – CAPÍTULOS

Esta tese está baseada na Resolução CCPG/001/98/UNICAMP que regulamenta o formato alternativo para teses de Mestrado e Doutorado e permite a inserção de artigos científicos de autoria ou co-autoria do candidato (Anexo 1). Por se tratarem de pesquisas envolvendo seres humanos, ou partes deles, os projetos de pesquisas destes trabalhos foram submetidos à apreciação do Comitê de Ética em Pesquisa da Faculdade de Odontologia de Piracicaba, tendo sido aprovados (Anexos 2, e 3). Assim sendo, esta tese é composta de dois capítulos contendo artigos em fase de redação, conforme descrito abaixo:

✓ Capítulo 1

"Chemical, morphological and thermal effects of 10.6 μm CO_2 laser on the inhibition of enamel demineralization." Steiner-Oliveira C, Rodrigues LKA, Soares LES, Martin AA, Zezell DM, Nobre dos Santos M. Este artigo será submetido para publicação no periódico *Lasers in Surgery and Medicine*.

✓ Capítulo 2

"Carbon dioxide laser combined with fluoridated products on the inhibition of enamel subsurface demineralization." Steiner-Oliveira C, Rodrigues LKA, Nobre dos Santos M. Este artigo será submetido para publicação no periódico *Photomedicine and Laser Surgery.*

CHEMICAL, MORPHOLOGICAL AND THERMAL EFFECTS OF 10.6 µm CO_2 LASER ON THE INHIBITION OF ENAMEL DEMINERALIZATION

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ABSTRACT

Background and Objective: Studies have shown that enamel can be modified by pulsed CO_2 laser to form a more acid-resistant substrate. This study evaluated the effects of 10.6μm CO_2 laser on enamel surface morphology and chemical composition and monitored intrapulpal temperatures during irradiation. **Study Design:** Human teeth were irradiated with 1.5-11.5J/cm^2 and pulpal thermal, chemical and morphological modifications were assessed. The teeth were submitted to a pH-cycling model and the mineral loss was determined by cross-sectional microhardness. **Results:** For all irradiated groups, intrapulpal temperature changes were below 3°C. FT-Raman spectroscopy and scanning electron microscopy indicated that fluencies as low as 6.0J/cm^2 were sufficient to induce chemical and morphological changes in enamel. Laser-induced inhibitory effects on demineralization were observed from 10.0J/cm^2 fluencies. **Conclusions**: In this study, the laser parameters for the efficient thermal modification of enamel and capable of producing chemical and morphological changes to reduce acid reactivity is 10.0J/cm^2.

INTRODUCTION

Since the development of a ruby crystal laser by Maiman in 1960 (1), different lasers have been studied for use in dentistry. Many studies were performed to examine the effects of lasers on hard dental substrates with several different applications (2). Thus, in the last 30 years many studies have demonstrated the potential of laser pre-treatment on dental tissues to inhibit enamel dissolution or artificial caries-like challenge in the laboratory (3-12). The effects of laser on enamel substrate are due to temperature changes which can be extremely high at the interaction site even for a short action time. This quick local temperature rise on enamel prompts melting and cooling of apatite crystals up to a 5 μm-depth (6), and can cause undesirable effects, such as cracking, pitting and pulpal damage (12). Carbon dioxide lasers proved to be effective without any significant damaging side effect, provided that care was taken to maintain temperatures at a safe level. Temperature changes exceeding 5°C within the pulp chamber could result in permanent damage to the dental pulp (13). There have been reports of degenerative pulp changes and necrosis following accidental or intentional laser irradiation of teeth during oral surgical procedures, suggesting a potential hazard of laser use in the oral cavity.

There is still no consensus about the exact action mechanism of CO_2 laser in the inhibition of enamel demineralization. Most theories focus on enamel mineral phase changes, such as surface melting and hydroxyapatite crystal fusion. It is well known that irradiation of dental hard tissue with lasers of sufficient power leads to a variety of structural and ultrastructural changes in the tissue near the surface (14, 15) and several studies have shown that irradiation by CO_2 laser light at 10.6μm can produce surface changes in enamel (7, 15, 16). In view of the uncertain mechanism of interaction between

CO_2 laser and enamel, a more sensitive analysis should be performed in order to clarify the enamel modifications induced by laser irradiation, mainly as regards the chemical aspect. Raman spectroscopy is a non-destructive information-rich and highly selective technique for investigating molecular species (17), which can be applied to almost any biomolecule (18). The Raman spectrum of any mineral structure, such as human teeth, can reveal the chemical composition and structure of mineral and organic contents.

The successful use of lasers for preventive dental procedures in the mouth is dependent upon the application of specific CO_2 laser power levels at a 10.6 μm wavelength that does not cause pulpal harm, and that is still able to produce demineralization preventive effects on dental enamel. However, as far as is known, there are no studies reported in the literature combining the chemical, morphological and thermal aspects of pulsed 10.6 μm CO_2 laser with regard to inhibitory effects on demineralization.

Thus, the aims of this study were two-fold: 1 - to establish the lowest CO_2 laser energy power that can be applied to dental enamel capable of reducing the acid reactivity of the enamel, without causing pulpal damage. 2 – to investigate physical and chemical changes promoted by a pulsed CO_2 laser at 10.6 μm wavelength.

MATERIAL AND METHODS

Tooth Selection and Sample Preparation

Ninety extracted impacted human third molars were used to perform this *in vitro* study, in conformity to the norms of the Research and Ethics Committee of the Dental School of Piracicaba (protocol No. 102/2005) (Anexo 2). The teeth were stored in 0.1% thymol solution for one month and after that had their pulpal content removed with a Kerr type file (Dentsply, Maillefer Instruments; Ballaigues, Switzerland) and their apical foramen enlarged by a Gates-Glidden rotary instrument (Dentsply Maillefer) in order to receive a thermocouple inside their pulp chambers. The teeth were then coated with an acid-resistant varnish leaving a window (16 mm^2) of exposed enamel including the central fissure.

Experimental Design

The experiment involved six groups (n=15), which differed from each other in the power of laser irradiation. The teeth for each group were randomly selected by lottery method (19) and the groups were named as follows: Control, 1.5 J/cm^2, 3.0 J/cm^2, 6.0 J/cm^2, 10.0 J/cm^2 and 11.5 J/cm^2. During the laser irradiation, thermal changes were monitored by a thermocouple. Before and after the laser treatment, 5 teeth from each group were evaluated through Fourier Transformed Raman Spectroscopy. After irradiation, the same teeth were also analyzed by the Scanning Electron Microscopy (SEM). The remaining teeth (n=10) were submitted to a pH-cycling model (Anexo 4).

Laser Irradiation

Irradiation was carried out by the scanning of the occlusal central fissure of the teeth exposed enamel of each tooth for approximately 10 s and then at a distance of ten mm from the tip of the hand piece to the tooth by manual movement of the laser tip. A pulsed CO_2 laser at 10.6 μm wavelength (Union Medical Engineering Co. Model UM-L30, Yangju-si, Gyeonggi-Do, Korea) was used for irradiation with the following parameters: 10 ms pulse duration, 10 ms of time off, 50 Hz repetition rate, beam diameter of 0.3 mm and 2W, 4W, 6W, 8W and 10W, according to the treatment groups. Using a power meter (Scientech 373 Model-37-3002, Scientech Inc., Boulder, CO, USA) the average power outputs were measured and found to be 0.1 W, 0.2 W, 0.4 W, 0.7 W and 0.8 W, for the correspondent lased groups. Thus, the laser fluencies applied on enamel were approximately of 1.5, 3.0, 6.0, 10.0 and 11.5 J/cm^2.

Thermal Analysis

Thermal data were acquired from the beginning of the CO_2 laser irradiation to fifteen seconds after the end of this procedure. Thermal changes were monitored by means of a cromel-alumel (K-type) thermocouple (OMEGA Eng. Inc. Stanford, CT-USA) (Anexo 5); the 125 μm tick tip was put into contact with the dentinal tissue inside the pulp chamber and the teeth were kept at a temperature of 37°C. A thermal paste was used to enable good thermal contact between the thermocouple tip and the internal pulp wall. Data were obtained during laser irradiation, and the internal pulp temperature increase was determined by calculating the difference between the maximum and the initial temperature values.

Raman Spectroscopy and Scanning Electron Microscopy

In order to verify the chemical and morphological effects of the CO_2 laser irradiation, five teeth were kept for evaluation through Fourier Transform Raman Spectroscopy followed by the SEM.

Spectra of the teeth were obtained using a FT Raman Spectrometer (RFS 100/S – Bruker Inc., Karlsruhe, Germany) both before and after irradiation with one Ge diode detector cooled by liquid N_2. To excite the spectra, the focused λ = 1,064.1 nm line of an air cooled Nd:YAG laser source was used. The maximum laser power incidence on the sample surface was about 100 mW and the spectrum resolution was 4 cm^{-1}. The teeth were positioned in the sample-holder and the IR354 lens collected radiation scattered over 180°

from the occlusal exposed surface. The FT-Raman spectra were obtained using 100 scans. The explored frequency ranged from 300 to 4,000 cm^{-1} and allowed a characterization of both mineral (hydroxyapatite) and organic (essentially collagen) constituents.

The teeth used for SEM analysis were longitudinally fractured through the enamel window and the surface, as well as the cut side were coated with a thin layer of gold (approximately 10-12 nm in thickness). The observations were then performed in a JEOL JSM-5600 LV Scanning Electron Microscope (JEOL, Tokyo, Japan) at 15 kV and magnifications up to 800 × (Anexo 6).

pH-Cycling Process

The pH-cycling model used in this study was based on the one described by Feastherstone et al. (20) and modified by Argenta et al. (21). Each tooth was kept in a demineralizing solution (5 mL/mm^2 exposed enamel) containing 2.0 mmol/L calcium, 2.0 mmol/L phosphate in 75 mmol/L acetate buffer pH 4.6 for 3 h and in a remineralizing solution (2,5 mL/mm^2 exposed enamel) containing 1.5 mmol/L calcium, 0.9 mmol/L phosphate, 150 mmol/L KCl in 20 mmol/L cacodylic buffer pH 7.0 for an average of 21 h each day. During 12 days, the cycle was repeated and after that, the teeth remained in the remineralizing solution for 2 days (37°C). Between the demineralizing and remineralizing stages and at the end of the pH-cycling regime, the teeth were washed with deionized and distilled water for 10 s and wiped with tissue paper. The de- and remineralizing solutions were changed after 5 cycles and both contained thymol to prevent microorganism growth (Anexo 7).

Cross-Section Microhardness Testing (CSMH)

After the pH-cycling, the teeth were longitudinally sectioned through the border of the exposed enamel. Each cut section was embedded in acrylic resin and serially flattened and polished up to the occlusal central fissure area. The hardness profile was determined using a Future-Tech (FM-ARS) hardness tester and a Knoop diamond under a 25-g load for 5 s. Thirty six indentations (three rows of 12 indentations each) were made with the long axis of the Knoop diamond parallel to the outer surface, maintaining a 10-μm interval between 10-μm and 60-μm and then a 20-μm interval from 60-μm to 180-μm across the lesion and into the underlying enamel (Anexo 8). The mean values of the Knoop hardness numbers (KHN) at each distance were obtained and converted into volume percent mineral by using the relationship proposed by Featherstone et al. (22). Volume percent mineral was plotted against depth for each tooth and the integrated mineral content of the lesion was calculated. A mean of volume percent mineral for depths > 80 μm was used as a measure of the integrated mineral content of inner sound enamel. To compute ΔZ parameters, the integrated mineral content of the lesion was subtracted from that obtained for sound enamel (23). Based on the mean ΔZ parameter, the percent inhibition of caries-lesion was calculated for the irradiated groups as follows:

$$\text{Percent Inhibition} = \frac{\Delta Z\ \text{Control} - \Delta Z\ \text{Treatment}}{\Delta Z\ \text{Control}} \times 100$$

Statistical Analysis

For Raman data analysis, average spectra were obtained from each treatment group treatment before and after irradiation. The spectrum fluorescence was removed with a polynomial fitting from the spectra, with varying degree in the Microcal Origin5.0® software. Relative areas of the peaks were calculated by the Microcal Origin5.0® software. The changes in mineral and organic structures were evaluated by comparing the relative areas of the peaks in enamel both before and after irradiation. Statistical analysis of the Raman results was performed by the paired t test at a 95% level of confidence using the Instat® software. The Kolmorogov and Smirnov test verified the normal distribution of the sample data.

In order to assess the effect of the treatments, the dependent variable ΔZ data were transformed (linear transformation a+bx) and tested by analysis of variance (ANOVA). Next, the Tukey test was chosen to evaluate the significance of all pair-wise comparisons, using the software SAS (SAS Institute Inc., 2001). Values of $p < 0.05$ were accepted as statistically significant. Comparisons between the dependent variable ΔT were also carried out and tested with analysis of variance (ANOVA) and Tukey test at a 0.05 alpha error.

RESULTS

Figure 1 shows the temperature distribution of each group (standard deviation = SD), according to the time (s). Figure 2 shows the temperature variations ΔT (°C) according to the laser fluencies applied. A low temperature variation, below 3°C, can be observed for all groups.

Average Raman spectra of all groups are shown in Figure 3. Spectral analysis showed two characteristic parts (figure 3a): first, a region spanning from 300 to 1.100 cm^{-1} with an intense broad band at 962 cm^{-1}, characteristic of phosphate groupings and representative of the mineral phase of the enamel; another region, representative of the collagen phase, shows organic grouping vibration modes (amide and CH) in the 1.200-3.000 cm^{-1} region. The FT-Raman bands at ν_2 (430-450 cm^{-1}), ν_4 (585-612 cm^{-1}), ν_1 (960 cm^{-1}) and ν_3 (1026-1072 cm^{-1}) represent the phosphate vibrations in hydroxyapatite. The band in the range of 1026-1072 cm^{-1} can also represent the ν_1 carbonate vibration. Before and after irradiation no statistically significant difference in enamel spectrum could be observed ($p < 0.05$) for energy densities of 1.5 and 3.0 J/cm^2. After irradiation, there was a statistically significant decrease ($p < 0.05$) in the intensity of 585 and 1045 cm^{-1} bands in the 6.0 J/cm^2 group. In the 10.0 J/cm^2 group, there was a statistical significant decrease ($p < 0.05$) in the intensity of 585, 612, 960 and 1072 cm^{-1} bands and peaks ranging from 430-612 cm^{-1} have disappeared. For 11.5 J/cm^2 group all peaks have disappeared, except at the band of 960 cm^{-1}. In enamel irradiated with 10.0 and 11.5 J/cm^2, a new peak appeared at the region of 740 cm^{-1} (Figure 3e,f)

The SEM observations showed evidence of melting and recristalization only in slabs irradiated by power intensities of 6.0 J/ cm^2, 10.0 J/cm^2 and 11.5 J/cm^2 (Figure 4) (Anexo 9).

The results of the enamel mineral loss (ΔZ) for laser-treated samples and non-irradiated control group are given in Table 1. The mean ΔZ represents the severity of the average caries-like lesion that has developed in each group. The lower the mean ΔZ, the less caries that developed in these teeth. The best demineralization inhibition result was found in the group irradiated with 10 J/cm^2.

DISCUSSION

The results of this study showed minimal temperature changes in the dental pulp when the occlusal surface was irradiated by CO_2 laser and also chemical and morphological changes in enamel capable of inhibiting enamel demineralization.

Elevation of intrapulpal temperature through the production of surface heat has been reported to be the most severe stress imparted to the living pulp (13). Therefore, the pulpal response to injury can be assumed to be directly proportional to the intensity of the damage (24). This study examined pulpal thermal changes in teeth during CO_2 laser exposure operated in a pulsed mode. Few thermal investigations were conducted using pulsed CO_2 laser, making it difficult to compare the reports of thermal effects on enamel. The results of this study showed a low range of temperature variation, not exceeding 3°C even for the highest laser fluency applied (11.5 J/cm^2). This is in accordance with the study performed by Malmström et al. (25) showing that the low energy levels used should not have a detrimental effect on the pulpal tissue (13, 24).

In the FT-Raman spectroscopy results, the strongest bands of phosphate v_1 (962 cm^{-1}), v_2 (430-450 cm^{-1}), v_4 (585-612 cm^{-1}), and v_3 (1070 cm^{-1}) modes, which have been previously reported (26,27) were immediately identified. For 6.0 J/cm^2 group, changes in enamel Raman spectrum were observed. The decrease in carbonate content by CO_2 laser irradiation is in agreement with previous studies that correlate this carbonate loss in laser treated dental enamel and a corresponding reduction in the rate of acid dissolution (28). With regard to the absence of the well known Raman bands related to hydroxyapatite minerals and the appearance of the 740 cm^{-1} band, similar results were found by Tudor et al. (29) who analyzed a thermally sprayed hydroxyapatite. These authors assigned this band to an overlap of hydroxide bands and concluded that FT-Raman spectrum of hydroxyapatite arises mainly from the hydroxide part. In addition, Rehman et al. (30,31) assigned this band to asymmetric P=O stretching vibration and a dense hydroxyapatite Raman band. However, as reported by Aminzadeh et al. (32), the appearance of a peak at the band 740 cm^{-1}, after irradiation with the highest laser fluencies (10.0 and 11.5 J/cm^2) most likely reflects a fluorescence band. The authors have suggested that this fluorescence band may arise from the presence of tricalcium phosphate (TCP) when hydroxyapatite is heated (27,32). On the other hand, Barnes (33) pointed out that locality and chemical environment in a particular mineral is very important in its luminescence, indicating that this fluorescence could be related to the irradiation of rare earth impurities, rather than to TCP. This calcium phosphate phase is formed when enamel is heated to temperatures of about 800 °C and it is considered to be a more soluble phosphate form, increasing the surface solubility of enamel (34). In the authors view, the hypothesis stating that fluorescence could be related to the irradiation of rare earth impurities would appear to be the most appropriated for explaining the results, since a reduction in demineralization was found, as evidenced by microhardness analysis. However, further studies are necessary to identify the 740 cm^{-1} band appearance and the characteristic hydroxyapatite bands disappearance.

Moreover, the laser action mechanism remains unclear, even though several studies about it have been published. The most frequently mentioned hypothesis for laser effect states that caries inhibition is due to the melting and fusion of hydroxyapatite crystals (35,36). The SEM data in the present study suggest that the fusing and melting phenomena (Figure 2) are related to the inhibition of demineralization found in the irradiated groups. These features are in agreement with data reported by Nelson et al. (6), Kantorowitz et al.

(37), Mccormack et al. (38) and Klein et al. (39), who used energy densities similar to or higher than those used in the present study.

The CO_2 laser inhibitory effects on enamel demineralization could only be observed using laser fluencies of 10.0 J/cm^2 and 11.5 J/cm^2 when compared to control group (Table 1). Previous studies have also shown significant inhibition of enamel demineralization by CO_2 laser ranging from 17 to 98% and varying according to the type of laser beam, wavelength, operational mode and energy output (6,11,40-42). In the present study, a maximum of 67% inhibition was achieved, which is lower than the 87% reported by Kantorowitz et al. (37) who used similar parameters and laser wavelength. This discrepancy might have been caused by the higher susceptibility of occlusal surfaces to caries development than enamel smooth surface, making caries inhibition more difficult (43,44). In contrast to the results of the present study, are the findings of Hsu et al. (45) who used very low energy density (0.3 J/cm^2) and found almost complete inhibition of enamel demineralization. This might have occurred due to an energy loss during the irradiation procedure because the occlusal surface forms an angle between the walls of the pit and fissures, thus requiring higher laser fluencies than smooth surface to produce similar inhibition effects on the substrate (46).

CONCLUSION

In conclusion, the findings of the present investigation indicate that 10.0 J/cm^2 is the lowest CO_2 laser energy fluency that can be applied to dental enamel capable of producing chemical and morphological changes to reduce acid reactivity without exposing pulp vitality to danger.

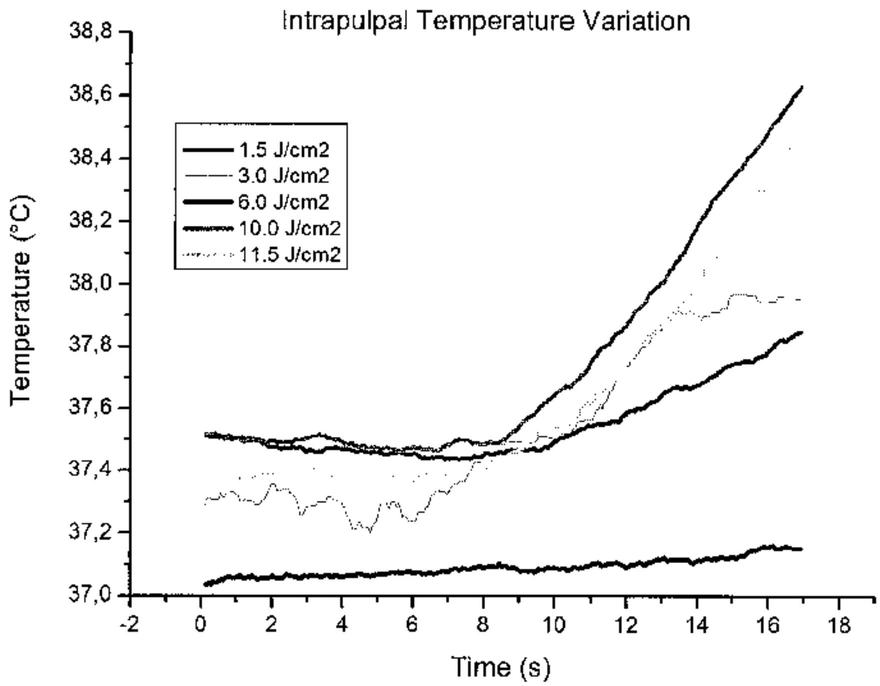

Figure 1. Pulp chamber temperature changes during irradiation of occlusal surfaces.

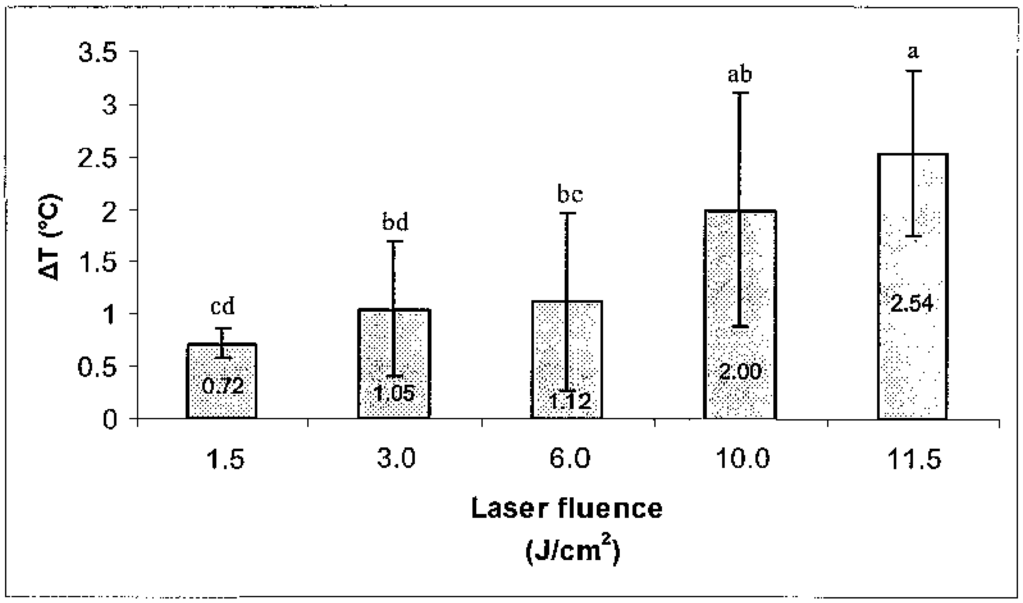

Figure 2. Averages of intrapulpal temperature variations (ΔT) according to the laser fluences (J/cm^2) applied on enamel. Distinct letters are statistically different by the Tukey test ($p<0.05$).

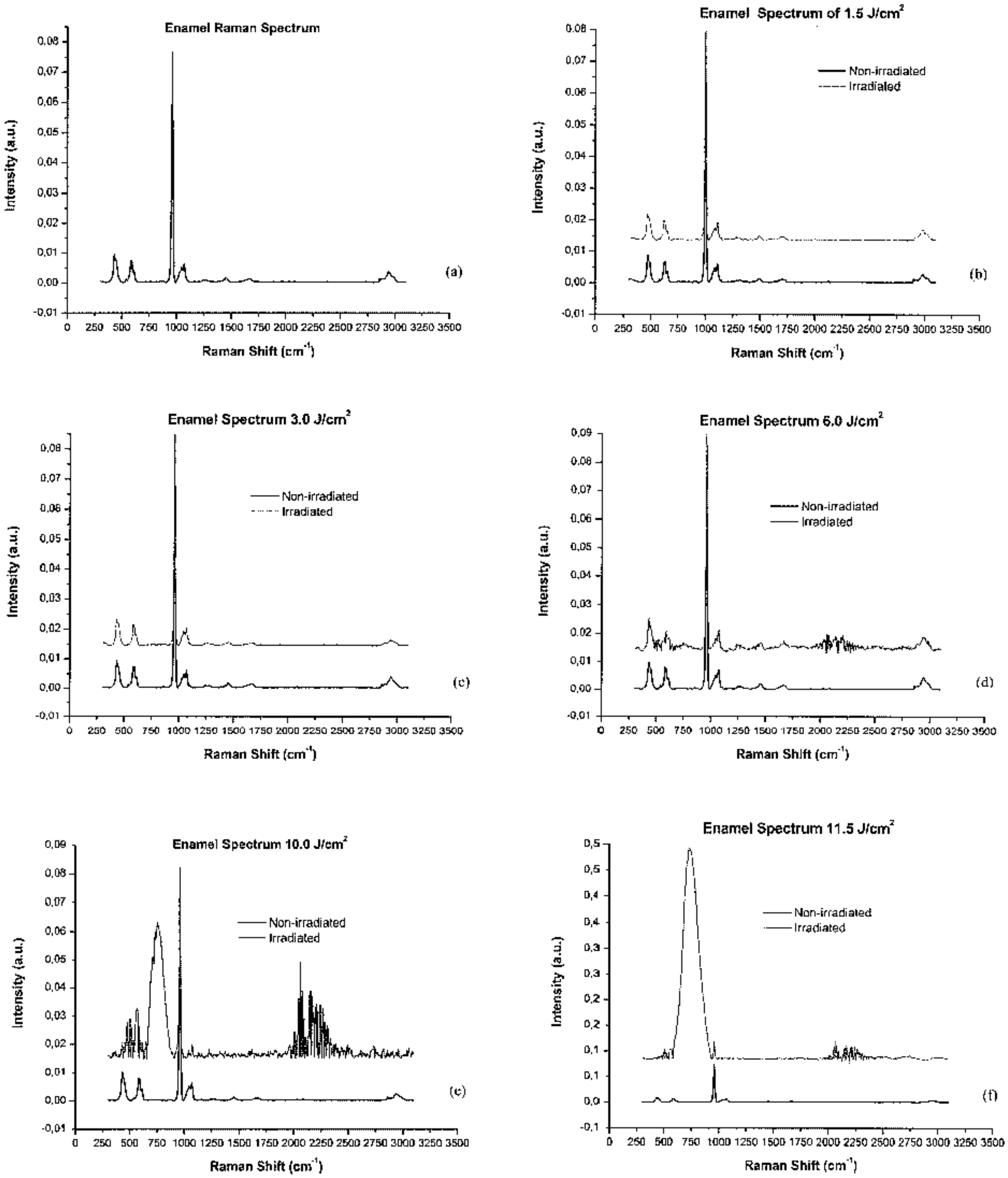

Figure 3. Raman Spectra of (a) the average of non-irradiated enamel, (b) irradiated enamel with 1.5 J/cm^2, (c) irradiated enamel with 3.0 J/cm^2, (d) irradiated enamel with 6.0 J/cm^2, (e) irradiated enamel with 10.0 J/cm^2 and (e) irradiated enamel with 11.5 J/cm^2.

Group	Mineral Loss - ΔZ (%vol x μm)
Control	2325.83 ± 1000.9 a
1.5 J/cm^2	1295.74 ± 346.0 ac
3.0 J/cm^2	1690.33 ± 914.8 ab
6.0 J/cm^2	1707.92 ± 762.5 ab
10.0 J/cm^2	761.66 ± 373.9 c
11.5 J/cm^2	950.67 ± 342.9 bc

Means followed by distinct letters are statistically different by the Tukey test ($p<0.05$).

Table 1. Enamel mineral loss (vol% x μm) of each intensity laser treatment when compared with control group (mean ± SD, n = 10).

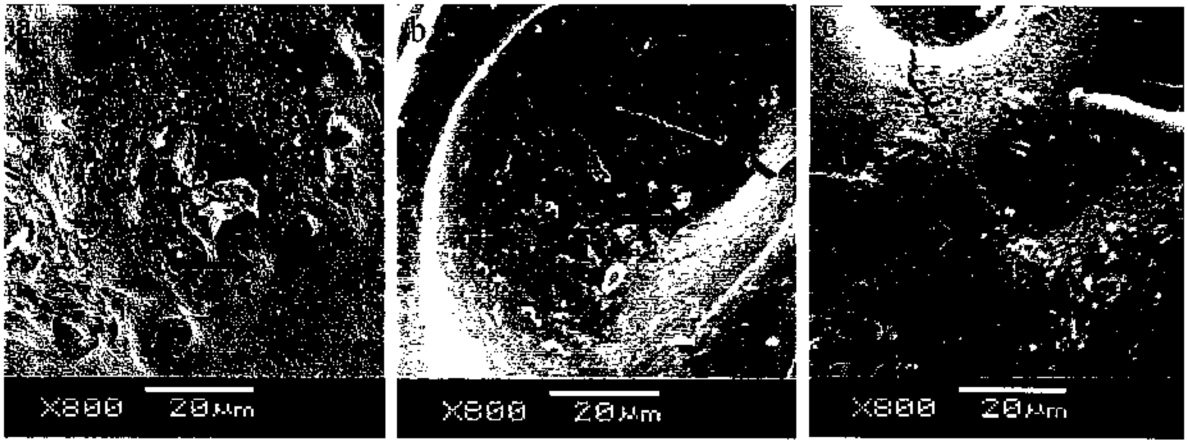

Figure 4. SEM micrographs of irradiated enamel of 6.0 J/cm^2(a), 10.0 J/cm^2(b) and 11.5 J/cm^2(c) groups at 800 X magnification. Arrows indicate areas of melted enamel. Bar = 20 µm.

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CAPÍTULO 2

CARBON DIOXIDE LASER COMBINED WITH FLUORIDATED PRODUCTS ON THE INHIBITION OF ENAMEL SUBSURFACE DEMINERALIZATION

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ABSTRACT

Dental caries is still an oral health problem of school-aged children and adults, so the combination of preventive treatments should be recommended. Thus, the purpose of this *in vitro* study was to assess the combined effects of a 10.6 μm CO_2 laser, fluoridated dentifrice and fluoridated mouthrinse in the reduction of lesion progression in human carious dental enamel. Slabs of previously demineralized dental enamel were randomly assigned to nine groups ($n = 10$) and treated or not with CO_2 laser and with/without fluoridated dentifrice and with/without fluoridated mouthrinse. After a pH-cycling regime, fluoride concentrations were determined in the de and remineralizing solutions and qualitative polarized light analysis was performed. In addition, cross-sectional microhardness test was done to quantify changes in mineral content. All treatments were able to decrease mineral loss when compared to control group ($p < 0.05$). Moreover, except for the association of laser with fluoridated mouthrinse, all combined treatments have caused enamel remineralization. In the present study, demineralization progression inhibition ranged from 48% to 60%. In conclusion, the results suggested that the CO_2 laser irradiation alone or combined with fluoridated products produced an effective protection against demineralization progression in dental enamel.

INTRODUCTION

Dental caries is still an oral health problem in most industrialized countries as it affects 60–90% of school-aged children and the vast majority of adults. In most developing countries, the levels of dental caries were low until recent years but caries prevalence rates and dental caries experience are now tending to increase. [1] In addition, in spite of the widespread use of fluoride, the manifestation of this disease is still high in some individuals or groups.[2,3] Large scale epidemiological studies in children show that 50% are caries free, 25% have 25% of the lesions and 25% have 75% of the total number of lesions.[4-8] On the face of it, we should concentrate our attention on those individuals ('high-caries-risk' group), for whom the combination of preventive treatments should be recommended .[9-10]

Several investigations have demonstrated that treatment with various lasers can reduce the rate of subsurface demineralization in enamel.[11-14] For this purpose, CO_2 lasers appear to be the most efficient due to absorption coefficient of the enamel, which closely corresponds to the wavelength of CO_2 laser emission.[15]

A recent study pointed out the need to focus on combined laser-fluoride treatment of incipient carious lesions to investigate whether lesion progression can be influenced by these treatments.[16] Fluoridated dentifrices and mouthrinses are widely used products that deliver fluoride to oral cavity and the use of these products has contributed substantially to the widespread decline in caries incidence in some Western countries. [17-19] However, there is evidence that the cariostatic effects of fluoride are, in part, related to the sustained presence of low levels of ionic fluoride in the oral environment [19-22] such as in plaque [23] and saliva,[24] making it dependent on the patient's ability to keep fluoride levels constant in the oral cavity. Thus, in order to be effective, therapies that do not depend on the patient's compliance should be more advantageously applied for high caries risk individuals. Regarding this, CO_2 laser associated or not with fluoride can represent a good alternative for these patients.

The efficacy of fluoride treatments combined with CO_2 laser irradiation in caries inhibition has been demonstrated by several investigations.[25-29] However, none of them have attempted to investigate the combined effects of dentifrice and mouthrinse with a clinical 10.6 μm CO_2 laser in the inhibition of demineralization progression in human carious dental enamel. Furthermore, most studies have been carried out with TEA (transversely excited atmospheric pressure) laser technology at 9.6 μm wavelength, which is a prototype laser, not commercially available.

Thus, the purpose of this *in vitro* study was to assess the combined effects of a 10.6 μm CO_2 laser with fluoridated dentifrice and mouthrinse in the reduction of lesion progression in human carious dental enamel.

MATERIALS AND METHODS

Tooth Selection and Sample Preparation

This study was approved by the Research and Ethics Committee of the Dental School of Piracicaba-UNICAMP (Protocol No. 58/2001) (Anexo 3).

Forty five extracted impacted human third molars, which were sterilized by gamma radiation, were used to perform this *in vitro* study. Ninety enamel slabs (5 x 5 x 2 mm) were obtained and coated, using an acid-resistant varnish, leaving a window (4 mm^2) of exposed enamel.

Caries-like Lesion Formation and Grouping

The caries-like lesion formation was performed, in all slabs, according to Paes Leme *et al* (2003)[30]. Early caries lesions were produced by individual immersion in an acetate buffer (6.25 mL of solution/mm^2 of exposed enamel) 0.05 mol/L pH 5.0, 50% saturated with hydroxyapatite (ref. F500) for 48 h at 37ºC. The slabs were then randomly assigned to one of the following groups (n = 10): Carious (Ca); Control (C); Dentifrice (D), Mouthrinse (M), Laser (L); Dentifrice+Mouthrinse (DM), Laser+Dentifrice (LD), Laser+Mouthrinse (LM) or Laser+Dentifrice+Mouthrinse (LDM), wherein the first group (Carious) was demineralized by acetate buffer, however, differently from all the other groups, was not submitted to the pH-cycling regime. The control group was demineralized not only by acetate buffer, but also by the posterior pH-cycling model applied. Mineral loss due to acetate buffer was assessed in Carious group to determine the initial mineral content and then, the effect of the treatments in inhibition of lesion progress (Anexo 10).

Laser Treatment

A pulsed CO_2 laser at 10.6 μm wavelength (Union Medical Engeneering Co. Model UM-L30, Yangju-si, Gyeonggi-Do, Korea) was used with the following parameters: 1.6 W, 10 ms pulse duration, 10 ms of time off, 50 Hz repetition rate and a beam diameter of 0.3 mm. For these conditions, a power meter (Model - 201, Coherent Radiation, Palo Alto, CA, United States) indicated a 0.7 W peak power, thus determining an incident fluence of approximately 10 J/cm^2 per pulse. A 10 mm distance from the tip of the hand piece to the slab was maintained during irradiation which was carried out through the scanning of each slab exposed enamel for approximately 30 s by an X-Y positioning platform, in order to provide a uniform coverage of each window (Anexo 11).

Fluoride Treatment

Before the daily immersion in the de and remineralizing solutions, the enamel slabs from groups Dentifrice, Dentifrice+Mouthrinse, Laser+Dentifrice, and Laser+Dentifrice+Mouthrinse were treated with a 1:3 (w/w) slurry made with deionized and distilled water and fluoridated dentifrice (1100 ppm F). This was performed twice a day, for 5 minutes while being agitated on an orbital shaker (Cientec CT-165, Piracicaba, SP, Brazil) (Anexo 12). After this, slabs from groups Mouthrinse, Dentifrice+Mouthrinse, Laser+Mouthrinse and Laser+Dentifrice+Mouthrinse were submitted to a single mouthrinse treatment (0.05% w/v NaF), before being immersed in the demineralizing solutions, without any dilution, for 1 minute, under agitation on an orbital shaker. After the treatments all slabs were washed in deionized and distilled water (Anexo 13).

pH-Cycling Process

The pH-cycling model used in this study was based on that described by Feastherstone *et al.* (1986)[31] and modified by Klein *et al.* (2005)[32]. Each slab was kept in a demineralizing solution (5 mL/mm^2 exposed enamel) containing 2.0 mmol/L calcium, 2.0 mmol/L phosphate in 75 mmol/L acetate buffer pH 4.6 for 3 h and in a remineralizing solution (2.5 mL/mm^2 exposed enamel) containing 1.5 mmol/L calcium, 0.9 mmol/L phosphate, 150 mmol/L KCl in 20 mmol/L cacodylic buffer pH 7.0 for an average of 21 h each day. Both solutions were changed daily and the cycle was repeated during 10 days. After that the slabs remained in the remineralizing solution for 2 days (37ºC). Between the demineralizing and remineralizing stages and at the end of the pH-cycling the slabs were washed with deionized and distilled water for 10 s and wiped with tissue paper. Both solutions contained thymol to prevent microorganism growth (Anexo 14).

Chemical Analysis

Fluoride concentrations in the de and remineralizing solutions used in the pH-cycling model were analyzed in days 1-5 and 8-12 . For this analysis, duplicate aliquots of the solutions were mixed with TISAB III at a ratio of 1:0.1. Fluoride determination was performed by means of an ion-selective electrode, Orion 96-09 (Orion Research Inc., Boston, MA, USA) and a digital ion-analyzer, Orion EA-940, previously calibrated with various standard solutions (0.015 to 0.5 μgF/mL) (Anexo 15). Fluoride concentrations of the de-and remineralizing solutions before the pH-cycling were 0.019 and 0.015 μg/mL, respectively, measured immediately after preparation.

Polarized Light

Three slabs of each group were cut with a Silverstone-Taylor hard-tissue microtome (series 1000 Deluxe, Sci Fab, Littleton, CO, USA) in the middle of the exposed enamel window (Anexo 16) to obtain sections of 200 μm thickness. After that, the sections were polished with 600 and 1200-grit sand-paper to obtain sections of 100 ± 20 μm thickness. The sections were imbibed in water and were observed with a polarized light microscope (Leica DMLP, Leica Mic rosystems, Wetzlar, Germany) coupled to a digital system (Leica FFC 280) and standard 10 X magnification photomicrographs were taken.

Cross-Section Microhardness Testing (CSMH)

After pH-cycling, the remaining portion of the slabs were embedded in self-cured acrylic resin (Pre-30, Arotec SA Ind. E Com, Cotia, SP, Brazil) and serially flattened and polished. The hardness profile was determined using a microhardness Future Tech FM-

ARS and a Knoop diamond under a 25-g load for 5 s. Thirty six indentations (three rows of 12 indentations each) were made with the long axis of the Knoop diamond parallel to the outer surface, maintaining a 10-μm interval between 10-μm and 60-μm and then a 20-μm interval from 60-μm to 180-μm across the lesion and into the underlying enamel (Anexo 8). The mean values of the Knoop hardness numbers (KHN) at each distance were obtained and converted into volume percent mineral by using the relationship proposed by Featherstone *et al.* (1983)[33]. Mineral volume was plotted against depth for each slab and integrated mineral content of the lesion was calculated. A mean of volume percent mineral for depths > 80 μm was used as a measure of the integrated mineral content of inner sound enamel. To compute ΔZ parameters, the integrated mineral content of the lesion was subtracted from that obtained for sound enamel. [34]

Statistical Analysis

First, a One-way Analysis of Variance (ANOVA) model was constructed to assess the treatment effects and fluoride concentration in de- and remineralizing solutions. Then, Tukey test was chosen to evaluate the significance of all pair-wise comparisons, using the software SAS (SAS Institute Inc., 2001). Values of $p<0.05$ were accepted as statistically significant.

RESULTS

Table 1 shows the mineral loss (ΔZ) for each group. The ΔZ values were significantly lower ($p<0.05$) for D, M, L, DM, LD, LM and LMD groups when compared with the Control group. Groups D, M, L and LM did not show statistically significant difference when compared with the Carious group and in contrast, groups DM, LD and

LDM have shown statistically significant difference when compared with the Carious group. Additionally, the combination of treatments with CO_2 laser and fluoride did not show an additional effect ($p>0.05$) against lesion progression.

Table 2 shows fluoride concentration in the demineralizing and remineralizing solutions used during pH-cycling to simulate the dynamics of caries development. A significantly higher concentration of fluoride was found both in de and remineralizing solutions for the groups treated with dentifrice (D, LD, DM and LDM). In the remineralizing solutions, the groups treated with fluoride from mouthrinse (M and LM) showed statically significant lower concentrations, not differing from the groups that did not receive any fluoride treatment (C and L).

In the qualitative polarized light analysis, Figure 1 shows patterns of demineralization in 9 sections, belonging to the 9 groups. It can be observed a remineralizing line in the groups treated with combined therapies (Figure 1.f, g and i), except for LM group.

Group	Mineral Loss - ΔZ (%vol x μm)
Carious (Ca)	3651.33 ± 609.0 b
Control (C)	5288.42 ± 971.4 a
Dentifrice (D)	2497.97 ± 624.1 bc
Mouthrinse (M)	2764.43 ± 544.6 bc
Laser (L)	2594.23 ± 634.9 bc
Dentifrice + Mouthrinse (DM)	2288.18 ± 771.4 c
Laser + Dentifrice (LD)	2130.36 ± 625.7 c
Laser + Mouthrinse (LM)	2577.20 ± 308.2 bc
Laser + Dentifrice + Mouthrinse (LDM)	2364.79 ± 498.9 c

Means followed by distinct letters are statistically different by the Tukey test ($p<0.05$).

Table 1. Mineral loss (vol% x μm) of each treatment when compared with control and carious group (mean ± SD, n = 10).

Group	Demineralizing solutions (μg F/mL)	Remineralizing solutions (μg F/mL)
Control (C)	0.016 ± 0.002 c	0.015 ± 0.001 c
Dentifrice (D)	0.040 ± 0.012 ab	0.136 ± 0.132 a
Mouthrinse (M)	0.038 ± 0.025 b	0.028 ± 0.028 bc
Laser (L)	0.016 ± 0.002 c	0.015 ± 0.001 c
Dentifrice + Mouthrinse (DM)	0.057 ± 0.003 a	0.131 ± 0.126 a
Laser + Dentifrice (LD)	0.041 ± 0.009 ab	0.120 ± 0.154 a
Laser + Mouthrinse (LM)	0.037 ± 0.015 b	0.025 ± 0.028 c
Laser + Dentifrice + Mouthrinse (LDM)	0.044 ± 0.012 ab	0.109 ± 0.117 ab

Means followed by distinct letters are statistically different by the Tukey test ($p<0.05$).

Table 2. Fluoride concentration (μg/mL) in the de and remineralizing solutions according to treatments (mean ± SD).

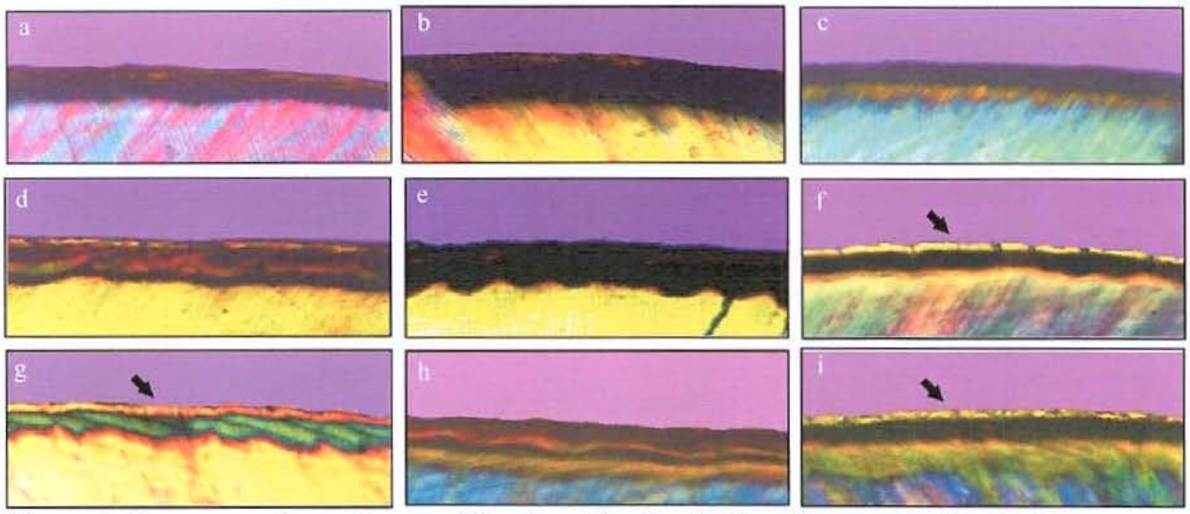

Figure 1. Representative patterns of demineralization in 9 sections according to the groups - 10X magnification.: (a) Carious, (b) Control, (c) Dentifrice, (d) Mouthrinse, (e) Laser, (f) Dentifrice + Mouthrinse, (g) Laser + Dentifrice, (h) Laser + Mouthrinse and (i) Laser + Dentifrice + Mouthrinse. Arrows indicate areas of remineralization.

DISCUSSION

Many *in vitro* and *in vivo* studies have been carried out to define the optimal fluoride therapy for prevention of dental caries.[35] In situations of high-caries risk, the combination of preventive measures should be indicated, such as the use of agents with high-frequency and low concentrations of fluoride and CO_2 laser applications.[25,29,30]

The results of the present study (Table 1) showed that all treatments applied were capable of reducing the caries lesion progression in dental enamel ($p<0.05$) when compared to control group. The results showed that caries inhibition ranged from 48% to 60% and they are in line with other previously reported results.[16,25-28,36-38] Our data revealed that after a high cariogenic challenge, the enamel mineral loss in groups D, M, L and LM did not show statistically significant difference ($p > 0.05$) from that found in enamel with carious lesion only (Carious group), indicating that the isolated treatments as well as the combined regime of laser and mouthrinse avoided the additional enamel demineralization promoted by the pH-cycling regime. With regard to the fluoride treatments, our results are consistent with Paes Leme *et al.* (2003)[30] and Damato *et al.* (1990)[39] who also found caries progression inhibition using fluoridated dentifrice and fluoridated mouthrinse in a pH-cycling model, respectively. With respect to the laser treatment, our findings are in line with several investigations that have shown that treatment with CO_2 laser can reduce the rate of subsurface demineralization in enamel.[32,36,38,40] Considering our data of the lack of synergism of the laser and mouthrinse treatment, Tepper *et al.* (2004)[16] also could not show a synergic effect when using a 2% amine fluoride solution and CO_2 laser therapy in subsurface enamel layers. In addition, groups treated with dentifrice showed better remineralizing capacity compared with the ones treated with mouthrinse (Figure 1). This could be explained not only by the fluoride treatment regime which was twice[30] and once[41] a day for the dentifrice and mouthrinse, respectively, but also by the different fluoride concentrations in the vehicles making the enamel slabs less exposed to fluoride on the second treatment.

When compared to the carious group, a statistically significant mineral loss reduction was found for all combined therapies, except for the LM group. Then, it can be suggested that these treatments were not only able to avoid the additional enamel demineralization promoted by the pH-cycling regime, but were also capable of remineralizing the softened enamel, as evidenced by the microhardness recovery. Corroborating these findings, polarized light (Figure 1) have also shown a remineralizing line for the same groups. Since laser treatment does not enhance remineralization in the absence of fluoride,[42] the remineralizing effect found in the present study for the combined laser fluoride therapies, may be related to the fluoridated treatments. This could be due to the increased capability of fluoride diffusion into the enamel promoted by cracks and roughness created by the laser treatment, as evidenced by Tagomori & Morioka (1989)[43] who used similar irradiation parameters.

It must be emphasized that mouthrinse remineralization effect was not observed either when used alone or combined with laser treatment. Thus, it may be suggested that the remineralizing effect found in DM group was much more related to fluoride from the dentifrice than from the mouthrinse. In addition, these results can be confirmed considering the other results shown in Table 2. Part of the products formed on enamel surface was lost during the subsequent treatment (pH cycling). This fluoride released from enamel was found in remineralizing and demineralizing solutions. The mean value found for fluoride concentration in both solutions was higher only when fluoridated dentifrice was applied. The results of the fluoride concentration in these solutions are in agreement with those described by Paes Leme *et al.* (2004)[30], even though they had used different pH cycling model and fluoridated daily dentifrice exposure.

CONCLUSION

In conclusion, the results suggested that the CO_2 laser irradiation alone or combined with fluoridated products produced an effective protection against demineralization progression in dental enamel.

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IV – CONCLUSÃO GERAL

1. Os resultados indicam que densidades de energia do laser de CO_2 iguais ou inferiores a 11,5 J/cm^2 são seguras para ser empregadas na superfície oclusal do esmalte dentário humano.

2. A menor densidade de energia do laser de CO_2 que pode ser aplicada sobre o esmalte dentário, capaz de produzir mudanças químicas e morfológicas e também efeito na prevenção da desmineralização, sem oferecer danos para à polpa dentária, é 10,0 J/cm^2.

3. O laser de CO_2, combinado ou não a compostos fluoretados, é capaz de reduzir a progressão da desmineralização do esmalte dentário humano em situações de alto desafio cariogênico *in vitro*.

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* De acordo com a norma da UNICAMP/FOP, baseada no modelo Vancouver. Abreviatura dos periódicos em conformidade com o Medline.

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ANEXO 1

DELIBERAÇÃO CCPG – 001/98

Dispõe a respeito do formato das teses de Mestrado
e de Doutorado aprovadas pela UNICAMP

Tendo em vista a possibilidade, segundo parecer PG Nº 1985/96, das teses de Mestrado e Doutorado terem um formato alternativo àquele já bem estabelecido, a CCPG resolve:

Artigo 1º - Todas as teses de mestrado e de doutorado da UNICAMP terão o seguinte formato padrão:

I) Capa com formato único, dando visibilidade ao nível (mestrado e doutorado), e à Universidade.
II) Primeira folha interna dando visibilidade ao nível (mestrado ou doutorado), à Universidade, à Unidade em foi defendida e à banca examinadora, ressaltando o nome do orientador e co-orientadores. No seu verso deve constar a ficha catalográfica.
III) Segunda folha interna onde conste o resumo em português e o Abstract em inglês.
IV) Introdução Geral.
V) Capítulo.
VI) Conclusão geral.
VII) Referências Bibliográficas.
VIII) Apêndices (se necessários).

Artigo 2º - A critério do orientador, os Capítulos e os Apêndices poderão conter cópias de artigos de autoria ou de co-autoria do candidato, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, escritos no idioma exigido pelo veículo de divulgação.

Parágrafo único – Os veículos de divulgação deverão ser expressamente indicados.

Artigo 3º - A PRPG providenciará o projeto gráfico das capas bem como a impressão de um número de exemplares, da versão final da tese a ser homologada.

Artigo 4º - Fica revogada a resolução CCPG 17/97.

COMITÊ DE ÉTICA EM PESQUISA

FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa "**Segurança no emprego do laser de CO_2 para prevenção de cárie**", protocolo nº **102/2005**, dos pesquisadores **CAROLINA STEINER OLIVEIRA e MARINÊS NOBRE DOS SANTOS UCHÔA**, satisfaz as exigências do Conselho Nacional de Saúde – Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 15/09/2005.

The Research Ethics Committee of the School of Dentistry of Piracicaba - State University of Campinas, certify that project "**Safe use of CO_2 laser in caries prevention**", register number **102/2005**, of **CAROLINA STEINER OLIVEIRA and MARINÊS NOBRE DOS SANTOS UCHÔA**, comply with the recommendations of the National Health Council – Ministry of Health of Brazil for researching in human subjects and was approved by this committee at 15/09/2005.

Cinthia Pereira Machado Tabchoury
Secretária
CEP/FOP/UNICAMP

Jacks Jorge Júnior
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
Notice: The title of the project appears as provided by the authors, without editing.

COMITÊ DE ÉTICA EM PESQUISA

FACULDADE DE ODONTOLOGIA DE PIRACICABA

UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa "**Efeito in vitro de laser de CO2 associado a bochecho e dentifricio fluoretados na redução de cárie no esmalte humano**", protocolo nº **058/2001**, dos pesquisadores **MARINÊS NOBRE DOS SANTOS UCHÔA e CAROLINA STEINER OLIVEIRA**, satisfaz as exigências do Conselho Nacional de Saúde – Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 02/05/2001.

The Research Ethics Committee of the School of Dentistry of Piracicaba - State University of Campinas, certify that project "**Effect in vitro of CO2 laser associated with fluoridated dentifrice and mouth rinse in the reduction of caries on human dental enamel**", register number **058/2001**, of **MARINÊS NOBRE DOS SANTOS UCHÔA and CAROLINA STEINER OLIVEIRA**, comply with the recommendations of the National Health Council – Ministry of Health of Brazil for researching in human subjects and was approved by this committee at 02/05/2001.

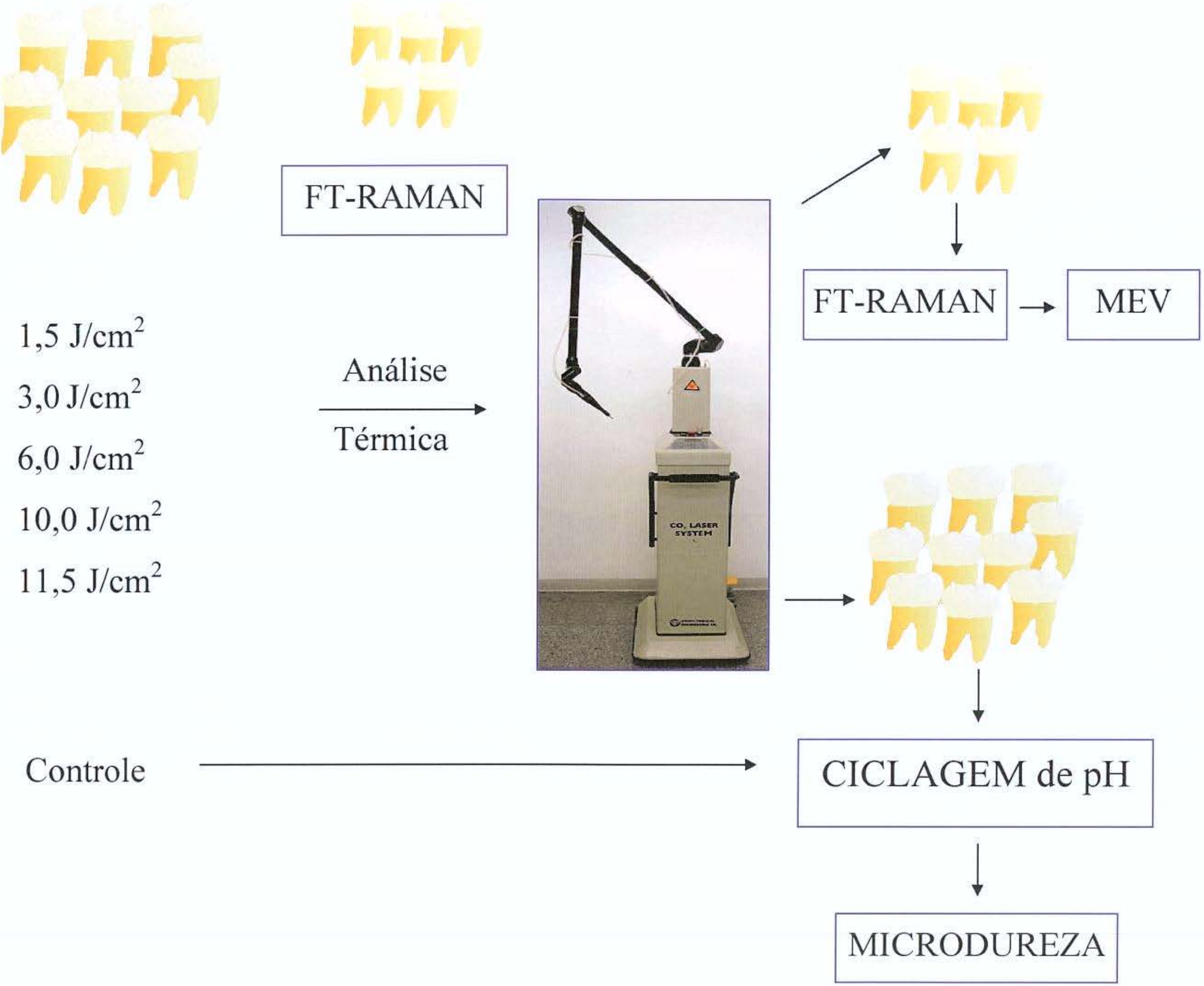
Piracicaba, SP, Brazil, May 03 2005

Cinthia Pereira Machado Tabchoury
Secretária
CEP/FOP/UNICAMP

Jacks Jorge Júnior
Coordenador
CEP/FOP/UNICAMP

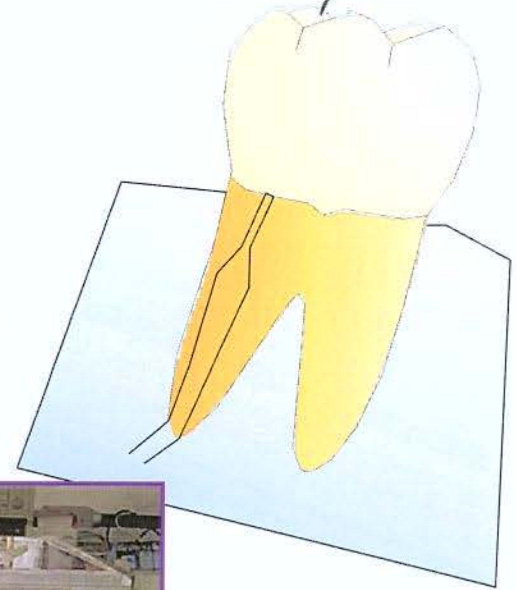
Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição
Notice: The title of the project appears as provided by the authors, without editing

ANEXO 4


FT-RAMAN
1,5 J/cm²
3,0 J/cm²
6,0 J/cm²
10,0 J/cm²
11,5 J/cm²
Análise
Térmica
CO₂ LASER SYSTEM
FT-RAMAN
MEV
Controle
CICLAGEM de pH
MICRODUREZA

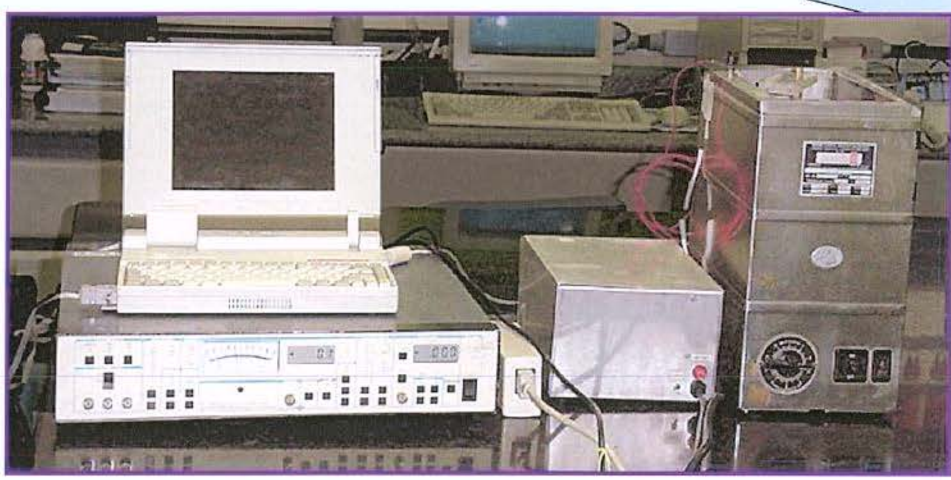
a

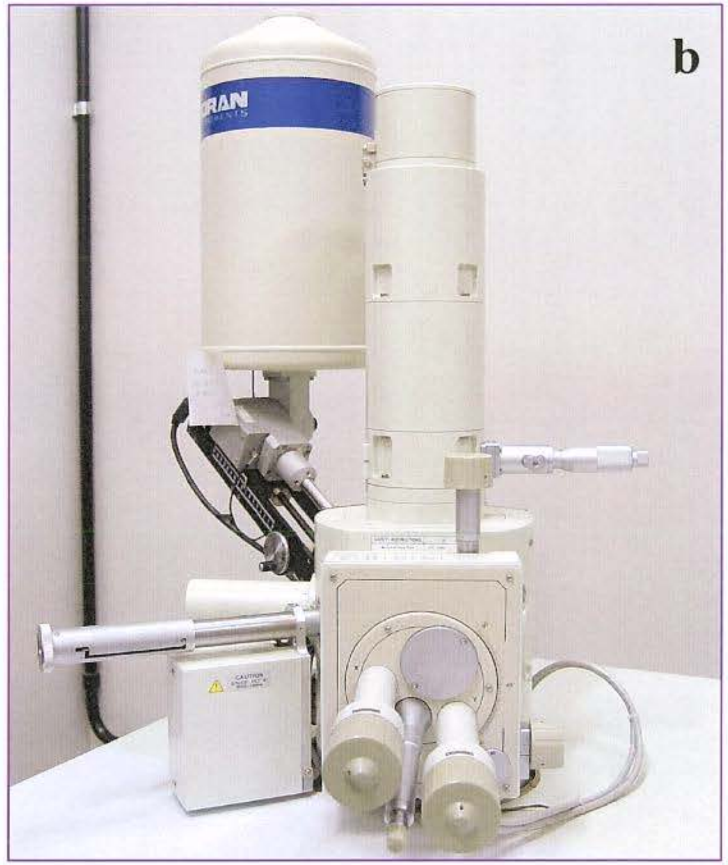
a) Irradiação com laser de CO_2; b) Dente em banho-maria com superfície oclusal irradiada com termopar acoplado no interior da câmara pulpar; c) Aparelho de mensuração da temperatura intrapulpar acoplado a computador.



b

c

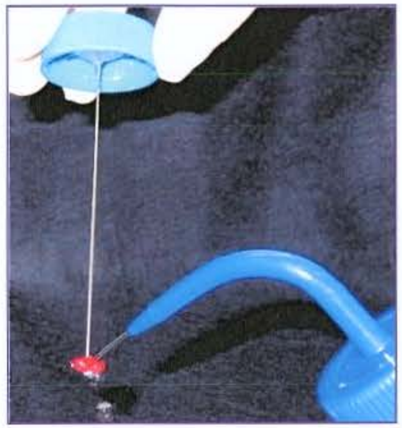




a) Metalizadora; b) Microscópio Eletrônico de Varredura (MEV); c) Computador acoplado ao MEV.

Cálcio 1,5 mM
Fosfato 0,9 mM
KCl 150 mM
Cacodilato 20 mM
pH 7,0

2,5 mL/mm²



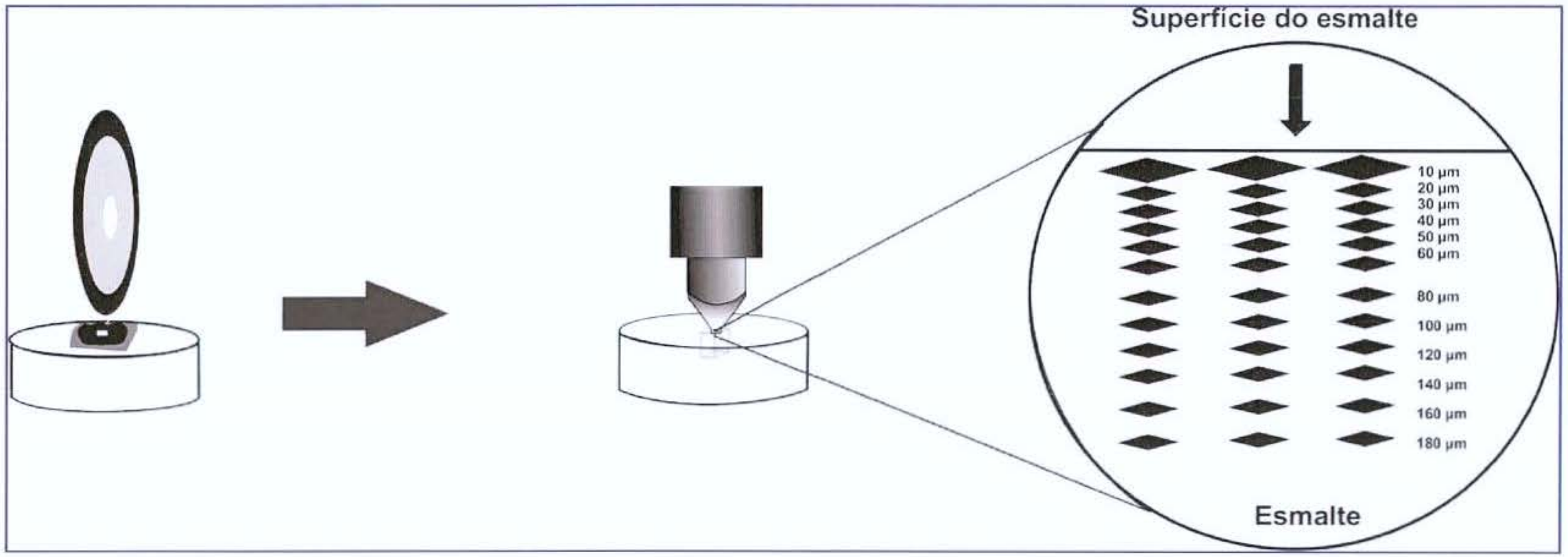
5,0 mL/mm²

Cálcio 2 mM
Fosfato 2 mM
Acetato 75 mM
pH 4,6

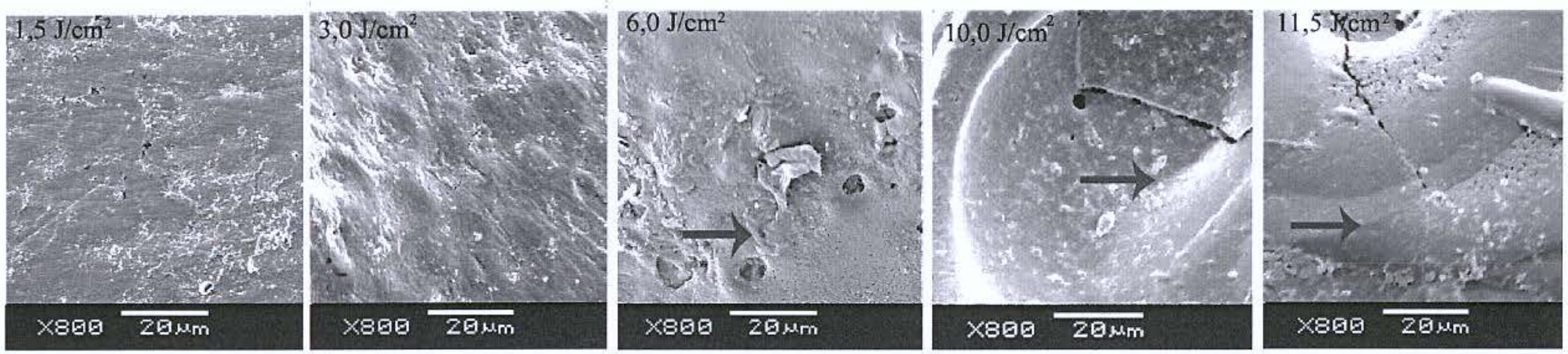
5 ciclos - 37°C

Featherstone *et al.*, 1986; Argenta *et al.*, 2003

ANEXO 8

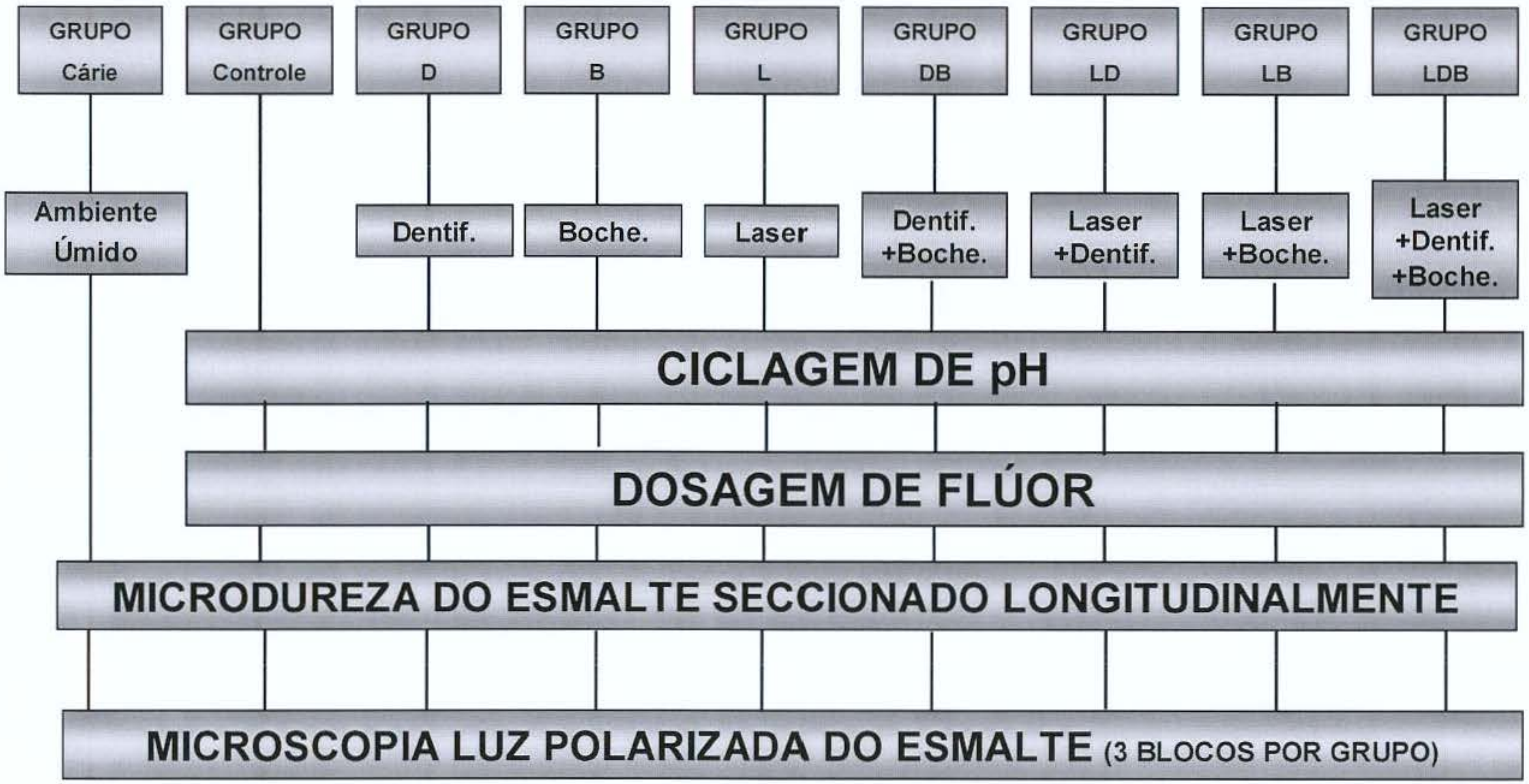


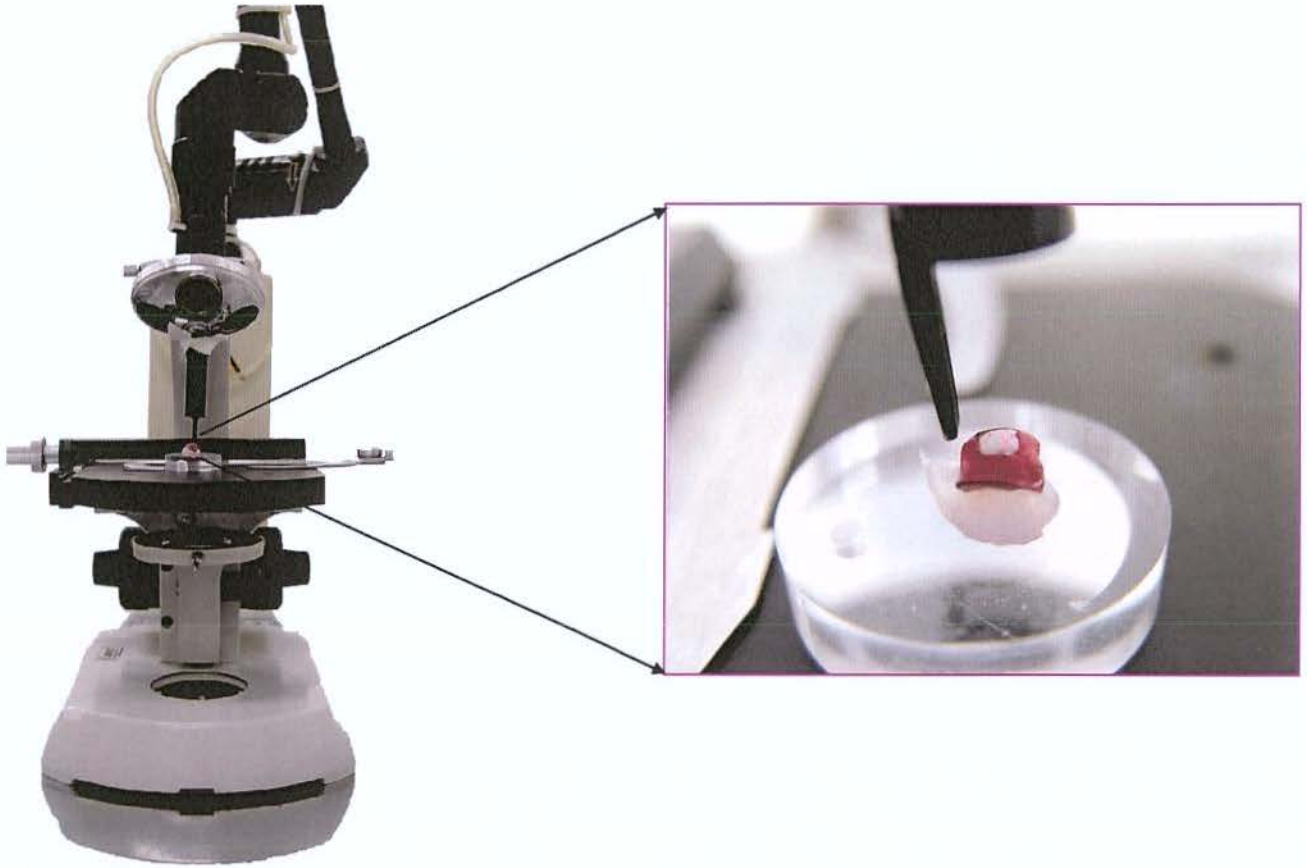
Esquema de identações de microdureza
em corte longitudinal em esmalte.



Micrografias eletrônicas do esmalte irradiado com 1,5 J/cm^2, 4,0 J/cm^2, 6,0 J/cm^2, 10,0 J/cm^2 e 11,5 J/cm^2 em aumento de 800X. Setas indicam áreas de esmalte derretido.

Barra = 20 µm.


GRUPO Cárie
GRUPO Controle
GRUPO D
GRUPO B
GRUPO L
GRUPO DB
GRUPO LD
GRUPO LB
GRUPO LDB
Ambiente Úmido
Dentif.
Boche.
Laser
Dentif. +Boche.
Laser +Dentif.
Laser +Boche.
Laser +Dentif. +Boche.
CICLAGEM DE pH
DOSAGEM DE FLÚOR
MICRODUREZA DO ESMALTE SECCIONADO LONGITUDINALMENTE
MICROSCOPIA LUZ POLARIZADA DO ESMALTE (3 BLOCOS POR GRUPO)

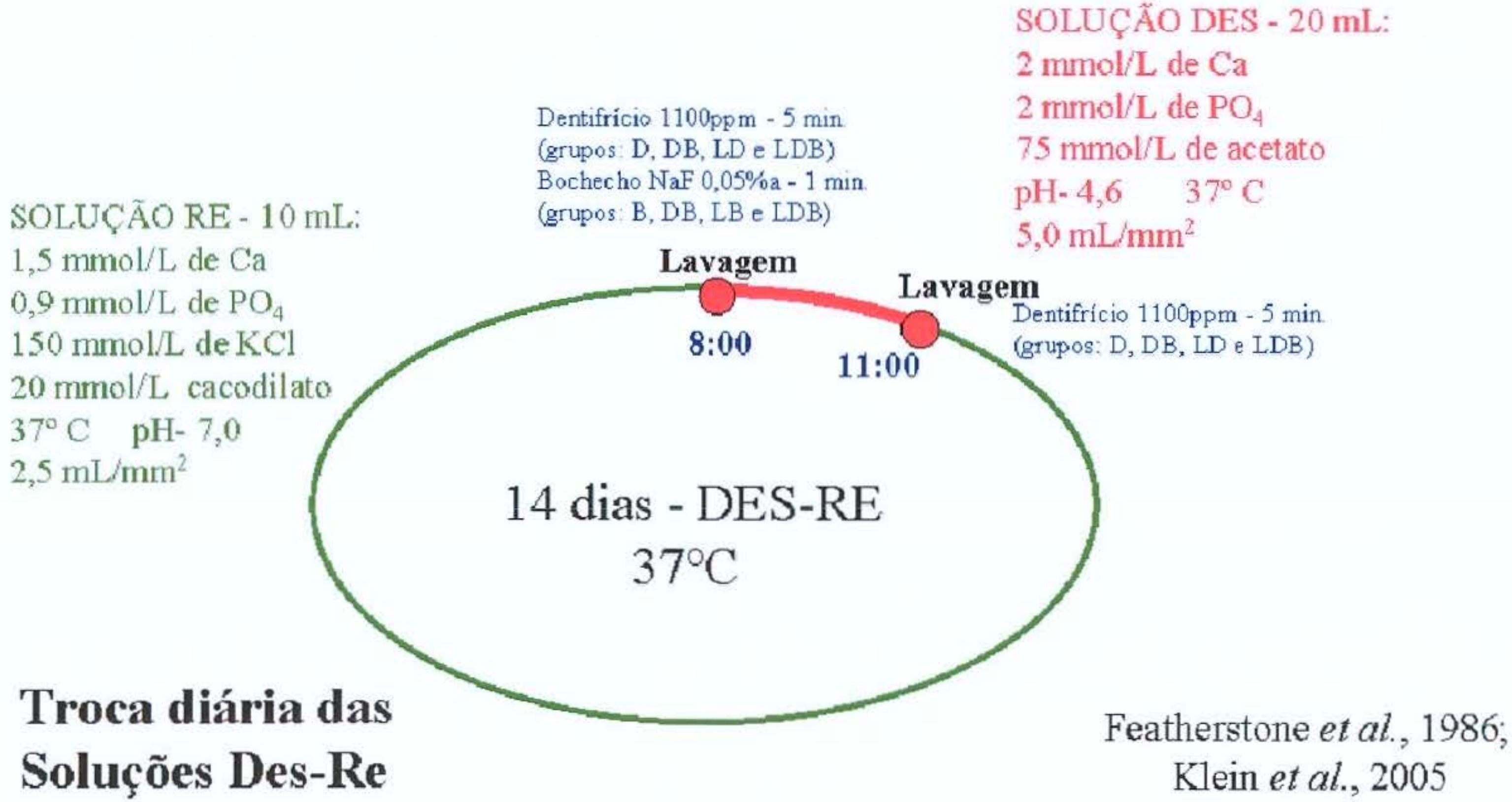


Laser de CO_2 acoplado ao microscópio para varredura da área de esmalte (4 mm^2) e luz guia do laser de CO_2 para facilitar irradiação.

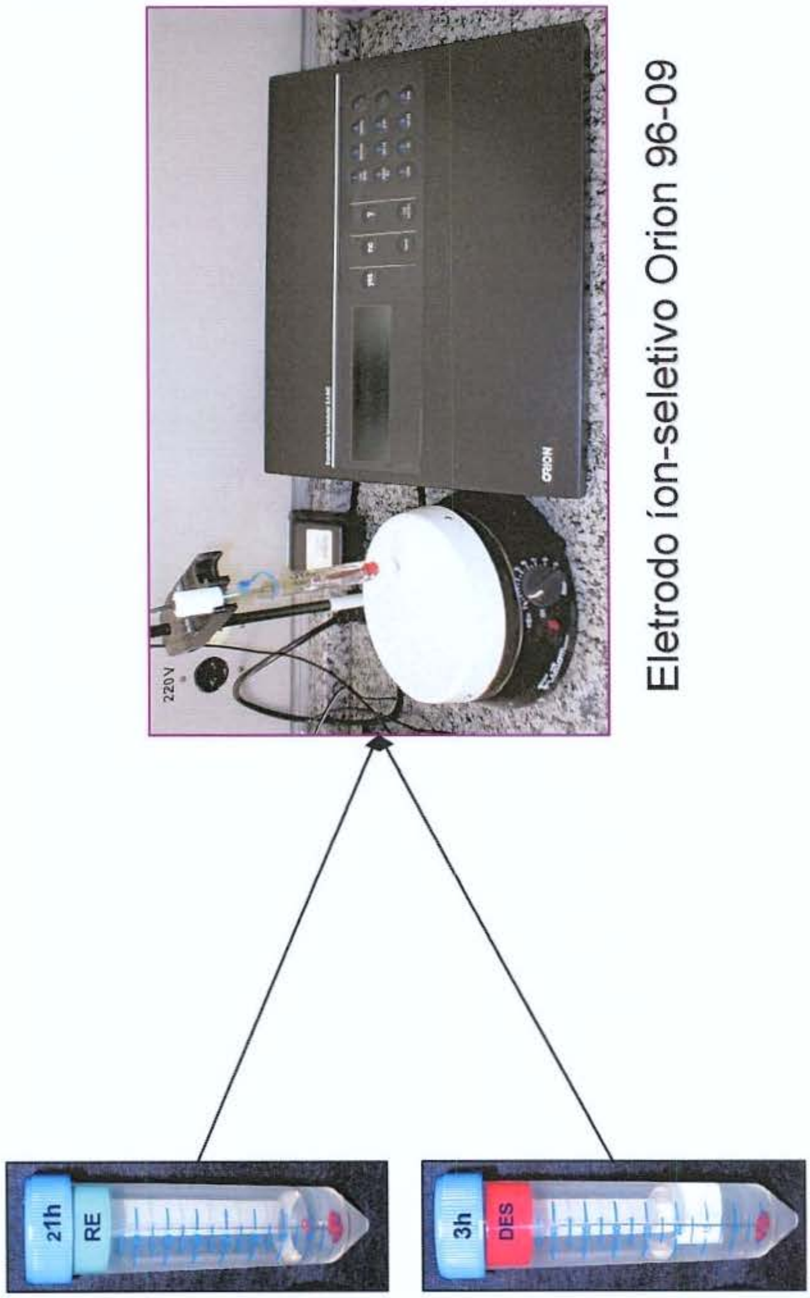
(a) Dentifrício com 1100 ppm de flúor Crest®-Cavity Protection; (b) Recipiente para agitação dos blocos de esmalte na diluição de dentifrício com água destilada (1:3); (c) Mesa agitadora Cientec CT a b c

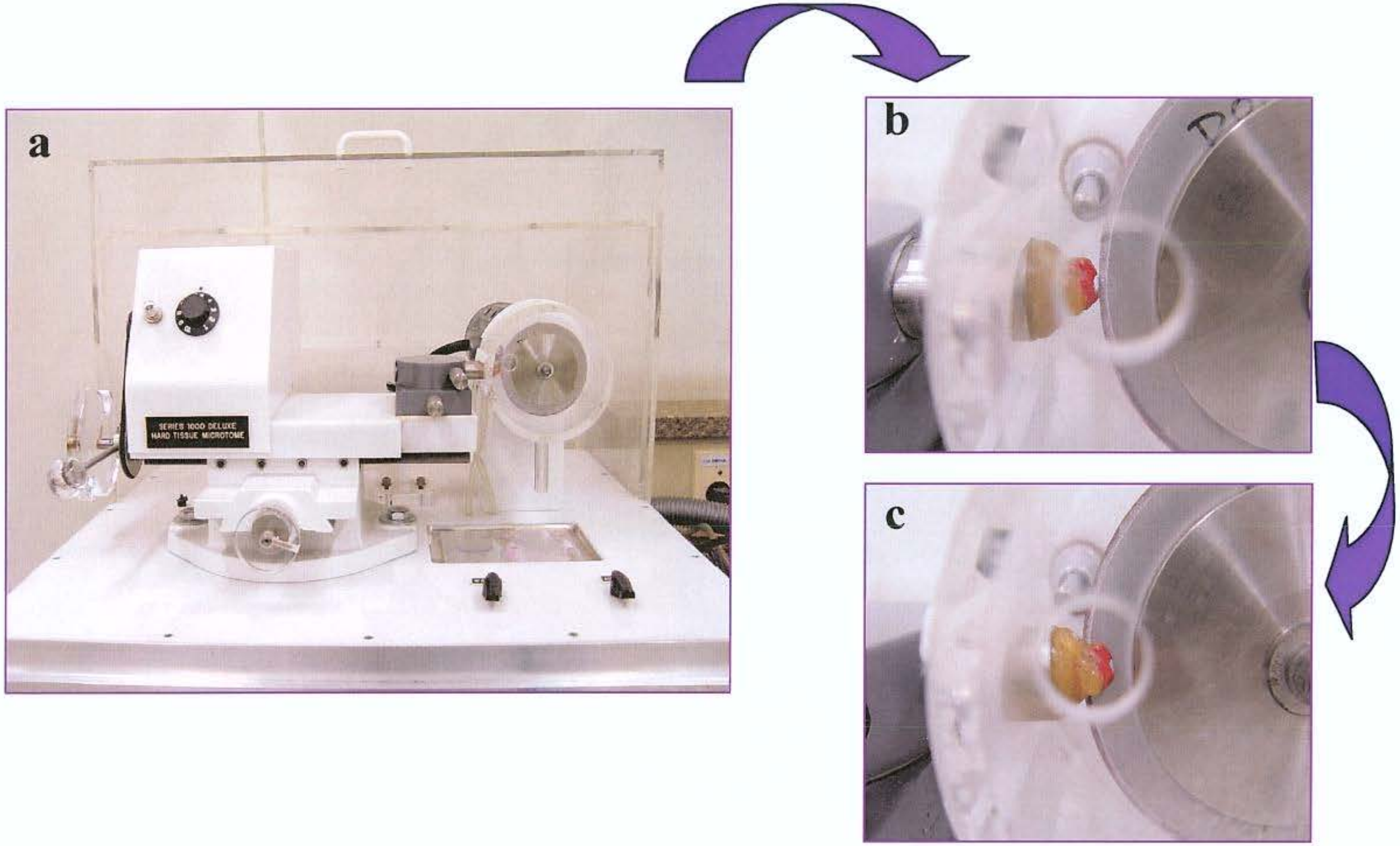
(a) Enxaguatório bucal com 0,05% de flúor Johnson & Johnson® (b) Recipiente para agitação dos blocos de esmalte na solução do enxaguatório bucal fluoretado; (c) Mesa agitadora Cientec CT 165.

ANEXO 14


SOLUÇÃO DES - 20 mL:
2 mmol/L de Ca
2 mmol/L de PO_4
75 mmol/L de acetato
pH- 4,6 37° C
5,0 mL/mm²
Dentifrício 1100ppm - 5 min.
(grupos: D, DB, LD e LDB)
Bochecho NaF 0,05%a - 1 min.
(grupos: B, DB, LB e LDB)
SOLUÇÃO RE - 10 mL:
1,5 mmol/L de Ca
0,9 mmol/L de PO_4
150 mmol/L de KCl
20 mmol/L cacodilato
37° C pH- 7,0
2,5 mL/mm²
Lavagem
8:00
Lavagem
11:00
Dentifrício 1100ppm - 5 min.
(grupos: D, DB, LD e LDB)
14 dias - DES-RE
37°C
Troca diária das
Soluções Des-Re
Featherstone *et al.*, 1986;
Klein *et al.*, 2005

ANEXO 15





a) Micrótomo de alta potência Silverstone-Taylor; b) Início da secção do bloco de esmalte transversalmente; c) Bloco de esmalte seccionado.