UNIVERSIDADE DE SÃO PAULO

FACULDADE DE ODONTOLOGIA DE RIBEIRÃO PRETO

DANIELA THOMAZATTI CHIMELLO DE SOUSA

Influência da irradiação com laser de Er:YAG na resistência ácida dos tecidos dentais

Ribeirão Preto
2008

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Tese apresentada à Faculdade de Odontologia de Ribeirão Preto da Universidade de São Paulo, para obtenção do título de Doutor em Odontologia.

Área de concentração: Odontologia Restauradora - Dentística
Orientadora: Profa. Dra. Silmara Aparecida Milori Corona

Ribeirão Preto
2008

FICHA CATALOGRÁFICA

Chimello-Sousa, Daniela Thomazatti
Influência da irradiação com laser de Er:YAG na resistência ácida dos tecidos dentais. Ribeirão Preto, 2008.

101 p.: il. ; 30cm

Tese de Doutorado, apresentada à Faculdade de Odontologia de Ribeirão Preto/USP. Área de concentração: Odontologia Restauradora - Dentística.

Orientadora: Corona, Silmara Aparecida Milori.

1. Lasers. 2. Preparo da cavidade dentária. 3. Suscetibilidade à cárie dentária. 4. Desmineralização do dente. 5. Testes de dureza. 6. Microscopia de Polarização.

FOLHA DE APROVAÇÃO

Daniela Thomazatti Chimello de Sousa

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Aprovada em: _____/_____/_____

Banca Examinadora

Prof (a). Dr (a)._____

Instituição:_____Assinatura:_____

Prof (a). Dr (a)._____

Instituição:_____Assinatura:_____

Prof (a). Dr (a)._____

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"Graças vos dou, Senhor, por serdes a fonte de que dimana todo o bem que me sucede. Os que esperam no Senhor renovam suas forças, sobem com asas de águia, correm e não se cansam, caminham e não se fatigam."

Isaías

DEDICATÓRIA

Dedico esse trabalho:

Aos meus pais, **Roque** e **Maria Alice**, pela formação, dedicação e pelo amor incondicional. Serei grata a vocês por toda minha vida.

Ao meu marido, **Régis**, pela presença em **todos** os momentos e pela felicidade de poder viver um amor verdadeiro.

À minha filha, **Maria Júlia**, por simplesmente existir em minha vida, e por compreender as inevitáveis ausências.

"Uma mãe nunca deixa seu filho em casa, mesmo quando ela não o carrega consigo."

Margaret Culkin Banning

À minha irmã, **Juliana**, pelo apoio nos momentos de adversidade.

Aos meus sogros, **Raimundo** e **Rosalina**, por me acolherem em sua família.

AGRADECIMENTOS

À Profa. Dra. Silmara Aparecida Milori Corona, pela orientação, pelo auxílio durante o desenvolvimento desse trabalho, e pela compreensão nos momentos de dificuldade.

À Profa. Dra. Mônica Campos Serra, pela co-orientação, inestimável contribuição na realização desse trabalho, e pelo constante incentivo.

À Profa. Dra. Regina Guenka Palma Dibb, quem primeiro me acolheu no Departamento de Odontologia Restauradora, pela amizade e pelos valiosos ensinamentos transmitidos.

Às amigas Juliana Jendiroba Faraoni Romano e Michelle Alexandra Chinelatti, pela convivência ao longo de todos esses anos, compartilhando sonhos, alegrias e dificuldades. Desejo a vocês um futuro brilhante.

"Na prosperidade nossos amigos nos conhecem; na adversidade conhecemos os nossos amigos."

Collins

À Faculdade de Odontologia de Ribeirão Preto da Universidade de São Paulo, na pessoa do diretor Prof. Dr. Osvaldo Luiz Bezzon, ao curso de pós-graduação da FORP/USP, na pessoa da presidente Profa. Dra. Léa Assed Bezerra da Silva, e ao programa de pós-graduação em Odontologia Restauradora da FORP/USP, na pessoa do coordenador Prof. Dr. Jesus Djalma Pécora, pela oportunidade de realização do curso de doutorado.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pelo apoio financeiro concedido durante todo o curso de doutorado.

Ao Prof. Dr. Antonio Luiz Rodrigues Júnior, pela disponibilidade em realizar a análise estatística desse trabalho, e paciência em esclarecer minhas dúvidas.

À Profa. Dra. Karina Fittipaldi Bombonato Prado, pelos longos anos de amizade e cumplicidade.

À Profa. Dra. Maria Cristina Borsatto, pelo carinho e por ter permitido de forma tão generosa a utilização do Laboratório de Pesquisa em Odontopediatria.

Ao Prof. Dr. Luiz Antonio Salata, por ter permitido a utilização do microscópio de luz polarizada.

À Profa. Dra. Raquel Fernanda Gerlach, pelo apoio e pela orientação no uso do microscópio de luz polarizada.

À Profa. Dra. Marisa Semprini, minha primeira orientadora na Iniciação Científica, que sempre acreditou em mim e me incentivou a prosseguir.

Ao Prof. Dr. Jesus Djalma Pécora, por permitir a utilização do laser de Er:YAG.

A todos os professores do Departamento de Odontologia Restauradora da FORP/USP, em especial às Profas. Dras. Izabel Cristina Fröner e Telma Nunes do Nascimento, pelo acolhimento e carinho.

À Patrícia Marchi, técnica do Laboratório de Pesquisa em Dentística da FORP/USP, pela amizade e disponibilidade.

Ao Carlos Feitosa dos Santos, secretário do programa de pós-graduação em Odontologia Restauradora da FORP/USP, pelo auxílio constante e pela eficiência.

Ao Reginaldo Santana da Silva, pelo auxílio no uso do laser de Er:YAG.

Às secretárias do Departamento de Odontologia Restauradora da FORP/USP, Maria Amália Viesti de Oliveira e Maria Izabel C. Francisco Miguel, e aos funcionários do Departamento de Odontologia Restauradora, Rosângela Angelini, Luiza Godoi Pitol e Fernando Pina Perez, pela atenção, carinho e solicitude.

Às secretárias da Seção de pós-graduação da FORP/USP, Isabel Cristina Galino Sola e Regiane Cristina Moi Sacilotto, pela atenção dispensada e disponibilidade.

A todos os voluntários que participaram do experimento *in situ*, e aos examinadores que realizaram a análise das imagens em microscopia de luz polarizada, sem os quais essa pesquisa não seria possível, pela disponibilidade e desprendimento.

À amiga Cristina Yoshie Garcia Takeuchi, pela troca de experiências.

À Cecília Pedroso Turssi, pelo esclarecimento de várias dúvidas durante a realização desse trabalho.

A todos os colegas do curso de pós-graduação em Odontologia Restauradora, em especial aos colegas do Laboratório de Pesquisa em Dentística, Francisco Carlos Rehder, Pedro Turrini, Walter Raucci, César Lepri, Alessandra Marques, Aline Evangelista, Danielle Messias, Vivian Colucci, Sílvia Domiciano, Juliane Ciccone, Cristiane Savaris, Daniel Galafassi, Fernando Maeda, Renato Schiavoni, Flávia Amaral, Carolina Amorim e Thaise Carrasco, pela boa convivência, tornando o trabalho mais agradável.

À protética Andréa Zanella, pela confecção dos dispositivos intra-bucais.

Ao Nicholas John Bewick, pela revisão do texto em inglês.

À Central do Óxido de Etileno do Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto/USP, pela esterilização dos fragmentos utilizados nesse trabalho, com disponibilidade e presteza.

Enfim, a todos que, de algum modo, me ajudaram na realização desse trabalho.

"A verdadeira medida de um homem não se vê na forma como se comporta em momentos de conforto e conveniência, mas em como se mantém em tempos de desafio e controvérsia."

Martin Luther King Jr.

“Diz-me, e eu esquecerei; ensina-me e eu lembrar-me-ei; envolve-me, e eu aprenderei.”

Provérbio chinês

RESUMO

CHIMELLO-SOUSA, D. T. **Influência da irradiação com laser de Er:YAG na resistência ácida dos tecidos dentais**. 2008. 100f. Tese (Doutorado) - Faculdade de Odontologia de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, 2008.

A irradiação dos substratos dentais com laser de Er:YAG, utilizando parâmetros sub-ablativos ou ablativos, gera um efeito térmico nas camadas mais superficiais dos tecidos, podendo levar a alterações morfológicas, estruturais e químicas. A resistência ácida dos tecidos dentais tem sido relacionada às alterações induzidas pela irradiação. Os objetivos do presente estudo foram: 1) Revisar criticamente as alterações induzidas pela irradiação com laser de Er:YAG, sua influência na resistência ácida dos tecidos dentais, e as variáveis de resposta empregadas nessas pesquisas; 2) Avaliar a microdureza do esmalte adjacente a preparos cavitários realizados com laser de Er:YAG, restaurados com resina composta e submetidos a um modelo intra-bucal de desafio cariogênico; 3) Analisar, por meio de microscopia de luz polarizada, as alterações no esmalte ao redor de preparos cavitários confeccionados com laser de Er:YAG e restaurados, após desafio cariogênico *in situ*, através de medidas quantitativas da área desmineralizada e largura da zona de inibição, e da análise qualitativa da ocorrência de trincas e de escores atribuídos à desmineralização e zona de inibição. A correlação entre os dados quantitativos e seus respectivos escores também foi avaliada. Baseado nos estudos realizados, foi possível concluir que a revisão crítica das alterações induzidas pelo laser de Er:YAG e dos resultados relacionados à resistência ácida dos tecidos dentais permitiu melhor entendimento da real influência da irradiação na perda mineral dos substratos. As variáveis de resposta utilizadas refletem o estágio atual das pesquisas. De modo geral, a análise do esmalte adjacente a restaurações submetidas a desafio carigênico *in situ* mostrou que a técnica de preparo cavitário com laser de Er:YAG foi semelhante àquela com turbina de alta-rotação, com relação à microdureza e às alterações analisadas em microscopia de luz polarizada. Os escores relacionados à zona

de inibição sugeriram menor desmineralização nas margens das cavidades preparadas com laser. A correlação entre os dados quantitativos e qualitativos sugeriu que a análise por escores pode ser considerada uma alternativa viável às variáveis de resposta quantitativas, para análise de lesões de cárie ao redor de restaurações, através de microscopia de luz polarizada.

Palavras-chave: Lasers. Preparo da cavidade dentária. Suscetibilidade à cárie dentária. Desmineralização do dente. Testes de dureza. Microscopia de Polarização.

ABSTRACT

CHIMELLO-SOUSA, D. T. **Influence of irradiation with Er:YAG laser on acid resistance of dental tissues**. 2008. 100f. Thesis (Doctoral) - Faculdade de Odontologia de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, 2008.

The irradiation of dental substrates with Er:YAG laser, using sub-ablative or ablative parameters, generates a thermal effect in the most superficial layers, which might lead to morphological, structural and chemical changes. Acid resistance of dental tissues has been related to the laser-induced alterations. The aims of the present study were: 1) Critically review the Er:YAG laser-induced changes, their influence on tooth acid resistance, and the response variables used in these researches; 2) Assess the microhardness of enamel adjacent to Er:YAG laser-prepared cavities, which was filled with composite resin and submitted to an intra-oral model of cariogenic challenge; 3) Analyze, by means of polarized light microscopy, the alterations in enamel around restored Er:YAG laser-prepared cavities, after cariogenic challenge *in situ*, by the quantitative measurement of demineralized area and inhibition zone width, and the qualitative analysis of cracks occurrence and scores attributed to demineralization and inhibition zone. The correlation between the quantitative data and their respective scores was also evaluated. Based on the conducted studies, it was feasible to conclude that the critical review of the Er:YAG laser-induced alterations and the results related to tooth acid resistance allowed a better understanding of the real influence of laser irradiation on tooth mineral loss. The response variables used reflects the current stage of researches. Generally, the analysis of enamel adjacent to restorations submitted to cariogenic challenge *in situ* showed that cavity preparation technique with Er:YAG laser was similar to that with high-speed handpiece, with regard to microhardness, and alterations analyzed by means of polarized light microscopy. The scores related to inhibition zone suggested less demineralization at the margins of laser-prepared cavities. The correlation between the quantitative and qualitative data suggested that the analysis by scores could be

considered a viable alternative to the quantitative response variables, when assessing caries lesion around restorations, by means of polarized light microscopy.

Keywords: Lasers. Dental cavity preparation. Dental caries susceptibility. Tooth demineralization. Hardness tests. Microscopy, Polarization.

SUMÁRIO

1. INTRODUÇÃO GERAL

O emprego do laser de Er:YAG (Erbium:Yttrium-Aluminum-Garnet) em Odontologia iniciou-se no final da década de 80, com os estudos realizados por Hibst e Keller (1989) e Paghdiwala et al. (1993). Originalmente, o laser de Er:YAG tem sido indicado para remoção de tecido cariado e preparo cavitário, utilizando parâmetros ablativos que removem estrutura dental, na tentativa de superar algumas limitações apresentadas pelos instrumentos rotatórios, tais como o alto nível de ruído e vibração, principais causas de desconforto aos pacientes (KELLER et al., 1998). A cavidade resultante pode apresentar níveis reduzidos de bactérias (SHARON-BULLER et al., 2003), constituindo uma vantagem da técnica.

Com a valorização cada vez maior da Odontologia Preventiva, a aplicação do laser de Er:YAG passou a ser investigada também para o retardo da desmineralização, através da irradiação da superfície com parâmetros sub-ablativos (APEL et al., 2002; APEL, et al., 2004; APEL et al., 2005; CASTELLAN et al., 2007; CECCHINI et al., 2005; DELBEM et al., 2003; LIU et al., 2006), na tentativa de manter a estrutura dental e promover apenas modificações superficiais capazes de aumentar a resistência dos substratos à ação dos ácidos (APEL et al., 2002).

Alterações morfológicas, estruturais e químicas nas superfícies irradiadas, geradas pelo efeito térmico do laser de Er:YAG (MATSUMOTO et al., 2003), têm sido relacionadas à resistência ácida dos tecidos dentais. Porém, a influência do laser de Er:YAG depende de vários parâmetros, que, associados, resultam na sua ação sobre os substratos. A necessidade de uma análise crítica dos estudos existentes na literatura sobre as modificações induzidas pelo laser de Er:YAG, sua influência na desmineralização dental, bem como das variáveis de resposta empregadas nessas pesquisas, resultou na redação do artigo científico intitulado “Er:YAG laser-induced changes: influence on tooth acid resistance - a review”, enviado para publicação no periódico *Lasers in Medical Science* (Capítulo 1).

Sugere-se que as margens das cavidades preparadas com laser de Er:YAG, utilizando parâmetros ablativos, poderiam apresentar uma certa resistência ácida, influenciando o desenvolvimento de lesões de cárie ao redor de restaurações (APEL et al., 2002). Pesquisas envolvendo irradiação com laser de Er:YAG e desmineralização ao redor de restaurações são escassas, sendo que o único estudo reportado utilizou o laser de Er:YAG para pré-tratamento das superfícies de cavidades preparadas de maneira convencional, e um modelo de indução de cárie *in vitro* (CEBALLOS et al., 2001). A realização de estudos envolvendo modelos *in situ* de desafio cariogênico é de suma importância na tentativa de simular as reais condições bucais, já que a cárie é uma doença dependente da associação de múltiplos fatores existentes no indivíduo. A desmineralização ao redor de restaurações tem sido avaliada através da análise da microdureza (BENELLI et al., 1993; HARA et al., 2000; MAGALHÃES et al., 2005; SERRA; CURY, 1992), a qual apresenta correlação com o conteúdo mineral, em esmalte (FEATHERSTONE et al. 1983). Com o propósito de verificar as alterações ocorridas no esmalte ao redor de preparos confeccionados com laser de Er:YAG e restaurados com resina composta, após alto desafio cariogênico *in situ*, foi realizada a pesquisa que deu origem ao artigo “Influence of Er:YAG laser on microhardness of enamel adjacent to restorations submitted to cariogenic challenge *in situ”,* aceito para publicação no periódico *Photomedicine and Laser Surgery* (Capítulo 2).

A microdureza constitui um método de avaliação útil para a obtenção de informações importantes sobre as alterações resultantes do processo de des-remineralização, com análises pontuais, o que gera a necessidade de múltiplas endentações (VIEIRA et al., 2005). A necessidade de métodos complementares à análise da microdureza tem sido sugerida (MCINTYRE et al., 2000), como a microscopia de luz polarizada, que permite a avaliação da lesão de cárie através da diferença de birrefringência entre tecido dental sadio e desmineralizado, fornecendo detalhes da lesão, em relação à profundidade (APEL et al., 2003; CEBALLOS et al., 2001; DIONYSOPOULOS et al., 2003; GARCÍA-GODOY et al., 1998; HICKS et al., 2000; HICKS et al., 2002; KONISHI et al., 1999; SAVARINO et al., 2004), área (DONLY; INGRAM, 1997; HARA et al., 2005; LIU et al., 2006) e limites em

relação à restauração. O grau de perda mineral pode ser determinado qualitativamente, ou quantitativamente, através da correta interpretação da birrefringência (SOUSA et al., 2006). Com a finalidade de complementação da análise da microdureza, a desmineralização ao redor de cavidades preparadas com laser de Er:YAG foi avaliada por meio de microscopia de luz polarizada, através da análise quantitativa da área desmineralizada e da largura da zona de inibição, e da avaliação qualitativa da presença de trincas, e de escores atribuídos à desmineralização e zona de inibição. A correlação entre as medidas quantitativas e os escores também foi avaliada. Esse experimento resultou no artigo “Influence of cavity preparation with Er:YAG laser on enamel adjacent to restorations submitted to cariogenic challenge *in situ*: a polarized light microscopic analysis”, submetido para publicação no periódico *Lasers in Surgery and Medicine* (Capítulo 3).

2. PROPOSIÇÃO

O presente estudo teve como objetivo avaliar a influência do laser de Er:YAG nos tecidos dentais, por meio de:

1. Revisão da literatura relacionada às alterações morfológicas, estruturais e químicas induzidas pela irradiação com laser de Er:YAG nos substratos dentais, sua influência na resistência ácida dos tecidos, e variáveis de resposta empregadas nesses estudos (Capítulo 1);

2. Avaliação da microdureza do esmalte adjacente a preparos cavitários confeccionados com laser de Er:YAG e restaurados com resina composta, após serem submetidos a um modelo intra-bucal de alto desafio cariogênico (Capítulo 2);

3. Análise, através de microscopia de luz polarizada, das alterações do esmalte ao redor de cavidades preparadas com laser de Er:YAG e restauradas com resina composta, após alto desafio cariogênico *in situ* (Capítulo 3).

3. CAPÍTULOS

3.1. CAPÍTULO 1

ARTIGO SUBMETIDO À PUBLICAÇÃO NO PERIÓDICO *LASERS IN MEDICAL SCIENCE* (ANEXO A)

Er:YAG laser-induced changes: influence on tooth acid resistance - a review

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Abstract

Er:YAG (Erbium:Yttrium-Aluminum-Garnet) laser has been investigated for caries removal and cavity preparation with ablative energy densities, with the aim of overcoming some limitations of rotary cutting instruments. The modification of tooth surface with Er:YAG laser, using sub-ablative parameters, has also been studied. Although it has been reported that this kind of laser generates less thermal damage to the irradiated tissues than other high-power lasers, morphological, structural and chemical changes in the irradiated sites have been described. It has been suggested that these alterations might influence tooth demineralization and caries development. The present article reviewed the caries process in enamel and dentin, and the action mechanism of Er:YAG laser on dental substrates. Er:YAG laser-induced morphological, structural and chemical changes, and their possible influence on tooth acid resistance were discussed. The methods for assessing demineralization in Er:YAG laser researches were also critically reviewed.

Keywords: lasers, dental caries susceptibility, tooth demineralization.

Introduction

The first studies involving the application of Er:YAG laser in dental tissues have been ascribed to Paghdiwala and Hibst & Keller in the late 80´s [1, 2], with the aim of overcoming some limitations of rotary cutting instruments, such as noise, vibration and the need for anesthesia. Thus, the primary indications of Er:YAG laser have been caries removal and cavity preparation with ablative energy densities that remove dental tissues, as demonstrated *in vitro* [1, 2, 3] and *in vivo* [4, 5].

Er:YAG laser has also been investigated to just modify the surfaces of dental structures and allow caries prevention, using sub-ablative energy densities [6, 7, 8, 9, 10, 11, 12]. It has been suggested that the margins of cavities prepared with Er:YAG laser with ablative energy densities might also present a certain resistance to the action of acids and thus to the development of secondary caries [6].

The present review aimed to discuss aspects of morphological, structural and chemical changes in tooth substrates after Er:YAG laser irradiation at ablative and sub-ablative modes, and their possible influence on tooth acid resistance. The methods for assessing demineralization of the irradiated substrates in Er:YAG laser researches were also critically reviewed.

Caries process affecting enamel and dentin structures

Enamel is composed of carbonated hydroxyapatite crystals [13], surrounded by water and organic material, basically proteins [14]. Trace elements, resulting from ion exchange during tooth development and after its eruption, can be found in dental structure [15]. Carbonate, mainly present when teeth erupt, destabilizes the crystals and leads to increased caries susceptibility [16]. The presence of acids inside the biofilm may cause the partial dissolution of the crystals, if they are not sufficiently protected by inhibitors (*e.g.* fluoride) [17]. The enamel organic portion, in the intercrystalline spaces, seems to represent the most important diffusion pathway in caries process [18]. In the lesion body, the crystals become reduced in diameter, in comparison with sound enamel [19]. Dissolution in the central core of

the crystals has also been reported, due to the presence of defects and a higher carbonate concentration [20].

Dentin is also mainly composed of carbonated hydroxyapatite [13], but its inorganic content is reduced, presenting higher amount of organic material (basically type I collagen) and water than enamel [21]. Histologically, dentin is composed of multiple dentinal tubules, which provide a degree of permeability for this substrate [21]. During the caries process, the mineral part of dentin is dissolved, exposing organic matrix to breakdown by bacterial acids, as well as by other host-derived enzymes, such as the matrix metalloproteinases [22].

Action mechanism of Er:YAG laser on dental substrates

Er:YAG laser emits photons at a wavelength of 2.94 μm, which is highly absorbed by water [1, 23] and the hydroxyl ions (OH^-) of hydroxyapatite [23]. For this reason, Er:YAG laser ablates dental hard substrates more effectively and efficiently than other high-power lasers, with minor thermal damage to the irradiated surfaces, surrounding tissues and pulp [24].

The Er:YAG laser mechanism consists on thermo-mechanical ablation [1]. In this process, evaporation of water and other hydrated organic components in dental substrates leads to internal pressure increase within the tissue, until micro-explosions that eject particles of hard substance [1]. When applied at sub-ablative mode, Er:YAG laser is expected not to remove dental substance, but only to modify the surface at structural and chemical levels [6]. The transition of sub-ablative to ablative energy densities, also called ablation threshold [25] stayed around 7.2 J/cm^2 [26] and 10 J/cm^2 [25], with 5 Hz pulse repetition rate, for sound enamel. Ablation threshold of dentin was lower (0.7 J/cm^2) [26], since this substrate is more efficiently ablated than enamel, due to a higher water content [26]. However, when pulse repetition rate was reduced to 2 Hz, the ablation threshold of enamel and dentin increased to 18.6 J/cm^2 and 1.2 J/cm^2, respectively [26].

For both ablative and sub-ablative energy densities, the irradiated energy is converted into heat, and this thermal effect, although not critical in deep layers [1], may result

in morphological, structural and chemical changes in the irradiated substrates [27], depending on the parameters employed and the degree of heat generated. Pulse repetition rate [28, 29], pulse length [25, 30, 31], energy per pulse [29], energy density [28, 32] and the spatial beam profile [32] are parameters that influence the interaction between Er:YAG laser and the target tissue. Moreover, the tissue optical properties [28, 30] and the distance between the probe's end and the target site [10, 33], which alters the energy density, also influence the laser action. Water flow applied during irradiation affects the ablation process [34], heat accumulation [30] and heat-induced alterations [27].

When the laser beam is applied to the target tissue, there is an increase in temperature at the beam impact point (reported to be from 50^{o}C to 400^{o}C) [1, 6, 28] and a residual heat deposition within the tissue, which is considerably lower (less than 15^{o}C) [35, 36], due to the high absorption of laser energy in the superficial layers of the irradiated substrates. For ablative energy densities, a large fraction of the absorbed energy is removed from the tooth as internal and kinetic energy of the ejected material, reducing residual heat deposition within the tissue, in comparison with sub-ablative energy densities [30]. With regard to the pulse repetition rate, the subsequent laser pulses contribute significantly to heat accumulation within the tissue, more evident with energy densities near the ablation threshold [30]. Pulse length in the order of nanoseconds contributes to less residual heat deposition, in comparison with longer pulses, in the order of microseconds [30]. However, shorter Er:YAG laser pulses can generate stronger acoustic damage (cracks) by waves propagation [30], which may be a concern.

The instantaneous temperature increase at the beam impact point seems to be the main responsible for the morphological, structural and chemical alterations in dental substrates, which might influence their resistance to the action of acids, affecting the caries process.

Morphological changes and acid resistance

Although the Er:YAG laser is reported to generate minor thermal injuries, morphological changes have been observed in dental substrates, varying widely, depending on the parameters used. Table 1 shows an overview of parameters used in some Er:YAG laser studies.

Sub-ablative parameters

The use of six different parameters considered sub-ablative, with water flow, resulted in no sign of enamel denaturing or disruption, under scanning electron microscopy [10]. Even with low energy densities, combinations of non-irradiated areas and ablated zones were observed, with roughness and exposure of enamel rods [10]. When the laser was applied with higher sub-ablative energy densities at contact mode, a less homogeneous morphological pattern and cracks were verified [10]. One aspect to be taken into consideration is that the irradiated surfaces from all the groups were demineralized prior to the scanning electron microscopy analysis [10]. Confocal laser scanning microscopy revealed no loss of enamel substrate irradiated without water flow [9], suggesting that the parameters were really below the ablation threshold. However, cracks covering the entire surface were observed, mainly at the prisms boundaries [9].

Ablative parameters

Enamel surfaces irradiated with ablative parameters and cooled with water, presented irregularities [23, 37] and a microretentive pattern [38]. The surfaces were slightly melted [23] or devoid of any signs of fusion, with minimal cracks and thermal injuries [34]. Fusion areas were present when no water mist was applied during irradiation [23, 34], indicating enamel melting and recrystallization. Signs of thermal and acoustic damage were also reported when water flow was absent [34].

Dentin irradiated with ablative parameters, under water mist, presented irregular [23, 27, 31], imbricate-patterned surfaces [39] and slight melting [23]. Smear layer was partially

[23] or totally [27, 31, 39] removed, and dentinal tubules were partially [23] or clearly [27, 31, 39] visible. Intertubular dentin suffered more ablation than peritubular dentin, making tubules protuberant [27]. Dentin irradiation without water mist created sites with severe melting and recrystallization, cracks, and an irregular structure with microholes [27]. Dentinal tubules were not clearly visible, due probably to the smear layer melting [23].

The melting point of hydroxyapatite is reported to be above 1200^{o}C [40]. Although temperatures at the beam impact site have been reported to be less than 400^{o}C, well below the melting point of hydroxyapatite, areas of dental substrates apparently melted and recrystallized have been described. These areas are suggested to be less permeable and more resistant to the action of acids, due to the surface sealing [23]. Enamel and dentin, thermally degenerated by irradiation without water mist, were almost unchanged after acid demineralization [23]. However, the melting and fusion of tooth structures seems to be inhomogeneous, which might limit a possible acid resistance to isolated areas. Roughness and cracks caused by the ablation process could increase the area exposed to demineralization [9, 10, 37], acting as open channels for acids to reach the subsurface [9, 37]. So, the hypothesis of acquired acid resistance by laser-induced morphological alterations should be seen with reservations.

Structural and chemical changes and acid resistance

The most accepted theory for the possible acid resistance of the irradiated dental substrates is the occurrence of structural and chemical alterations due to heating. Dental enamel heated in furnace at temperatures between 100 and 650^{o}C resulted in structural and chemical alterations, such as loss of water and reduction in carbonate content. Besides, acid phosphate (HPO_4^{2-}) ions condensed to form pyrophosphate ($P_2O_7^{4}$) at temperatures between 200 to 400^{o}C. Between 650 and 1100^{o}C, thermal recrystallization and crystal size growth occurred, and the formation of tricalcium phosphate was observed, concomitant to the reduction of $P_2O_7^{4}$ ions. Tetracalcium phosphate was formed at temperatures above 1100^{o}C [41]. A lower amount of carbonate provides less solubility to hydroxyapatite, since carbonate

causes crystal defects and fits less well in the lattice, generating unstable and acid-soluble apatite phases [20]. Pyrophosphate is able to inhibit the dissolution of hydroxyapatite crystals, whereas tri and tetracalcium phosphates are potentially more susceptible to acid dissolution than hydroxyapatite [41].

The “organic blocking theory” [40, 42] has also been considered an explanation for the reduction in acid solubility of the irradiated substrates, with the sealing of diffusion pathways and prevention of demineralization [42], due to proteins decomposition (at about 350^{o}C) [41] located in the intra and inter-prismatic spaces.

Structural and chemical alterations in the irradiated dental substrates have been investigated with regard to calcium [6, 10, 23, 39, 43] and phosphorus [10, 43] loss, calcium and phosphorus concentration on the surfaces [27, 44], alterations in hydroxyapatite crystals [34, 45] and organic matrix [28, 45, 46], and carbonate content [28, 45].

Sub-ablative parameters

Irradiation of dental enamel with energy densities considered sub-ablative, followed by demineralization, resulted [10] or not [6] in reduction of calcium [6, 10] and phosphorus [10] loss from the surfaces, in comparison with non-irradiated samples. Despite using the same demineralizing solution, differences in the methodology, such as the type of substrate (human [10] and bovine [6]), the energy densities, and the immersion time in the solution, might have led to different results.

The spectrum analysis showed no damage in the crystal phase of the irradiated enamel, since no alteration of the phosphate bandwidth was identified [45]. Signs of organic matrix decomposition were observed in the enamel [45] and dentin [28] spectra, after irradiation. Carbonate concentration in dental substrates may decrease [45] or not [28], after laser treatment. Although both studies analyzed the spectrum of irradiated dental structures, the comparison between them is limited by differences in the substrates and experimental conditions.

Ablative parameters

Er:YAG laser was employed to treat enamel [23, 43] and dentin [23, 39] surfaces with parameters above the ablation threshold, with [23, 39, 43] and without [23] water flow. Calcium [23, 39, 43] and phosphorus [43] loss from the irradiated surfaces was reduced [23, 43] or not [39]. Dentin surfaces lased under water mist presented higher concentration of calcium and phosphorus ions than non-irradiated samples [27, 44], due probably to the heterogeneous deposition of the ablated material on the surface, with increase in calcium and phosphorus densities in certain areas.

The analysis of dentin spectrum revealed that organic matrix suffered degeneration after cavity preparation with laser, under water flow [46]. Enamel spectrum revealed non-hydroxyapatite phases, apparently similar to tri and tetracalcium phosphates, when this substrate was irradiated with high energy densities, without water mist [34].

Water flow seems to play an important role in enamel and dentin changes during Er:YAG laser irradiation, although the results still remain inconclusive for both substrates. Thus, additional investigation into the most suitable ablative and sub-ablative parameters should be conducted.

Assessing tooth acid resistance in Er:YAG laser researches

Acid resistance of dental substrates after Er:YAG laser irradiation has been investigated by different qualitative and quantitative methods, such as scanning electron microscopy [10, 23], confocal laser scanning microscopy [9], polarized light microscopy [11, 37, 47], microhardness [7, 8, 12] and atomic spectroscopy [6, 10, 23, 39, 43]. The experimental design and results of these studies are described in Table 2. Table 3 displays the *in vitro* demineralization protocols used in Er:YAG laser researches.

Conventional scanning electron microscopy is mainly a qualitative method, allowing a wide range of magnifications, high resolution and focus depth (giving a three-dimensional appearance), by detection of electrons emitted by metallized surfaces. However, it is considered a destructive method, being sample preparation a sensitive step in the technique,

due to the need for completely dry specimens. The photomicrography provides a representative analysis of the sample surface.

Confocal laser scanning microscopy is a non-destructive method, allowing image acquisition without any sample preparation, by incidence of a laser light on the specimen and detection of its fluorescence. The resultant image presents high resolution and contrast, due to the selection of focused light only. Qualitative surface inspection and non-invasive optical sections of samples are possible with this device. However, its relatively high cost constitutes a limitation for its use.

Another type of microscopy, with different principles, is the polarized light microscopy, in which a polarized light passes through a birefringent sample, resulting in polarized light waves with different refraction indexes, depending on the material, allowing qualitative and quantitative analysis. Differences in the birefringence of sound and demineralized tissues allow delimiting the caries lesion and measuring its area [11] or depth [47]. For this purpose, samples must be immersed in a solution with a known refraction index (*e.g.* water) that penetrates into the pores. Quantitative assessment of the mineral content is also possible, requiring the correct interpretation of the substrate birefringence [48]. Polarized light microscopy is a sensitive technique with regard to sample preparation, due to the need for thin sections (in the order of 100 μm) with standardized thickness, to allow correct comparisons.

Microhardness is a quantitative method used in Er:YAG laser researches to assess alterations occurred in enamel after demineralization. The action of acids is associated with softening and weakening of dental structures, which are reflected in the substrate hardness. It is considered a relatively low-cost and easy-to-use evaluation technique. Despite having correlation with microradiography in enamel [49], microhardness does not allow the direct measurement of the mineral content. Multiple indentations are required, since a single indentation cannot reflect precisely the microhardness of a sample as a whole [50]. Considering that this technique evaluates isolated points of the altered area, complementary investigations should be conducted to a more detailed analysis.

Quantitative chemical analysis of the irradiated substrates is possible by atomic spectroscopy (atomic *absorption* spectroscopy and atomic *emission* spectroscopy). Since every element has a unique electronic structure, the wavelength absorbed or emitted by its atoms is a unique property of each individual element. Basically, this technique consists on specimen demineralization, with ions loss to the solution. The solution is atomized, and the atoms are submitted to a light source (atomic *absorption*) or excited (atomic *emission*). The wavelength absorbed or emitted by the atoms is detected and quantified. Despite being a sensitive technique, it results in destruction of the samples, not allowing longitudinal studies.

Concerning the limitations of each method, when used isolated, the association of evaluation techniques in laser research is of paramount importance to more reliable and convincing results.

Conclusions

The comprehension of Er:YAG laser mechanism on dental hard tissues, with regard to morphological, structural and chemical changes, is essential to understand the real influence of such tool on tooth acid resistance. However, the wide range of parameters and methodologies employed in Er:YAG laser researches and the lack of studies involving the oral environment suggest that further investigations should be necessary for the establishment of the real influence of Er:YAG laser on dental caries process in a clinical point of view. The favorable results, with regard to the increase in acid resistance, should not be sufficient for the prompt indication of this laser in dental practice for primary or secondary caries retardation. Other aspects of the irradiated substrates (*e.g.* adhesion of restorative materials), outside the scope of the present review, should be taken into account.

Acknowledgements

The authors would like to thank Mr. Nicholas John Bewick for the English language review.

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Table 1. Parameters employed in some Er:YAG laser researches.

Author	Energy/pulse (mJ)	Pulse repetition rate (Hz)	Energy density (J/cm^2)	Irradiation distance	Water flow
Hibst; Keller, 1989	30 to 360	1	Variable	Focused beam	*
Li et al., 1992	25 to 365	2 and 5	10 to 140	Focused beam	With water mist
Paghdiwala et al., 1993	*	6	159 to 780	Focused beam	With and without water mist
Dostálová et al., 1997	345	2	*	*	50 mL/min
Mehl et al., 1997	250 to 400	3 to 15	*	*	With (0.19 mL/s) and without water mist
Armengol et al., 2000	140	4	*	10 mm	With (1.4 mL/min) and without water mist
Hossain et al., 2000	400	2	*	20 mm	With and without water mist
Kameyama et al., 2000	100	10	*	Contact mode	With water mist
Ceballos et al., 2001	300 (enamel) 250 (dentin)	2	*	> 15 mm	With water mist
Fried et al., 2001	Variable	Variable	Variable	Variable	With (5 and 15 μL) and without water mist
Apel et al., 2002a	*	5	4, 6 and 8	*	*
Apel et al., 2002b	*	5	2 to 20	*	Without water mist
Fried et al., 2002	*	*	Variable	*	With (5 μL) and without water mist

Table 1. Parameters employed in some Er:YAG laser researches. *(continuação)*

Author	Energy/pulse (mJ)	Pulse repetition rate (Hz)	Energy density (J/cm^2)	Irradiation distance	Water flow
Ishizaka et al., 2002	250 (enamel) 80 to 140 (dentin)	4	*	≈ 12 mm	3.0 mL/min
Apel et al., 2003	260	20	41	Contact mode	74 mL/min
Delbem et al., 2003	60	1	0.95	40 mm	Without water mist
Hossain et al., 2003	200	2	25.5	2 to 3 mm	1 mL/min
Matsumoto et al., 2003	200	2	25.5	2 to 3 mm	With (1 mL/min) and without water mist
Apel et al., 2004	*	5	6	Contact mode	*
Camerlingo et al., 2004	350	10	*	*	10 mL/min
Yamada et al., 2004	*	10	*	≈ 1 mm	With water mist
Ying et al., 2004	*	4	6.25	*	*
Apel et al., 2005	*	5	6	Contact mode	Without water mist
Aranha et al., 2005	250	4	80.6	12 mm	6 mL/min
Bachmann et al., 2005	*	2	0.365, 0.651, 0.937, 1.271, 1.581, 1.94	*	*
Cecchini et al., 2005	60, 80 and 120 (handpiece No. 2051); 64, 86.4 and 135 (handpiece No. 2055)	2	33.3, 44.4 and 66.6 (handpiece No. 2051) 20, 29.9 and 42.2 (handpiece No. 2055)	Contact mode and 12 mm	5 mL/min
Corona et al., 2005	80	2	*	11, 12, 14, 16, 17 mm	5 mL/min

Table 1. Parameters employed in some Er:YAG laser researches. *(continuação)*

Author	Energy/pulse (mJ)	Pulse repetition rate (Hz)	Energy density (J/cm^2)	Irradiation distance	Water flow
Kim et al., 2006	380	2	33	Noncontact mode	With water mist
Liu et al., 2006a	700	10	*	Noncontact mode	24 mL/min
Liu et al., 2006b	100, 200, 300	10	12.7, 25.5, 38.2	*	Without water mist
Castellan et al., 2007	60	2	40.3	Contact mode	*
Corona et al., 2007	200, 250, 300 and 350	2, 3, 4	64.16, 80.19, 96.24 and 112.28	12 mm	2 mL/min
Liu & Hsu, 2007	*	2	5.1	*	10 mL/min
Matsumoto et al., 2007	700	8	*	Noncontact mode	12 mL/min

* Information not explicit in the text

Table 2. Experimental design and results of Er:YAG laser researches on tooth demineralization.

Author	Experimental design	Results
Hossain et al., 2000	**n:** 40 **s:** human enamel and dentin **s.f.:** treatment of cavity surfaces **r.v.:** morphological alterations (s.e.m.); calcium dissolution (a.a.s.)	s.e.m.: -Lased (with water flow) and demineralized enamel: rough surface, visible enamel rods, signs of dissolution; -Lased (without water flow) and demineralized enamel: melted and recrystallized areas, markedly less dissolution; -Lased (with water flow) and demineralized dentin: absence of smear layer, open tubules, signs of dissolution; -Lased (with water flow) and demineralized dentin: irregularities, not clear dentinal tubules, markedly less dissolution; a.a.s.: Lased < non-lased samples
Kameyama et al., 2000	**n:** 7 **s:** bovine dentin **s.f.:** surface treatment **r.v.:** calcium and phosphorus dissolution (a.a.s.)	Lased ≅ non-lased samples
Ceballos et al., 2001	**n:** 5 **s:** human enamel and dentin **s.f.:** surface treatment **r.v.:** demineralization depth (p.l.m.)	Lased samples: less deep secondary caries
Apel et al., 2002a	**n:** 21 **s:** human enamel **s.f.:** surface treatment **r.v.:** calcium dissolution (a.a.s.)	Lased ≅ non-lased samples
Apel et al., 2003	**n:** 10 **s:** human enamel **s.f.:** cavity preparation **r.v.:** demineralization depth (p.l.m.)	Lased samples: deeper demineralization than control
Delbem et al., 2003	**n:** 10 **s:** human enamel **s.f.:** surface treatment **r.v.:** Knoop microhardness	Laser ≅ fluoride Laser < laser + fluoride

Table 2. Experimental design and results of Er:YAG laser researches on tooth demineralization. *(continuação)*

Author	Experimental design	Results
Apel et al., 2004	**n:** 3 (in triplicate) **s:** human enamel **s.f.:** surface treatment **r.v.:** Knoop microhardness	Decrease in microhardness after irradiation; Lased ≅ non-lased samples after intra-oral phase
Apel et al., 2005	**n:** 3 **s:** human enamel **s.f.:** surface treatment **r.v.:** surface morphology (c.l.s.m.)	Lased samples: less surface demineralization; cracks towards subsurface with signs of marked demineralization
Cecchini et al., 2005	**n:** 5 (s.e.m.); **n:** 10 (a.e.s.) **s:** human enamel **s.f.:** surface treatment **r.v.:** morphological alterations (s.e.m.); calcium and phosphorus dissolution (a.e.s.)	s.e.m.: irradiated and non-irradiated areas; enamel rods exposure; more irregularities and cracks with higher energy densities and contact mode. a.e.s.: lased samples (lower energy densities, non-contact) < non-lased and lased with higher energy densities and contact mode
Kim et al., 2006	**n:** 7 **s:** human enamel **s.f.:** surface treatment **r.v.:** calcium and phosphorus dissolution (a.e.s.)	Lased < non-lased samples
Liu et al., 2006b	**n:** 21 **s:** human enamel **s.f.:** surface treatment **r.v.:** demineralization depth (p.l.m.)	100 and 200 mJ energy: less deep primary caries; 300 mJ ≅ non-lased samples
Castellan et al., 2007	**n:** 10 **s:** deciduous enamel **s.f.:** surface treatment **r.v.:** Knoop microhardness (converted to mineral volume percent)	Mineral loss: Non-lased > Nd:YAG ≅ Er:YAG ≅ fluoride Caries inhibition: -Fluoride: 59.4% -Nd:YAG: 40% -Er:YAG: 35.7%

n: number of samples
s: substrate
s.f.: study factor
r.v.: response variable
s.e.m.: scanning electron microscopy
a.a.s.: atomic absorption spectroscopy
p.l.m.: polarized light microscopy
c.l.s.m.: confocal laser scanning microscopy
a.e.s.: atomic emission spectroscopy

Table 3. *In vitro* demineralization protocols in Er:YAG laser researches.

Author	Demineralization protocol
Hossain et al., 2000	0.1 M lactic acid, 0.2 mM methylene diphosphonic acid (pH 4.8), and 0.01% thymol, for 24 h
Kameyama et al., 2000	0.1 M lactic buffer (pH 4.0), for 24 h, changing solution after 1, 3, 6 and 12 h
Ceballos et al., 2001	2.2 mM calcium, 2.2 mM phosphate, 50 mM acetic acid, 5.0 mg/L fluoride (pH 4.25), for 10 days
Apel et al., 2002a	0.1 mol/L acetate buffer (pH 4.5), for 24 h
Apel et al., 2003	2 mM calcium nitrate, 2 mM sodium dihydrogen phosphate, 0.0075 M sodium acetate, pH 4.3 (demineralizing solution) for 6 h; 20 mM sodium cacodylate, 0.9 mM sodium dihydrogen phosphate, 150 mM potassium chloride, 1.5 mM calcium nitrate, pH 7 (remineralizing solution) for 17 h, during 11 days
Delbem et al., 2003	2.0 mM calcium, 2.0 mM phosphate in 0.075 M acetate buffer, pH 4.3 (demineralizing solution) for 3 h; 1.5 mM calcium, 0.9 mM phosphate, 150 mM of KCL in 0.1 Tris buffer, pH 7.0 (remineralizing solution) for 21 h, during 10 days
Cecchini et al., 2005	0.1 mol/L acetate buffer (pH 4.5), for 8 h
Kim et al., 2006	0.1 M lactic acid, 0.2 mM methylene diphosphonic acid (pH 4.8), for 24 h
Liu et al., 2006b	0.05 M acetic acid, 2.2 mM calcium, 2.2 mM phosphate ions, pH 4.5 (demineralizing solution) for 18 h; 0.15 M potassium chloride, 1.5 mM calcium, 0.9 mM phosphate, pH 7.0 (remineralizing solution) for 6 h, during 2 days
Castellan et al., 2007	Proposed by Featherstone et al. (1986) and modified by Argenta et al. (2003). Demineralizing solution (pH 4.6) for 3 h; remineralizing solution (pH 7.0) for 21 h, during 5 days

3.2. CAPÍTULO 2

ARTIGO ACEITO PARA PUBLICAÇÃO NO PERIÓDICO *PHOTOMEDICINE AND LASER SURGERY* (ANEXO B)

Influence of Er:YAG laser on microhardness of enamel adjacent to restorations submitted to cariogenic challenge *in situ*

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ABSTRACT

Objective: This study assessed the effect of Er:YAG laser on enamel adjacent to restorations submitted to cariogenic challenge *in situ*, by microhardness analysis. *Background data:* The influence of Er:YAG laser on secondary caries formation was investigated *in vitro*. However, no research involving demineralization around cavities prepared with Er:YAG laser under intra-oral conditions has yet been described. *Methods:* Slabs of enamel were randomly assigned to 7 groups (n=12), according to the cavity preparation technique: I, II, III - Er:YAG laser with 250 mJ, combined with 2, 3 and 4 Hz, respectively; IV, V, VI - 350 mJ, with 2, 3 and 4 Hz, respectively; VII. High-speed handpiece (control). Cavities were restored, and specimens were fixed in intra-oral appliances, worn by 12 volunteers for 14 days. Sucrose solution was applied to each slab 6 times/day. Samples were removed, sectioned and observed for microhardness at 100, 200 and 300 µm (factor distance) and 30, 60 and 90 µm (factor depth), from restoration and enamel surface, respectively. *Results:* Analysis of Variance according to a Split-Plot model showed no difference among the cavity preparation techniques, among distances or among depths, as well as no difference in the interaction between the factors cavity preparation and distance. Significant difference was found in the interaction of cavity preparation and depth ($p<0.0001$), identified by Contrast Technique. *Conclusion:* Results revealed that Er:YAG laser did not differ from conventional cavity preparation, with regard to enamel microhardness.

INTRODUCTION

Although rotary cutting instruments are widely used, they present problems such as vibration, noise and the need for anesthesia, constituting the major cause of discomfort to patients.[1] With the aim of overcoming these limitations, new caries removal and cavity preparation techniques have been studied, such as irradiation with Er:YAG (Erbium:Yttrium-Aluminum-Garnet) laser.

In vitro[2-8] and *in vivo*[1,9-14] researches have reported the ability of Er:YAG laser to remove carious tissues and prepare cavities by thermo-mechanical ablation, which can cause morphological, structural and chemical changes in the irradiated surfaces. Alterations resulting from the thermal effect of laser irradiation might influence the resistance of lased substrates to the action of acids.[15] It has been speculated that the margins of cavities prepared with Er:YAG laser with ablative energy densities might present a certain resistance to the action of acids and thus to the development of caries around restorations.[16]

Morphologically, the surfaces of cavities prepared in enamel with Er:YAG laser, using ablative parameters, presented irregular[5,17-21] and microretentive[6,22] patterns. Fusion areas, varying in extent,[18,19,23] indicated enamel melting and recrystallization, which might present less permeability and higher acid resistance.[19] On the other hand, roughness and cracks on the surface could increase the area exposed to demineralization, acting as open channels for acids to reach the subsurface.[15,20] Heat-induced structural and chemical alterations to dental substrates, such as decrease in carbonate content and formation of pyrophosphate,[24-26] and protein decomposition, with organic blocking of diffusion pathways of caries process,[27,28] have been also proposed as possible theories for the increased acid resistance of irradiated substrates. Enamel irradiated with ablative parameters showed less release of calcium[19,29] and phosphorus[29] from surfaces submitted to demineralization.

Caries lesion is the result of a dynamic process of tooth structure de-remineralization, with increased mineral loss.[30] Secondary caries, defined as a primary caries lesion around restorations, still constitutes the major cause of their replacement.[31] The influence of Er:YAG laser on secondary caries formation was investigated *in vitro* with parameters that promoted

the surface treatment of bur-prepared cavities.[32] However, research involving demineralization around cavities prepared with Er:YAG laser under intra-oral conditions has not yet been described in literature. Therefore, the aim of the present study was to assess the effect of cavity preparation with Er:YAG laser on alterations in enamel adjacent to composite resin restorations submitted to high cariogenic challenge *in situ*, by means of microhardness analysis.

MATERIALS AND METHODS

Volunteers

This study was approved by the Ethics Committee of the Ribeirão Preto School of Dentistry, USP on 2004 December 3rd. Twelve volunteers (11 women and 1 man, aged 20-33 years), who filled inclusion and exclusion criteria, took part in this experiment after signing an informed, written consent (Resolution No. 196 of the National Health Council, Health Ministry, Brasília, DF, 10/03/1996).

Experimental Design

The factors under study were: cavity preparation technique at 7 levels (6 experimental groups and 1 control) (Table 1); distances from the occlusal wall of the cavity at 3 levels (100 μm, 200 μm and 300 μm); and depths from enamel surface at 3 levels (30 μm, 60 μm and 90 μm). Experimental units were composed of 84 human enamel slabs, randomly assigned to the 7 groups (n=12). The study was conducted in one period of 14 days, in which all the groups were submitted to the oral conditions of each volunteer, according to a randomized complete block design. The experiment was triple-blinded (volunteer, researcher and statistician did not know which group was being tested). The response variable was Knoop microhardness of enamel adjacent to composite resin restorations. Figure 1 illustrates each experimental phase.

Preparation of dental slabs

Unerupted third molars (from the Human Tooth Bank of Ribeirão Preto School of Dentistry-USP), kept in 0.1% thymol solution, were used in this study. From each tooth, 3 mm thick disks were obtained, using a diamond saw (Buehler, Lake Bluff, IL, USA) mounted in a sectioning machine (Isomet 1000, Buehler, Lake Bluff, IL, USA), at low speed and water cooled. Next, the disks were sectioned with double-faced diamond disks (KG Sorensen, Barueri, SP, Brazil) in a low-speed handpiece, flattened and polished with 1.200-grit silicon carbide papers (Hermes Abrasives Ltd., VA, USA) and 0.3-μ alumina paste (Arotec S/A Indústria e Comércio, São Paulo, SP, Brazil) on cloth (ATM, Altenkirchen, Germany), resulting in slabs measuring 5 x 3 x 2 mm. These were ultrasonically cleaned in distilled water for 15 min and inspected for surface defects, using a magnifying lens.

Samples were sterilized with ethylene oxide[33] (at the Ethylene Oxide Sterilization Department, Clinical Hospital, Ribeirão Preto School of Medicine, University of São Paulo) and the surface microhardness of the slabs was determined (Shimadzu Corporation, Kyoto, Japan) by 3 indentations on the flattened portion, 500 μm from the edges, under a 25-g load for 15 s. The three readings were averaged and used as the microhardness value of each slab. Specimens with microhardness values 20% above or below the mean value of all slabs were discarded. Next, samples were randomly assigned to the groups and stored in 0.5 mL of deionized water (pH ≈ 6.5) at 37°C for 24 h until cavity preparation procedures, in order to prevent the enamel from drying out.[34]

Cavity preparation

To demarcate the region to be prepared, a piece of insulating tape was attached to the slab surface, leaving an exposed area of 3 x 1 mm in the central portion of the specimen.

Cavity preparations were standardized by means of a device (Marcelo Nucci ME, São Carlos, SP, Brazil) that allowed the handpieces to be fixed, and the samples to be manually moved along the *x* and *z* axes. Samples movement was controlled by digital electronic

indicators (Mitutoyo Corp., Kawasaki, Japan), attached to the device. For groups prepared with the high-speed handpiece (Dabi Atlante, Ribeirão Preto, SP, Brazil), diamond burs No. 1332 (KG Sorensen, Barueri, SP, Brazil) were used, under air/water spray. The samples were moved along the *x* and *z* axes, resulting in cavities with 3 mm in length and 1 mm deep. For cavity preparations with laser, an Er:YAG laser (Kavo Key Laser II, Kavo Corp., Biberach, Germany) was used in non-contact and focused modes (handpiece 2051), at a working distance of 12 mm[35] and a beam focal area of 0.4 mm^2. The beam was perpendicular to the surface, and a constant water flow of 1.5 mL/min was used, similar to the method proposed by ARMENGOL et al.[36]. The samples were horizontally moved, only along the *x* axis, for a controlled period of 20 s, resulting in cavities with the same length (3 mm) but different depths (ranging from 0.5 to 1.0 mm), according to the laser parameter. Energies, pulse repetition rates and energy densities for each experimental group are shown in Table 1.

Restorative procedure

The resinous restorative system Single Bond Adper/Z250 (3M-ESPE, St. Paul, MN, USA), without fluoride in its composition, was used for filling the cavities, in accordance with the manufacturer's recommendations. Light polymerization was performed (XL-3000, 3M-ESPE, St. Paul, MN, USA) at a light intensity of around 500 mW/cm^2, periodically controlled with the aid of a radiometer. Samples were stored for 24 h in 100% relative humidity at 37^oC and then ground manually with 1.200-grit silicon carbide papers under water, to remove excess restorative material.[37] After that, surfaces were polished with Sof-Lex Pop-on disks (3M-ESPE, St. Paul, MN, USA) at low-speed, and specimens were kept in 100% relative humidity at 37^oC for a maximum period of 24 h until they were fixed to the intra-oral appliances.

Preparation of removable intra-oral appliances

Removable palatal intra-oral appliances were prepared in plaster models. Seven boxes, positioned bilaterally, were created in each appliance, where the slabs were fixed with wax. Group distribution was randomized, and a new randomization was performed for each volunteer. After being fixed, slabs were covered with nylon mesh, leaving a space of 1 mm between the slab surface and the mesh, to allow biofilm accumulation, as described by BENELLI et al.[38] and HARA et al.[39]

Intra-oral procedures

The selected volunteers were asked to use the dentifrice (Colgate Refrescância Confiável, Colgate-Palmolive, Osasco, SP, Brazil) toothbrush (Oral-B Indicator Plus, Gillette do Brasil Ltda., Manaus, AM, Brazil) and dental floss (Sorriso Kolynos, Colgate-Palmolive, Osasco, SP, Brazil) supplied by the researchers for 7 days, before the intra-oral phase began. The purpose of this lead-in period was to try to standardize intra-oral conditions.[40]

Volunteers wore the appliances for 14 consecutive days, keeping to the usual methods of oral hygiene, exclusively with the fluoride-containing dentifrice, toothbrush and dental floss supplied by the researchers. The volunteers were asked to remove the appliances only to eat, drink and perform the oral hygiene. A drop of 20% sucrose solution, prepared daily, was applied to each slab to simulate the situation of high cariogenic challenge,[39,41] followed by a 5-minute wait before replacing the appliance in the mouth.[39,41] This procedure was carried out 6 times per day[42] (8:00, 10:00, 12:00, 14:00, 16:00 and 18:00 h), during the 14-day period.[41,42]

Microhardness test

After the intra-oral phase, the slabs were removed from the palatal appliances, ultrasonically cleaned (3L, Dabi Atlante, Ribeirão Preto, SP, Brazil) for 15 min and sectioned in the cervical-occlusal direction in the central portion of restoration, using a diamond disc

mounted in the sectioning machine and water cooled. The sections were coded according to the groups and volunteers. One of the sections was embedded in polyester resin (Milflex Indústria Química Ltda., São Bernardo do Campo, SP, Brazil) so that the sectioned surface was exposed and could be polished. The samples were finished and polished with 1.200, 2.400 and 4.000-grit silicon carbide papers (Hermes Abrasives Ltd., VA, USA) in polishing machine (Buehler, Lake Bluff, IL, USA) under water cooling, and 0.3 μ alumina paste on cloth, respectively. Next, the resin cylinders containing the slabs were ultrasonically cleaned.

Knoop microhardness test was performed on the enamel portion adjacent to restorations. In each section, 9 indentations were made at the occlusal margin of the restoration, at 100, 200 and 300 μm from the cavity wall, and 30, 60 and 90 μm from the outer enamel surface towards amelodentinal junction. Microhardness analysis was performed with 10 g static load applied for 20 s, based on preliminary tests, with the long axis of the indenter being positioned parallel to the enamel surface.[43]

Statistical Analysis

Data were submitted to the Analysis of Variance (ANOVA) according to a split-plot design, considering the cavity preparation techniques, distances and depths as factors under study, and microhardness values as response variables. Analysis was performed by using the Stata program (StataCorp).

RESULTS

The results of ANOVA detected no differences among the cavity preparation techniques, distances or depths, considering these factors separately. There was also no difference in the interaction between the factors cavity preparation and distance. Significant difference was found in the interaction of the factors cavity preparation and depth ($p<0.0001$), which was identified by the Contrast Technique.[44] Table 2 shows the mean microhardness values at each depth, within each cavity preparation technique.

DISCUSSION

In situ experiments have been used to study caries development around restorations,[38,45,46] presenting advantages over *in vitro* researches due to the more reliable reproduction of intra-oral conditions,[47] particularly when assessing dental caries, an entity dependent on multiple factors.[48] Only two studies involving Er:YAG laser irradiation and tooth demineralization using an intra-oral model have been reported.[15,34] They differed from the present study in the length of the intra-oral period (1 week, instead of 2 weeks), the number of samples (3, instead of 12), and the intra-oral appliances (mandibular, instead of palatal). Another difference was the sub-ablative energy density used, whereas the parameters used in the present study were ablative. The study, whose response variable was quantitative, verified that Knoop surface microhardness of irradiated enamel was similar to that of the control group after the intra-oral period, although the surface microhardness of irradiated samples was lower, before the cariogenic challenge.[34] The qualitative analysis of enamel surface and sub-surface by confocal laser scanning microscopy showed that the irradiated samples were less demineralized on the surface than the controls, although the cracks resulting from irradiation suffered marked demineralization in the sub-surface.[15]

The quantitative response variable used in the present study was the Knoop microhardness of dental enamel in cross section, a method considered adequate for assessing alterations that occur in this substrate, because it presents correlation with the mineral volume of caries lesion.[43] Er:YAG laser used for cavity preparation in enamel did not influence microhardness values adjacent to composite resin restorations, irrespective of the parameters, in comparison with conventional cavity preparation. There was also no difference among the distances from the cavity wall, when this factor was considered separately. Possible alterations in the substrate during Er:YAG laser irradiation appeared to be superficial, to the order of only a few microns,[49] as a result of its high absorption by dental tissues and low penetration, without critical temperature increase in deeper layers.[2] In addition, the higher concentration of photons at the core of laser beam, decreasing at the edges,[50] suggests a more intense action of Er:YAG laser on the cavity floor than at its

margins. The absence of significant differences among the cavity preparation techniques and distances might be due to the point chosen for the initial indentation (100 μm from the cavity wall). The conditions presented in the enamel after the cariogenic challenge were such, that if points closer to the cavity wall were selected, the indentation would also involve part of the restoration, thus hampering the correct reading of microhardness, as verified in preliminary tests. The longest diagonal of the indentation was parallel to the enamel surface, as proposed by FEATHERSTONE et al.,[43] as perpendicular as possible to the c-axis of enamel rods. Cross-sectional microhardness was also used to verify *in vitro* the effect of Er:YAG laser on enamel surface demineralization,[49,51] with promising results for Er:YAG laser alone[51] and associated with fluoride.[49] However, both studies used sub-ablative energy densities to treat the surfaces and converted the microhardness values to percentage of mineral volume, thereby limiting comparison with the present study.

ANOVA did not detect difference among the depths from the outer enamel surface, when this factor was analyzed separately. However, the interaction between the factors cavity preparation technique and depth revealed differences among microhardness values at the different depths from the enamel surface, suggesting the formation of caries lesions at an early stage. Microhardness values, lower at 30 μm than at 90 μm, might have reflected mineral loss, showing that the cariogenic challenge protocol might have generated detectable alterations resulting from the carious process. Clinically, constant and high cariogenic challenge leads to gradual enamel dissolution, more pronounced in the sub-surface, with diffusion of acids within enamel, following the direction of the rods.[52]

The depth of demineralization around composite resin restorations after Er:YAG laser irradiation was quantitatively assessed by polarized light microscopy, with a reduction in the depth of lesions induced *in vitro* in enamel adjacent to cavities prepared with a high-speed handpiece, which had been conditioned superficially with Er:YAG laser and restored.[32] In the present study, groups I (250 mJ/2 Hz), V (350 mJ/3 Hz) and VI (350 mJ/4 Hz) presented significantly lower microhardness values at 30 μm than at 60 μm, suggesting the formation of

shallower caries lesions in comparison with the control group. However, this finding deserves further investigation.

With regard to microhardness of enamel adjacent to composite resin restorations submitted to high cariogenic challenge *in situ*, Er:YAG laser, used for cavity preparation with different parameters, presented a similar performance to that of the high-speed handpiece. This suggests that irradiation with Er:YAG laser could be a promising technique for cavity preparation, since it did not predispose the irradiated enamel to acid attack, in comparison with conventional cavity preparation. The reduction of bacteria levels in cavities prepared with Er:YAG laser[53] would be a favorable point to this tool, with possible improvement of restoration and tooth prognosis. However, other aspects, outside the scope of the present study, should be taken into account before indicating this device for clinical use.

CONCLUSION

Within the conditions of the present study, it can be concluded that Er:YAG laser, used for cavity preparation, did not differ from the conventional cavity preparation with regard to microhardness of enamel adjacent to composite resin restorations submitted to high cariogenic challenge *in situ*.

ACKNOWLEDGEMENTS

The authors are grateful to CAPES for the financial support, to all the volunteers for participating in this study, to Ms. Patricia Marchi for the help with laboratory activities, and to Mr. Reginaldo Santana for the help with laser irradiation. The authors also thank Mr. Nicholas John Bewick for the English language review.

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Table 1. Groups distribution, according to the cavity preparation techniques.

Group	Cavity preparation technique	Energy (mJ)	Pulse repetition rate (Hz)	Energy density (J/cm^2)
I	Er:YAG laser	250	2	62.5
II	Er:YAG laser	250	3	62.5
III	Er:YAG laser	250	4	62.5
IV	Er:YAG laser	350	2	87.5
V	Er:YAG laser	350	3	87.5
VI	Er:YAG laser	350	4	87.5
VII	High-speed handpiece	-	-	-

Table 2. Mean values and standard deviation of Knoop microhardness at each depth within each cavity preparation technique.

Preparation	Depth 30 μm	60 μm	90 μm
I (250 mJ/2 Hz)	265.5 (± 145.7) **a**	306.2 (± 117.5) **b**	330,6 (± 71.6) **b**
II (250 mJ/3 Hz)	287.5 (± 122.2) **a**	306.7 (± 100.2) **ab**	327,4 (± 77.3) **b**
III (250 mJ/4 Hz)	249.4 (± 151.3) **a**	295.6 (± 133.9) **ab**	313,2 (± 89.7) **b**
IV (350 mJ/2 Hz)	265.1 (± 138.1) **a**	289.5 (± 105.6) **a**	324,0 (± 83.8) **b**
V (350 mJ/3 Hz)	263.2 (± 111.6) **a**	298.6 (± 67.2) **b**	325,4 (± 68.4) **b**
VI (350 mJ/4 Hz)	245.7 (± 103.5) **a**	314.8 (± 107.8) **b**	330,9 (± 82.3) **b**
VII (control)	259.7 (± 125.4) **a**	281.8 (± 103.0) **ab**	303,3 (± 88.8) **b**

Same letters indicate statistical similarity (in each line).

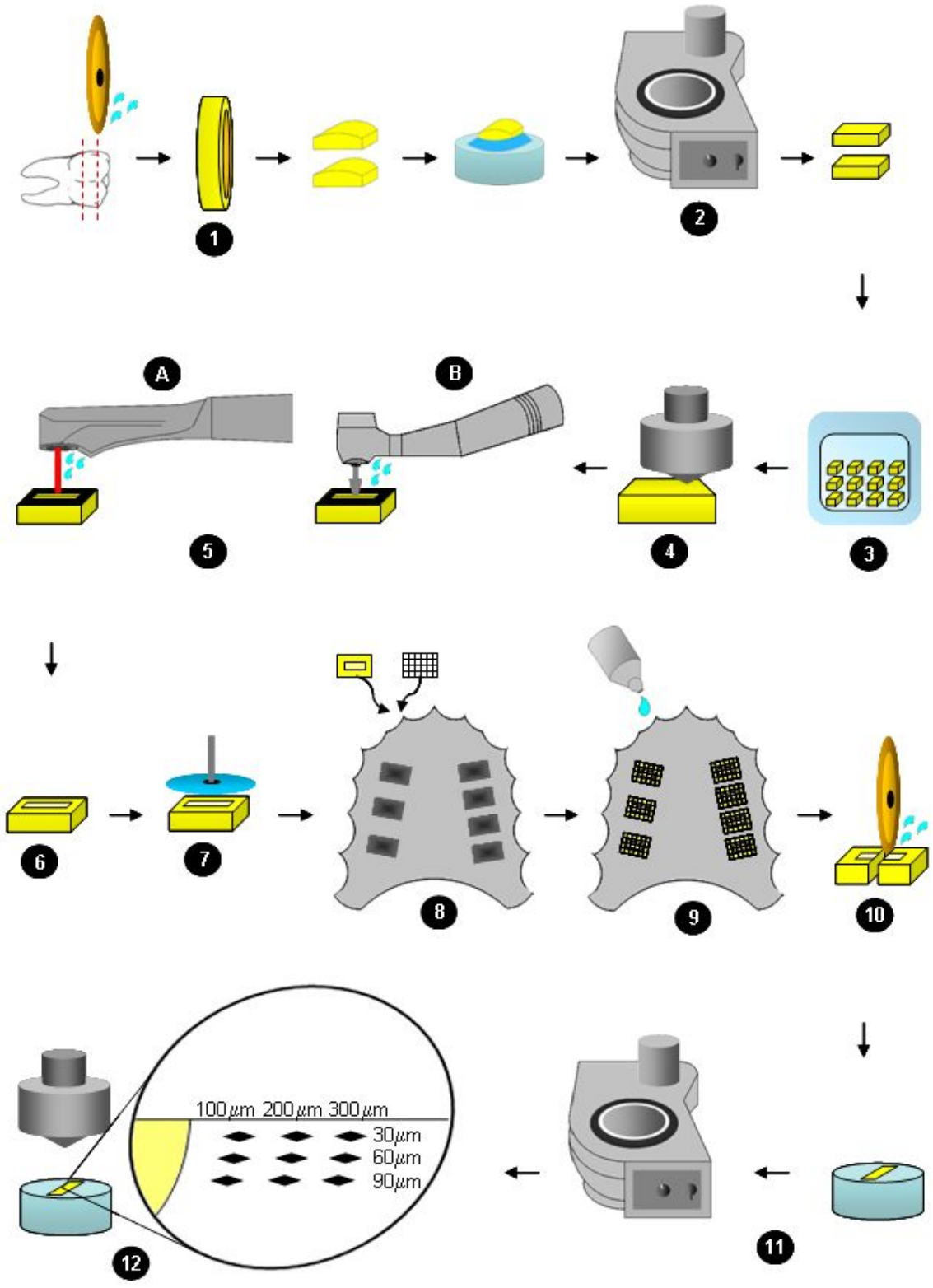

Figure 1. Experimental phases: 1 - Preparation of enamel slabs; 2 - Flattening and polishing; 3 - Sterilization with ethylene oxide; 4 - Selection of slabs by surface microhardness; 5 - Cavity preparations with Er:YAG laser (A) and high-speed handpiece (B); 6 - Restoration; 7 - Polishing; 8 - Fixing of slabs to intra-oral appliances; 9 - Intra-oral phase (application of 20% sucrose solution 6 times/day for 14 days); 10 - Sectioning of slabs; 11 - Inclusion in polyester resin, coding and polishing; 12 - Microhardness analysis (10 g, 20 s).

3.3. CAPÍTULO 3

ARTIGO SUBMETIDO À PUBLICAÇÃO NO PERIÓDICO *LASERS IN SURGERY AND MEDICINE* (ANEXO C)

Influence of cavity preparation with Er:YAG laser on enamel adjacent to restorations submitted to cariogenic challenge *in situ*: a polarized light microscopic analysis

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Key words: dental caries susceptibility dental cavity preparation, lasers, polarization microscopy, tooth demineralization.

ABSTRACT

Background and Objective: Er:YAG laser has been used for caries removal and cavity preparation, using ablative parameters. Its effect on the margins of restorations submitted to cariogenic challenge has not yet been sufficiently investigated. The aim of this study was to assess the enamel adjacent to restored Er:YAG laser-prepared cavities submitted to cariogenic challenge *in situ*, under polarized light microscopy.

Study design/Materials and Methods: Ninety-one enamel slabs were randomly assigned to 7 groups (n = 13): I, II, III - Er:YAG laser with 250 mJ, 62.5 J/cm^2, combined with 2, 3 and 4 Hz, respectively; IV, V, VI - Er:YAG laser with 350 mJ, 87.5 J/cm^2, combined with 2, 3 and 4 Hz, respectively; VII - High-speed handpiece (control). Cavities were restored and the restorations were polished. The slabs were fixed to intra-oral appliances, worn by 13 volunteers for 14 days. Sucrose solution was applied to each slab 6 times per day. Samples were removed, cleaned, sectioned and ground to polarized light microscopic analysis. Demineralized area and inhibition zone width were quantitatively assessed. Presence or absence of cracks was also analyzed. Scores for demineralization and inhibition zone were determined.

Results: No difference was found among the groups with regard to demineralized area, inhibition zone width, presence or absence of cracks, and demineralization score. Inhibition zone score showed difference among the groups. There was a correlation between the quantitative measures and the scores.

Conclusions: Er:YAG laser was similar to high-speed handpiece, with regard to alterations in enamel adjacent to restorations submitted to cariogenic challenge *in situ*. The inhibition zone score might suggest less demineralization at the restoration margin of the irradiated substrates. Correlation between the quantitative measures and scores indicates that scores could be an alternative to the quantitative assessment of caries lesion around restorations, under polarized light microscopy.

INTRODUCTION

The Er:YAG (Erbium:Yttrium-Aluminum-Garnet) laser, used with ablative parameters, has been widely investigated *in vitro* [1-9] and *in vivo* [10-16] for caries removal and cavity preparation, trying to overcome some limitations of rotary cutting instruments, such as vibration, noise, and the need for anesthesia [10]. Cavity preparation with Er:YAG laser could provide some benefits, such as less pain and discomfort [10,15,16] and the reduction of bacteria levels in cavities, with possible improvement of restoration and tooth prognosis [17].

The action mechanism of Er:YAG laser consists on thermo-mechanical ablation that removes dental tissues by microexplosions and generates a certain degree of heat in the most superficial layers [1]. The thermal effect of laser irradiation may cause morphological, structural and chemical alterations, which could influence the acid resistance of lased substrates [18], including the margins of Er:YAG laser-prepared cavities, using ablative energy densities [19].

Roughness and cracks observed on the irradiated surfaces might increase the area exposed to demineralization and act as open channels for acids to reach the subsurface [18,20]. The presence of fused areas [21-23], due probably to enamel melting and recrystallization, could result in a less permeable substrate to acid diffusion [22]. The increased acid resistance of the irradiated tissues has been also ascribed to the alterations induced by heat in structural and chemical levels, such as the decrease in carbonate content and formation of pyrophosphate [24-26], and protein decomposition located in the intra and inter-prismatic spaces, blocking the diffusion pathways of caries process [27,28].

The dynamic process of tooth de-remineralization, with a higher degree of mineral loss, may lead to caries lesion formation [29]. Secondary caries is a primary caries lesion around restorations, being the major cause of their replacement [30]. Caries formation around bur-prepared cavities, which were superficially conditioned with Er:YAG laser, was studied *in vitro* by means of polarized light microscopy [31], which is capable of detecting demineralization at an early stage [32] by the altered sign of birefringence of the

demineralized substrate [33]. However, no research involving polarized light microscopic evaluation of *in situ* demineralization around Er:YAG laser-prepared cavities has yet been described. Therefore, the aim of the present study was to assess the effect of cavity preparation with Er:YAG laser on alterations in enamel adjacent to composite resin restorations submitted to high cariogenic challenge *in situ*, by means of polarized light microscopy.

MATERIALS AND METHODS

Ethical aspects

The present study was approved by the Ethics Committee of Ribeirão Preto School of Dentistry, University of São Paulo. Thirteen volunteers (12 women and 1 man, aged 20-35 years) enrolled in this research, after an informed, written consent had been signed, according to the Resolution No. 196 of the National Health Council from Brazil.

Experimental Design

The factor under study was the cavity preparation technique at 7 levels (6 experimental groups and 1 control) (Table 1), and the experimental units were composed of 91 human enamel slabs, randomly assigned to the 7 groups (n=13). The intra-oral period lasted 14 consecutive days, in which all the groups were submitted to the oral conditions of each volunteer, according to a randomized complete block design. Volunteers, researcher and statistician did not know which group was being tested, characterizing a triple-blind experiment. The response variables were: the quantitative measurement of the demineralized area (μm^2) and the inhibition zone width (μm) adjacent to the restorations, the qualitative assessment of the presence or absence of cracks, and the qualitative analysis of demineralization and inhibition zone by scores determination. The experimental phases are illustrated in Figure 1.

Dental slabs preparation

Unerupted third molars (from the Human Tooth Bank of Ribeirão Preto School of Dentistry, University of São Paulo), stored in 0.1% thymol solution, were sectioned using a diamond saw (Buehler, Lake Bluff, IL, USA) mounted in a sectioning machine (Isomet 1000, Buehler, Lake Bluff, IL, USA), at low speed and water cooled, obtaining three-millimeters thick disks from each tooth. These were sectioned with double-faced diamond disks (KG Sorensen, Barueri, SP, Brazil) in a low-speed handpiece, flattened and polished with 1200-grit silicon carbide papers (Hermes Abrasives Ltd., VA, USA) and 0.3-μ alumina paste (Arotec S/A Indústria e Comércio, São Paulo, SP, Brazil) on cloth (ATM, Altenkirchen, Germany). The slabs, measuring 5 x 3 x 2 mm, were ultrasonically cleaned in distilled water for 15 min and viewed under a magnifying lens for surface defects.

Slabs sterilization with ethylene oxide was accomplished [34], and the surface microhardness was determined for each sample by 3 indentations on the flattened portion, 500 μm from the edges, under a 25-g load for 15 s (Shimadzu Corporation, Kyoto, Japan). The three readings were averaged and the specimens with microhardness values 20% above or below the mean value of all slabs were discarded. Samples were randomly assigned to the seven groups and stored in 0.5 mL of deionized water (pH ≈ 6.5) at 37°C for 24 h until cavity preparation procedures, in order to avoid the enamel desiccation [35].

Cavity preparation

A piece of insulating tape was attached to the slab surface to demarcate the region to be prepared, leaving an exposed area of 3 x 1 mm in the central portion of the specimen.

A special device (Marcelo Nucci ME, São Carlos, SP, Brazil) was used to standardize all the cavity preparations. Both the Er:YAG laser and high-speed handpieces were fixed, whereas the samples could be manually moved along the *x, y* and *z* axes, controlled by digital electronic indicators (Mitutoyo Corp., Kawasaki, Japan) attached to the device. Diamond burs No. 1332 (KG Sorensen, Barueri, SP, Brazil), mounted in a high-speed

handpiece (Dabi Atlante, Ribeirão Preto, SP, Brazil), were used to perform the conventional cavity preparations, under air/water spray. The samples were moved along the *x* and *z* axes, resulting in cavities with 3 mm in length and 1 mm deep. For the experimental cavity preparations, an Er:YAG laser (Kavo Key Laser II, Kavo Corp., Biberach, Germany), emitting light with a wavelength of λ=2.94 μm, was used. The handpiece 2051 was positioned in non-contact and focused modes, 12 mm from the target tissue [36]. The beam, which was perpendicular to the surface, presented a focal area of 0.4 mm^2. A constant water flow of 1.5 mL/min was used during the irradiation, similar to that reported by Armengol et al. [37]. The samples were moved horizontally, only along the *x* axis, for a controlled period of 20 s, resulting in cavities with the same length (3 mm) but different depths (ranging from 0.5 to 1.0 mm), depending on the laser parameter. Table 1 shows the energies, pulse repetition rates and energy densities used in each experimental group.

Cavity restoration

The cavities were filled with the restorative system Single Bond Adper/Z250 (3M-ESPE, St. Paul, MN, USA), presenting no fluoride in its composition, following the manufacturer's recommendations. Light polymerization was performed (XL-3000, 3M-ESPE, St. Paul, MN, USA) at a light intensity of ~ 500 mW/cm^2, periodically monitored with the aid of a radiometer. After being stored for 24 h in 100% relative humidity at 37^oC, the samples were manually ground with 1200-grit silicon carbide papers cooled with water, to remove excess restorative material [38], and polished with Sof-Lex Pop-on disks (3M-ESPE, St. Paul, MN, USA) at low-speed. The specimens were kept in 100% relative humidity at 37^oC for a maximum period of 24 h until they were fixed to the intra-oral appliances.

Removable intra-oral appliances

Removable palatal intra-oral appliances were prepared in plaster models, where seven boxes, bilaterally positioned, were created to allow the fixing of slabs. The groups

were randomly distributed to the retentive boxes, and a new randomization was performed for each volunteer. After being fixed with wax, the slabs were covered with nylon mesh, and a space of 1 mm was left between the slab surface and the mesh, to allow biofilm accumulation [39,40].

Intra-oral phase

Before the intra-oral phase began, the volunteers were submitted to a 7-day lead in period, in which they were asked to use the fluoride-containing dentifrice (Colgate Refrescância Confiável, Colgate-Palmolive, Osasco, SP, Brazil) toothbrush (Oral-B Indicator Plus, Gillette do Brasil Ltda., Manaus, AM, Brazil) and dental floss (Sorriso Kolynos, Colgate-Palmolive, Osasco, SP, Brazil) supplied by the researchers, trying to standardize the intra-oral conditions [41].

The volunteers wore the appliances for 14 consecutive days [42,43], keeping to the usual methods of oral hygiene, using only the dentifrice, toothbrush and dental floss supplied by the researchers. They were asked to remove the appliances only to eat, drink and perform the oral hygiene. A drop of 20% sucrose solution [40,42], daily prepared, was applied to each slab 6 times per day [43] (8:00, 10:00, 12:00, 14:00, 16:00 and 18:00 h), followed by a 5-minute wait before replacing the appliance in the mouth [40,42], to simulate the situation of high cariogenic challenge.

Polarized light microscopic analysis

The slabs were removed from the intra-oral appliances after the 14-day period, ultrasonically cleaned (3L, Dabi Atlante, Ribeirão Preto, SP, Brazil) for 15 min, and sectioned in the cervical-occlusal direction in the central portion of the restoration, using a diamond disc mounted in the sectioning machine and water cooled. One of the halves was coded according to the groups and volunteers, embedded in polyester resin (Milflex Indústria Química Ltda., São Bernardo do Campo, SP, Brazil) and sectioned in the cervical-occlusal

direction, using a diamond saw (Buehler, Lake Bluff, IL, USA), in order to obtain one section of ~ 600 μm thick, reduced to a thickness of 100 μm ± 20 μm [44-46], by hand, with 1200-grit silicon carbide papers (Hermes Abrasives Ltd., VA, USA), cooled with water.

The sections were soaked in 0.5 mL of deionized water for 24 h [33,38,44,47], mounted in microscope glass slides and sealed with cover glass slips. The microscopic analysis was performed by means of a polarized light microscope (Leica DMLB, Leica Microsystems), with the polarizer and analyzer being crossed, without using any compensator. The specimens were positioned so that they presented maximum brightness. The images were viewed under x20 and x40 objective lenses, and transferred to a computer by means of a digital camera (Leica DC 300F, Leica Microsystems), and a software (IM50, Leica Microsystems). The images under the x20 objective lens were used to the assessment of the demineralized area (μm^2), the presence or absence of cracks, and the demineralization and inhibition zone scores. The inhibition zone width (μm) was analyzed under the x40 objective lens.

Quantitative assessment of demineralized area and inhibition zone width, and qualitative evaluation of presence or absence of cracks

An area of 20,000 μm^2 (100 μm width [38,48] and 200 μm high) adjacent to the restorations was determined by means of the AxioVision 3.1 software (Carl Zeiss, Inc.). The demineralized area within these limits was measured by using the same software. The inhibition zone was characterized by a zone of non-demineralized enamel, between the restoration margin and the limit of the caries lesion, linearly measured (μm) (AxioVision 3.1 software). The presence or absence of enamel cracks adjacent to the restorations was qualitatively analyzed. The assessment was performed by one examiner, blinded to the groups.

Scores of demineralization and inhibition zone

The images were coded and randomly inserted into the Power Point program. A presentation was created, with the images presenting the same size and time for being viewed (30 s). Three examiners (all dentists and graduate students in Dentistry), blinded to the groups, analyzed the images, after a single calibration session to acquaint them with the scores criteria, by showing two images corresponding to each score, which would not be included in the examination. The presentation was exhibited in a dark room for the three examiners, at the same time. They were asked to perform the analysis independently, with no communication, and give scores to the demineralization and inhibition zone, according to the criteria:

Demineralization:

0 - Complete absence of demineralization;

1 - Slight or moderate demineralization;

2 - Intense demineralization, with presence of lesion body.

Inhibition zone:

0 - Absence of inhibition zone, due to the absence of demineralization;

1 - Absence of inhibition zone (when demineralization was in contact with the restoration);

2 - Presence of an inhibition zone.

Statistical analysis

The statistical analysis was performed by a randomized complete block design, considering the cavity preparation techniques as the factor under study. The response variables were the demineralized area and inhibition zone width, analyzed by nonparametric Friedman test; the dichotomy presence/absence of cracks in each experimental unit, analyzed by logistic model, using odds-ratio for pairwise comparison of groups; and the frequency distributions of scores of demineralization and inhibition zone, evaluated by the Fisher test, using the median of scores from triplicates (3 examiners). The inter-examiner reproducibility was tested by Kappa coefficient. The correlation between the demineralized

area and inhibition zone width and their respective scores was analyzed by the Spearman rank correlation coefficients.

RESULTS

The Friedman test showed no significant differences among the cavity preparation techniques, with regard to demineralized area (χ^2 = 9.3; *p-value* = 0.1583) and inhibition zone width (χ^2= 7.2; *p-value* = 0.3077) (Table 2 and Figure 2). There were not significant differences among the cavity preparation techniques for cracks occurrence (OR = 0.966; *p-value* = 0.747) (Figure 3). Fisher exact test did not show significant differences among the groups, with regard to the demineralization score (*p-value* = 0.432) (Figure 4a). The statistical evaluation of inhibition zone score showed significant differences among the cavity preparation techniques (*p-value* = 0.022) (Figure 4b); by excluding the control group from the dataset, the result of Fisher exact test was not significant (*p-value* = 0.238). The concordance evaluation by using Kappa coefficient showed two “moderate” (0.57 and 0.59) and one “substantial” (0.66) inter-examiner agreement for demineralization scores; for inhibition zone scores, the inter-examiner agreement was “substantial” (0.63, 0.62 and 0.71), according to the Landis & Koch criteria [49]. There was a significant correlation between demineralized area and demineralization score (ρ = 0.6982; *p-value* < 0.0001), and inhibition zone width and inhibition zone score (ρ = 0.5938; *p-value* < 0.0001). Figure 5 shows the representative photomicrographs of each studied group, under polarized light microscopy.

DISCUSSION

Caries development around restorations was induced by a high cariogenic challenge *in situ*, similar to the method proposed by other studies [39,50,51], with the aim of reproducing more reliably the intra-oral conditions [52]. In Er:YAG laser research, only two studies involving an intra-oral model and tooth demineralization could be found in literature [18,35]. Both studies presented some differences from the present research, since they were

conducted in one intra-oral period of one week, with a different sample size (n = 3), irradiated with a sub-ablative energy density and fixed to mandibular appliances. They used quantitative [35] and qualitative [18] response variables, which were, respectively, the Knoop surface microhardness of enamel [35], and the enamel surface and sub-surface analysis by confocal laser scanning microscopy [18].

The quantitative response variables used in the present study were the demineralized area and the inhibition zone width, observed under polarized light microscopy, a technique considered adequate for assessing alterations in enamel [31,32,47,53,54] and dentin [31-33,38,44,47,48,53,55] around restorations submitted to high cariogenic challenge. In this technique, a polarized light passes through a birefringent sample, resulting in polarized light waves with different refractive indexes, depending on the material [56]. Differences in the birefringence of sound and demineralized tissues allow delimiting the caries lesion and measuring its depth [20,31,33,47,53,54,55,57] or area [38,48,58], which results in a more detailed analysis of the caries lesion as a whole.

In the present study, Er:YAG laser, used for cavity preparation in enamel, did not influence the demineralized area or the inhibition zone width adjacent to composite resin restorations, irrespective of the parameters, in comparison with the conventional cavity preparation. The superficial action of Er:YAG laser, due to the high absorption of its wavelength by dental substrates [1], could justify the similarity among the tested groups. Polarized light microscopy was also used to verify the effect of Er:YAG laser on enamel surface demineralization, with promising results for Er:YAG laser with 100 and 200 mJ output energies (12.7 and 25.5 J/cm^2 energy densities, respectively) [58]. The comparison with the present research is limited by the use of parameters for surface treatment (instead of cavity preparation), and the cariogenic challenge protocol (*in vitro*, instead of *in situ*). The demineralization depth in enamel cavities prepared with Er:YAG laser was analyzed by polarized light microscopy, with unfavorable results for Er:YAG laser [20]. However, only unrestored cavities were assessed after an *in vitro* cariogenic challenge, thereby limiting comparison with the present research. Only one study assessed the demineralization around

cavities after Er:YAG laser irradiation by means of polarized light microscopy, showing a lesion depth reduction in enamel and dentin adjacent to bur-prepared cavities, which had been superficially conditioned with Er:YAG laser and restored [31]. It differed from the present research in the application mode of the Er:YAG laser (for cavity pretreatment, instead of cavity preparation) and the cariogenic challenge protocol (*in vitro*, instead of *in situ*).

The cavity preparation techniques showed similar proportion of enamel cracks adjacent to the restoration margin, although the control group has presented the lowest percentage of cracks. Usually, the cracks were at a short distance from the restoration, running from the enamel surface towards the amelodentinal junction, but their real cause remains not well-understood. Cracks formation might be ascribed to the laser irradiation itself [20], or to fractures produced by composite resin shrinkage during its polymerization [59]. In the present study, the occurrence of cracks did not influence the demineralized area in enamel.

There was no difference among the cavity preparation techniques, with regard to the scores attributed to demineralization, corroborating the results of the quantitative analysis. On the other hand, the results of the inhibition zone scores showed a significant difference among the groups, which was ascribed to the control group, with 7.7% of score 0 (absence of inhibition zone due to the absence of demineralization), 92.3% of score 1 (absence of inhibition zone), and 0% of score 2 (presence of inhibition zone), whereas no difference was found among the Er:YAG laser-prepared groups. The presence of inhibition zone in some samples of the irradiated groups could suggest less demineralization at the restoration margin, according to the score analysis. However, this finding deserves further investigation.

The significant correlation between the quantitative variables (demineralized area and inhibition zone width) and their respective categorized variables (demineralization score and inhibition zone score), when analyzed by polarized light microscopy, suggests that the assessment of caries lesion around restorations by scores should be a suitable alternative to the quantitative measures, with the advantage of being a simpler and faster method.

Er:YAG laser, used for cavity preparation with different parameters, presented similar performance to that of the high-speed handpiece, with regard to demineralized area, inhibition zone width, cracks occurrence, and demineralization score in enamel adjacent to composite resin restorations submitted to high cariogenic challenge *in situ*. The score analysis of the inhibition zone could suggest a lower degree of demineralization at the restoration margin of the irradiated samples. However, this result seems to be inconclusive and further studies on this aspect are required. Within the conditions of the present study, it could be suggested that Er:YAG laser irradiation might be a promising technique for cavity preparation, since it did not predispose the irradiated enamel to the action of acids from the biofilm formed *in situ*, in comparison with the conventional technique. However, other aspects of the irradiated tissues should be considered before indicating this device for clinical use.

CONCLUSION

Within the conditions of the present study, it was feasible to concluded that, in general, Er:YAG laser used for cavity preparation did not differ from the conventional cavity preparation, with regard to the alterations in enamel adjacent to composite resin restorations submitted to high cariogenic challenge *in situ*, under polarized light microscopy. The results of inhibition zone score might suggest less demineralization at the margins of Er:YAG laser-prepared groups. The correlation between the quantitative measures and their corresponding scores, analyzed under polarized light microscopy, indicates that scores determination could be an alternative to the quantitative assessment of caries lesion around restorations.

ACKNOWLEDGEMENTS

The authors are grateful to all the volunteers for participating in this study, and to the examiners who assessed the images. The authors also thank Ms. Patricia Marchi for the help with laboratory activities, Mr. Reginaldo Santana da Silva for the help with laser irradiation, and the Ethylene Oxide Sterilization Department from the Clinical Hospital of Ribeirão Preto

School of Medicine (University of São Paulo) for the slabs sterilization. The authors are indebted to Prof. Dr. Luiz Antonio Salata for allowing the use of the polarized light microscope, Prof. Dr. Raquel Fernanda Gerlach for the help with the microscope use, and Mr. Nicholas John Bewick for the English language review.

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TABLE 1. Groups distribution, according to the cavity preparation techniques

Group	Cavity preparation technique	Energy (mJ)	Pulse repetition rate (Hz)	Energy density (J/cm^2)
I	Er:YAG laser	250	2	62.5
II	Er:YAG laser	250	3	62.5
III	Er:YAG laser	250	4	62.5
IV	Er:YAG laser	350	2	87.5
V	Er:YAG laser	350	3	87.5
VI	Er:YAG laser	350	4	87.5
VII	High-speed handpiece	-	-	-

TABLE 2. Medians of demineralized area (μm^2) and inhibition zone width (μm) by cavity preparation techniques

Group	Demineralized area (μm^2) [a]		Inhibition zone width (μm) [b]	
	Median	n	Median	n
I	4371.7	13	0.0	13
II	2960.1	13	0.0	11
III	5933.2	13	2.2	12
IV	4883.4	13	0.0	11
V	5573.5	13	0.0	13
VI	6709.5	13	0.0	12
VII	6413.5	13	0.0	13

[a] Friedman test: $\chi^2 = 9.3$; *p-value* = 0.1583
[b] Friedman test: $\chi^2 = 7.2$; *p-value* = 0.3077

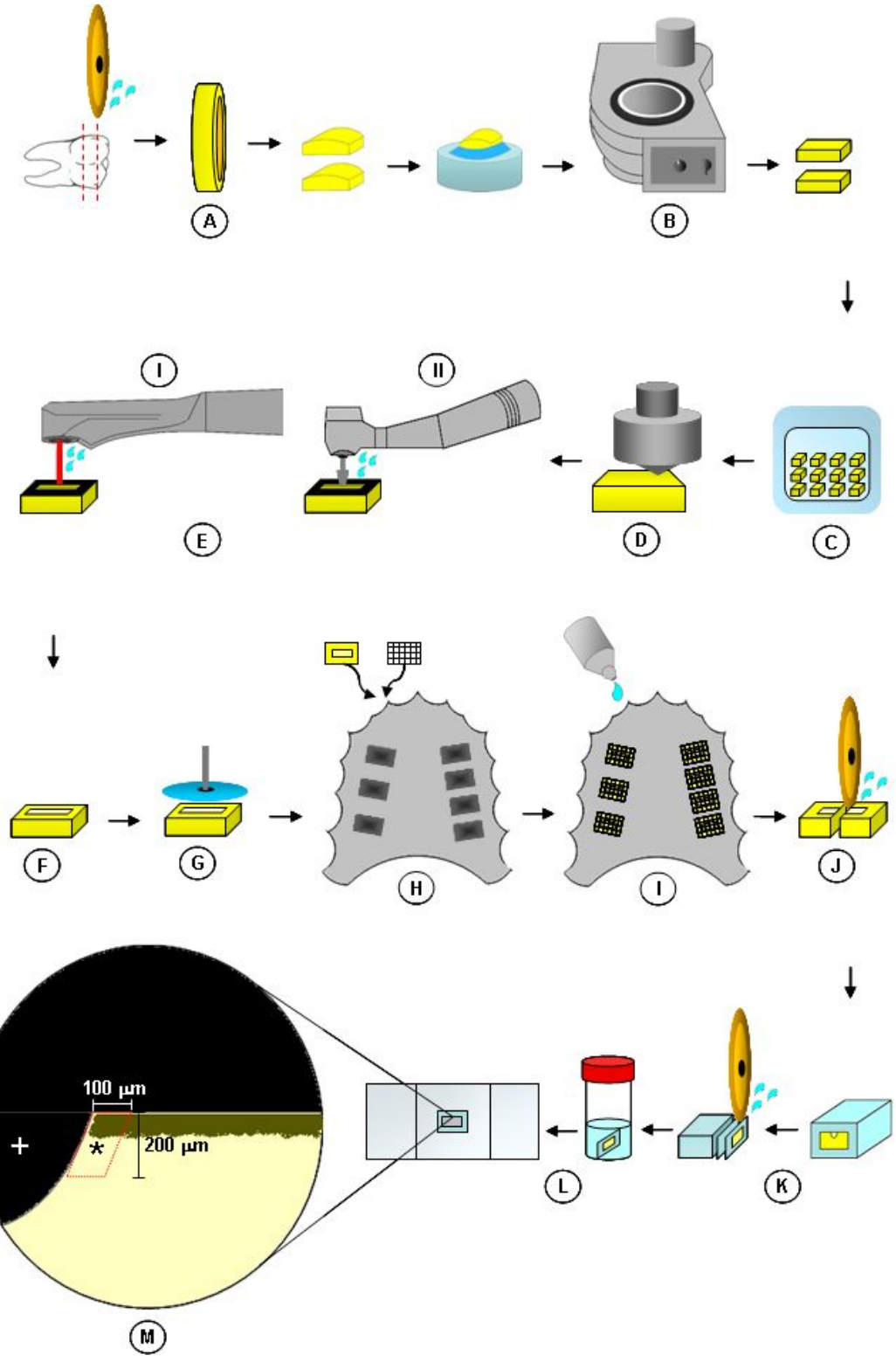
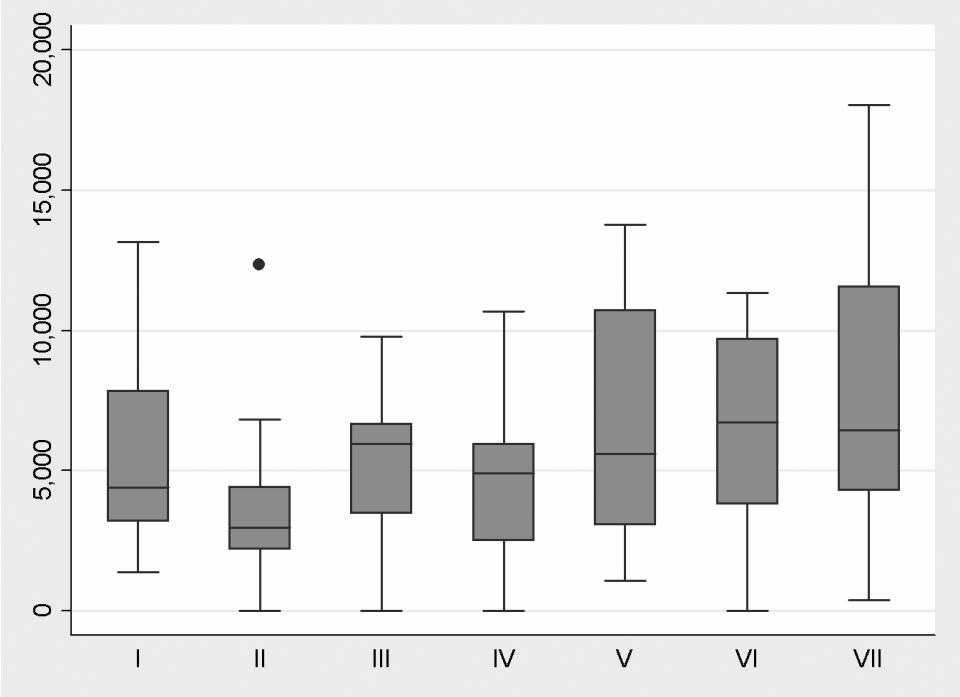


Fig. 1. Experimental phases. **A:** Preparation of enamel slabs; **B:** Flattening and polishing; **C:** Sterilization with ethylene oxide; **D:** Selection of slabs by surface microhardness; **E:** Cavity preparations with Er:YAG laser (I) and high-speed handpiece (II); **F:** Restoration; **G:** Polishing; **H:** Fixing of slabs to intra-oral appliances; **I:** Intra-oral phase (application of 20% sucrose solution 6 times/day for 14 days); **J:** Sectioning of slabs; **K:** Inclusion in polyester resin, sectioning and grounding; **L:** Soaking in deionized water and mounting in glass slides; **M:** Polarized light microscopic analysis for assessment of demineralized area, inhibition zone width, presence or absence of cracks, scores of demineralization and inhibition zone; *****: Schematic representation of the region delimited to the analysis of demineralized area; **+**: Restoration.

(a)



(b)

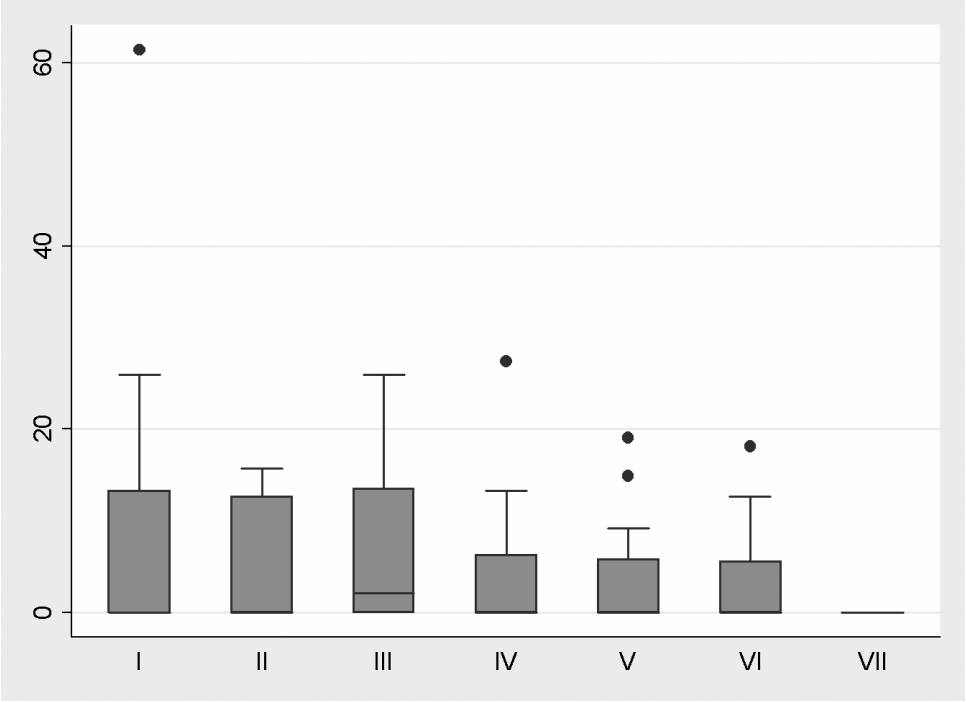


Fig. 2. Box-plot by cavity preparation techniques using demineralized area (μm^2) (a), and inhibition zone width (μm) (b).

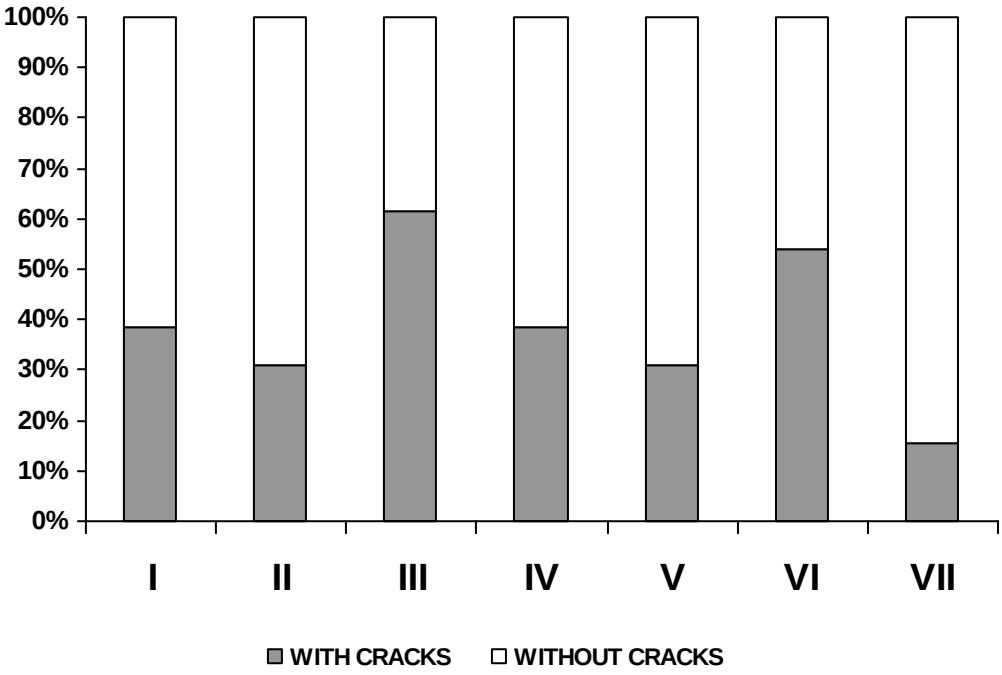
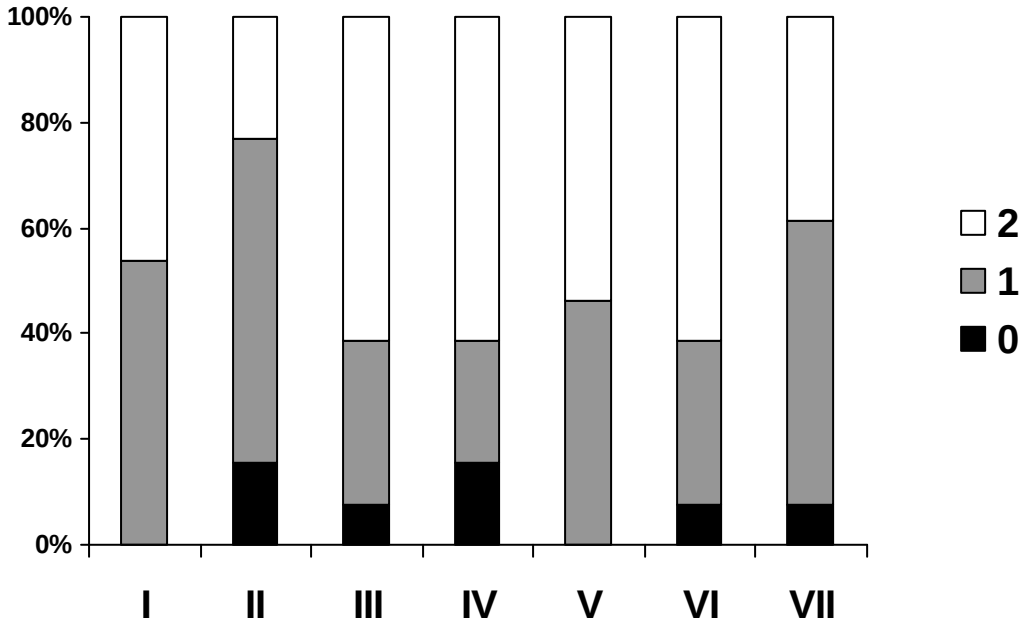


Fig. 3. Proportional distribution of cracks, in each group.

(a)



(b)

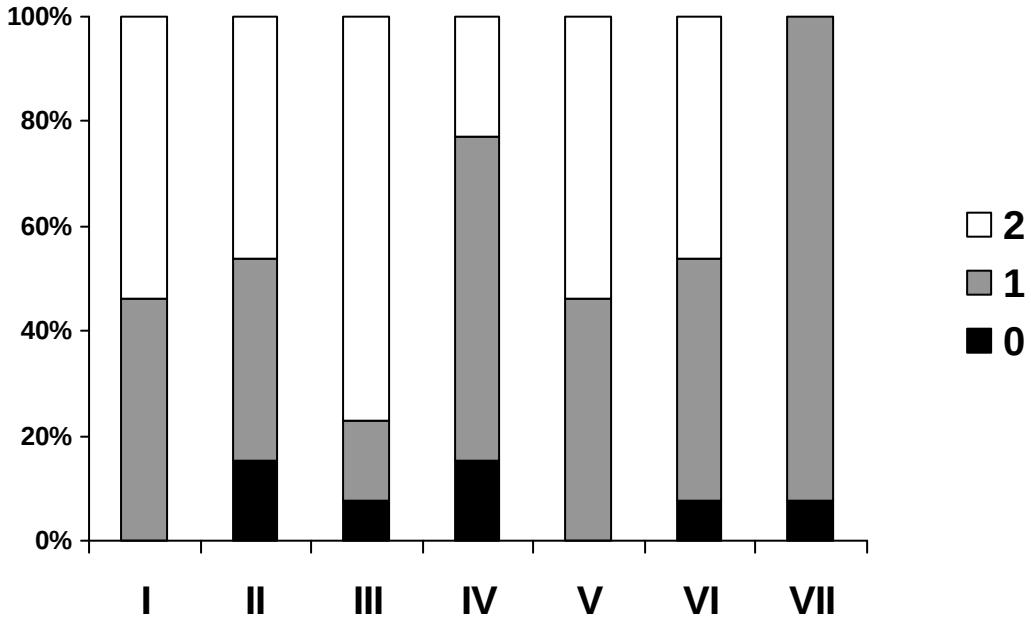


Fig. 4. Proportional distribution of demineralization scores (a), and inhibition zone scores (b), in each group.

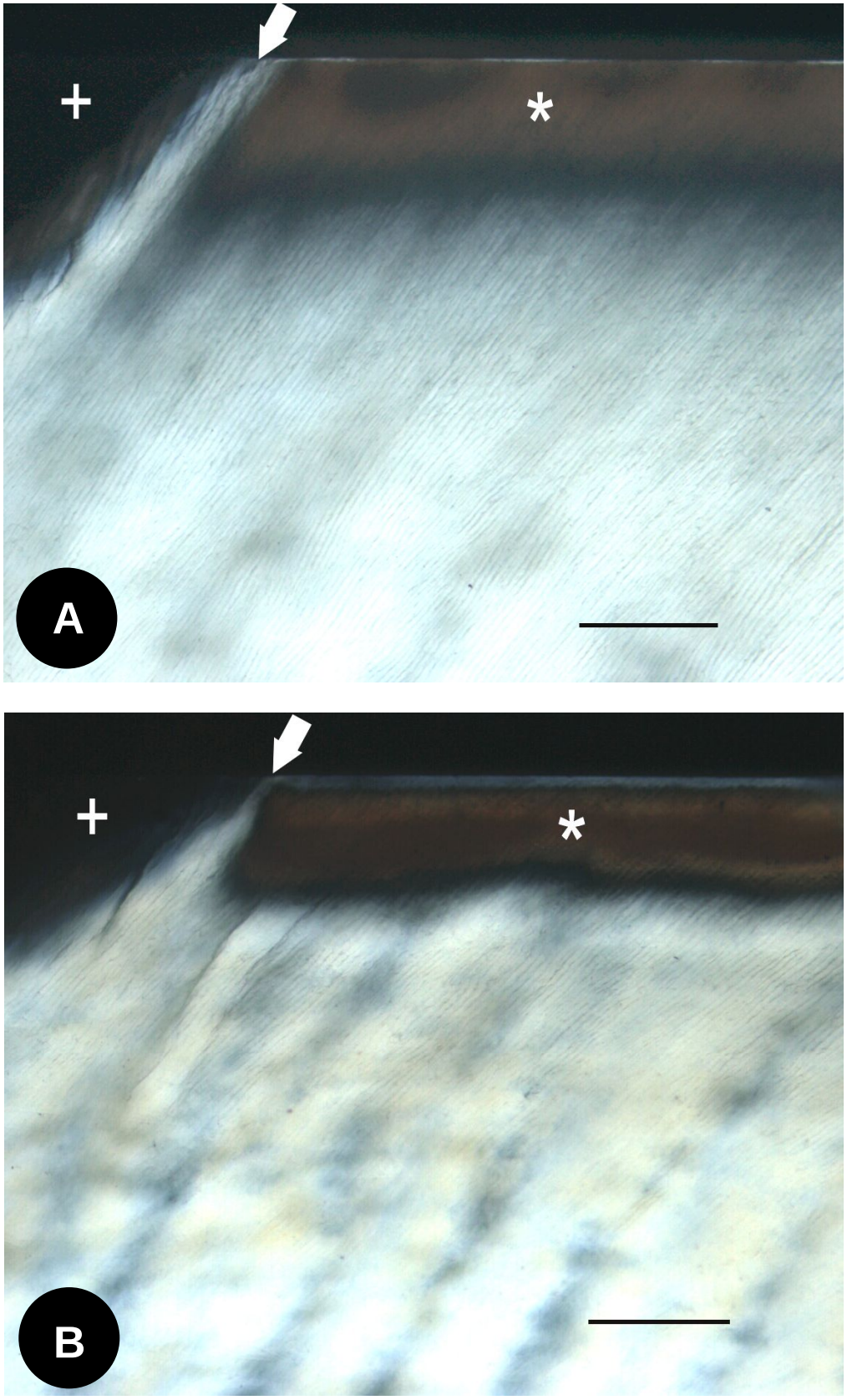


Fig. 5. Representative photomicrographs of the different cavity preparation techniques, under polarized light microscopy. **A:** Er:YAG laser with 250 mJ/2 Hz; **B:** 250 mJ/3 Hz. Restoration (+); Demineralization (*); Inhibition zone (arrow). x20. Bar represents 100 μm.

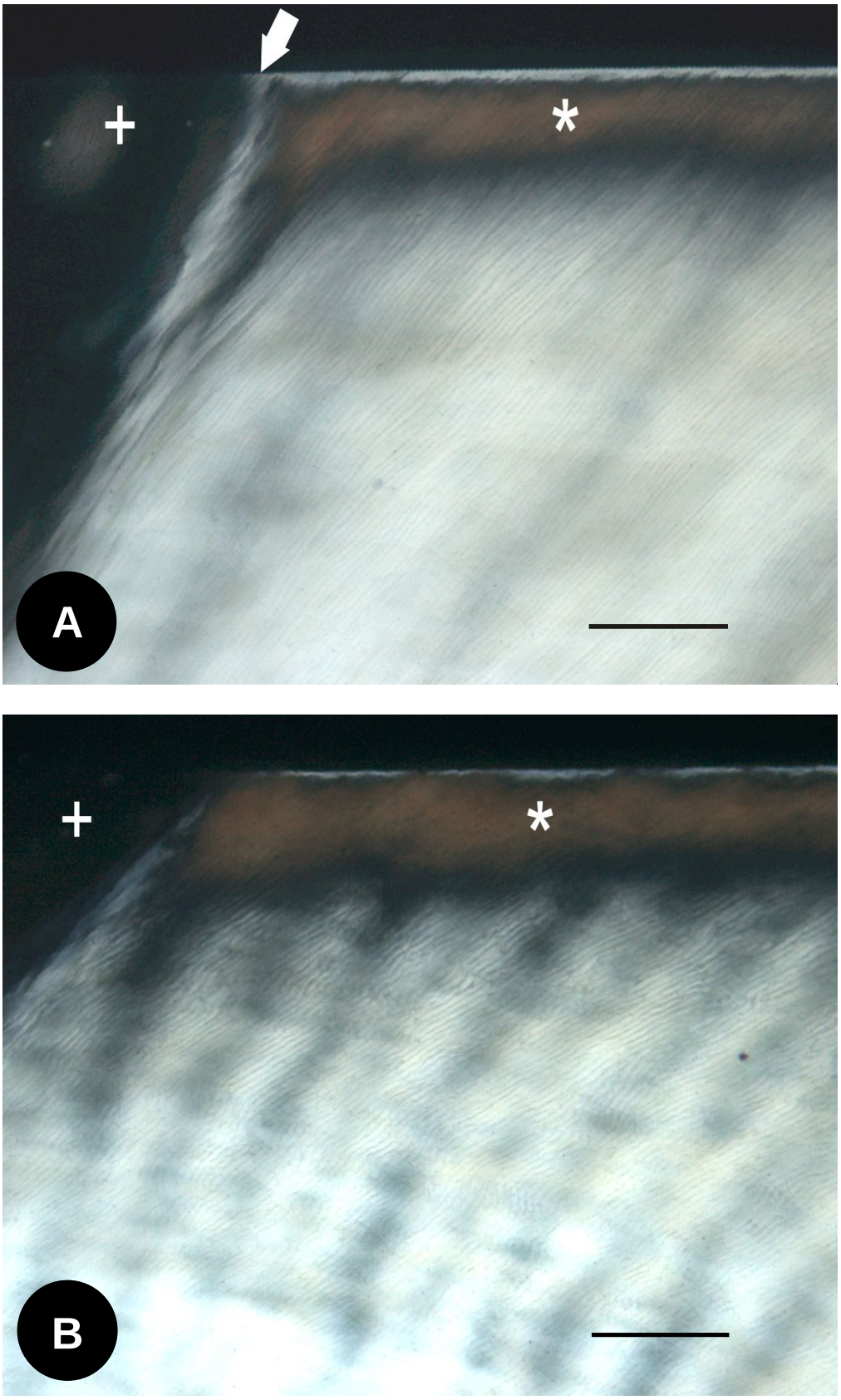


Fig. 6. Representative photomicrographs of the different cavity preparation techniques, under polarized light microscopy. **A:** Er:YAG laser with 250 mJ/4 Hz; **B:** 350 mJ/2 Hz. Restoration (+); Demineralization (*); Inhibition zone (arrow). x20. Bar represents 100 μm.

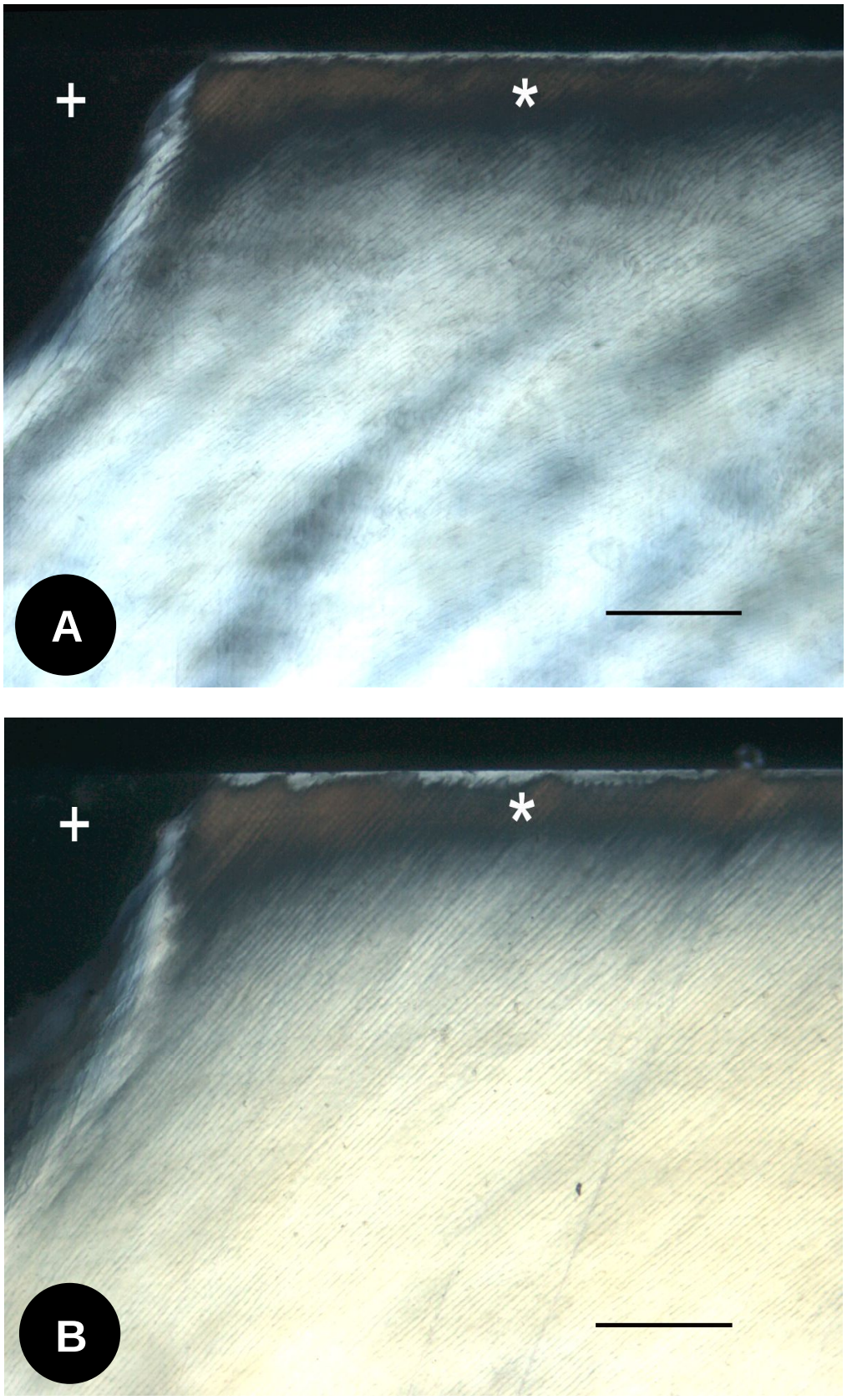


Fig. 7. Representative photomicrographs of the different cavity preparation techniques, under polarized light microscopy. **A:** Er:YAG laser with 350 mJ/3 Hz; **B:** 350 mJ/4 Hz. Restoration (+); Demineralization (*); x20. Bar represents 100 μm.

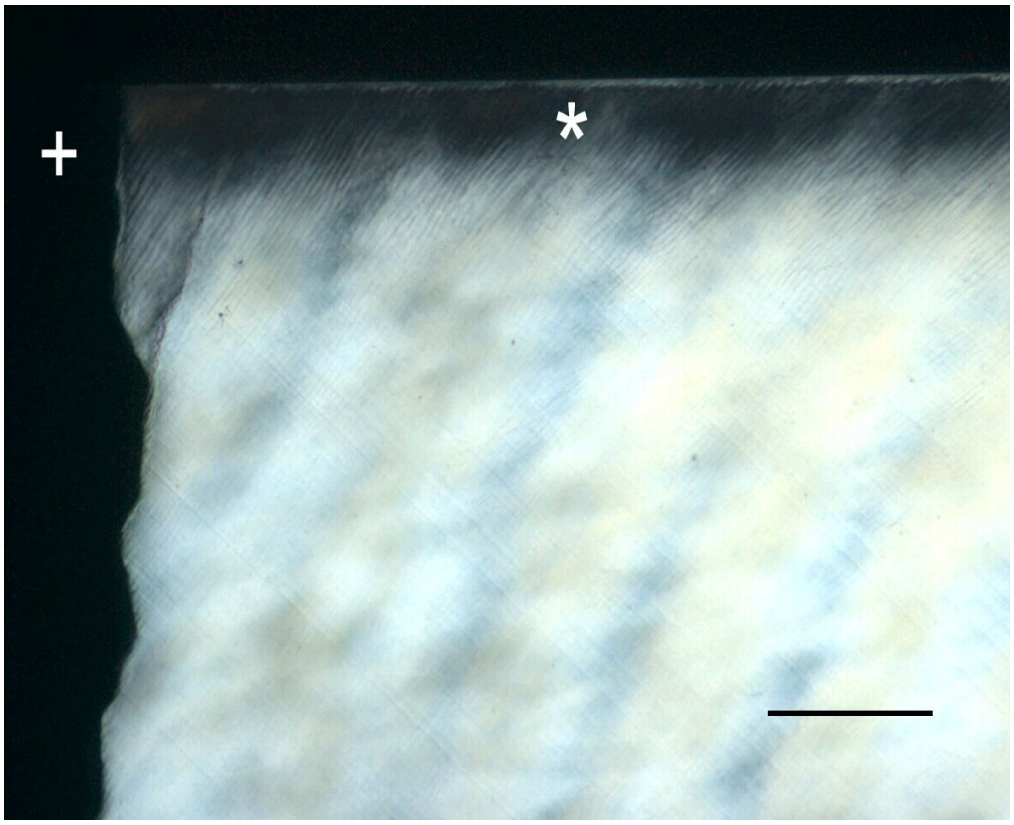


Fig. 8. Representative photomicrograph of the cavity preparation technique with high-speed handpiece (control group) under polarized light microscopy. Restoration (+); Demineralization (*); x20. Bar represents 100 μm.

4. CONCLUSÃO GERAL

Dentro das condições em que foram conduzidos, os estudos realizados permitiram concluir que:

1. O conhecimento das alterações morfológicas, estruturais e químicas induzidas pelo laser de Er:YAG no substrato dental, bem como dos resultados apresentados pelos estudos de resistência ácida, forneceu subsídios para o melhor entendimento da real influência da irradiação com esse equipamento na perda mineral dos tecidos dentais. A análise crítica das variáveis de resposta empregadas permitiu uma visão mais ampla do estágio atual das pesquisas;

2. A técnica de preparo cavitário com laser de Er:YAG não diferiu da técnica de preparo convencional, em relação à microdureza do esmalte adjacente à restaurações submetidas a alto desafio cariogênico *in situ*;

3. A análise das alterações do esmalte ao redor de restaurações submetidas a alto desafio cariogênico *in situ*, por meio de microscopia de luz polarizada, revelou que o preparo cavitário com laser de Er:YAG foi semelhante ao preparo convencional, de um modo geral. Os resultados dos escores referentes à zona de inibição sugeriram menor desmineralização nas margens dos grupos preparados com laser. A correlação entre as medidas quantitativas e os escores correspondentes indica que a análise das lesões de cárie ao redor de restaurações por atribuição de escores poderia ser uma alternativa viável à avaliação quantitativa, por meio de microscopia de luz polarizada.

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ANEXOS

ANEXO A - Comprovação de envio para publicação do artigo referente ao Capítulo 1

Submissions Being Processed for Author Silmara M. Corona, DDS, MS, PhD, ScD

Page: 1 of 1 (1 total submissions) Display 10 results per page.

Action	Manuscript Number	Title	Initial Date Submitted	Status Date	Current Status
Action Links View Submission Send E-mail	LIMS-230	Er:YAG laser-induced changes: influence on tooth acid resistance - a review	Dec 18, 2007	Jan 01, 2008	Under Review

Page: 1 of 1 (1 total submissions) Display 10 results per page.

ANEXO B - Comprovação de aceite para publicação do artigo referente ao Capítulo 2

Data:	Sat, 22 Dec 2007 09:10:44 -0300
Para:	"Daniela Chimello" <dtchimello@yahoo.com.br>
De:	"by way of Silmara Aparecida Milori Corona <silmaracorona@uol.com.br>" <PMLS.journal@gmail.com>
Assunto:	Photomedicine and Laser Surgery - Decision on Manuscript ID PHO-2007-2193.R1

21-Dec-2007

Dear Dr. Corona:

It is a pleasure to accept your manuscript entitled "Influence of Er:YAG laser on microhardness of enamel adjacent to restorations submitted to cariogenic challenge "in situ"" in its current form for publication in Photomedicine and Laser Surgery. The comments of the reviewer(s) who reviewed your manuscript are included at the foot of this letter.

The Copyright Agreement form attached to this email should be sent to the publisher as soon as possible. Manuscripts cannot be published without this form. The corresponding author is responsible for obtaining signatures of coauthors. Authors not permitted to release copyright must still return the form signed under the statement of the reason for not releasing the copyright. Please fax the Copyright Agreement form to 914-740-2101.

Thank you for your fine contribution. On behalf of the Editors of Photomedicine and Laser Surgery, we look forward to your continued contributions to the Journal.

Sincerely,
Dr. Raymond Lanzafame
Editorial Office, Photomedicine and Laser Surgery
PMLS.journal@gmail.com

Reviewer(s)' Comments to Author:
Reviewer: 1
Comments to the Author
The revisions have been done according to reviewers' coments and the manuscript reached a high quality to be published in the journal.

ANEXO C - Comprovação de envio para publicação do artigo referente ao Capítulo 3

Data:	Fri, 25 Jan 2008 05:44:00 -0500 (EST)
De:	lsm@manuscriptmgt.com
Para:	dtchimello@yahoo.com.br, dtchimello@ig.com.br
Assunto:	Lasers in Surgery & Medicine - Manuscript LSM-08-0013

25-Jan-2008

Manuscript number: LSM-08-0013

Dear Dr. Chimello-Sousa:

Thank you for submitting your manuscript entitled Influence of cavity preparation with Er:YAG laser on enamel adjacent to restorations submitted to cariogenic challenge in situ: a polarized light microscopic analysis by Chimello-Sousa, Daniela; Serra, Mônica; Rodrigues-Júnior, Antonio; Pécora, Jesus; Corona, Silmara. We will be passing it on to the editors for evaluation shortly. Please note, however, that if the format or any part of your paper to include text; abstract and/or references or figures are not styled according to our author instructions we will not be able to process your submission. In such case, we will contact you and we will unsubmit your paper back to you for proper formatting and re-submission.

To track the progress of your manuscript through the editorial process using our new web-based system, simply point your browser to:

http://mc.manuscriptcentral.com/lsm

May we also kindly ask you to always include the manuscript ID number LSM-08-0013 in any future correspondence regarding this article.
If you experience problems associated with the submission web site, please click on the "Get Help Now" link at
http://mc.manuscriptcentral.com/lsm

Thank you for your interest and your contribution to Lasers in Surgery & Medicine.

J. Stuart Nelson
Lasers in Surgery & Medicine Editor-in-Chief

ANEXO D - Aprovação do Comitê de Ética

UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE RIBEIRÃO PRETO
COMITÊ DE ÉTICA EM PESQUISA
Avenida do Café, s/nº - Telefone: (016) 602-3970
14040-904 - Ribeirão Preto - SP - Brasil
Fax: (016) 633-0999

CEP/38304/FORP/061204
MLCK/mlck

Prezada Professora,

Ref. Processo nº 2004.1.945.58.2

De ordem da Senhora Coordenadora do Comitê de Ética em Pesquisa desta Faculdade, informamos que o referido Comitê, em sua 55ª Sessão realizada no dia 03 de dezembro de 2004, deliberou **aprovar** o Projeto de Pesquisa envolvendo seres humanos intitulado: **"Avaliação da desmineralização ao redor de cavidades preparadas com laser de Er:YAG – Estudo *in situ*"**, e que será desenvolvido por Vossa Senhoria, na Faculdade de Odontologia de Ribeirão Preto, devendo o atestado para publicação final, ser expedido pelo Comitê de Ética em Pesquisa, após a entrega e aprovação do Relatório Final pelo referido Comitê.

Na oportunidade, lembramos da necessidade de apresentar a este Comitê, o **Relatório Parcial** no dia **30 de junho de 2006** e o **Relatório Final** no dia **30 de dezembro de 2007.**

Atenciosamente,

Maria Lúcia Câmara Kühl
Secretária do Comitê de Ética em Pesquisa

Ilma. Sra.
Profa. Dra. SILMARA APARECIDA MILORI CORONA
Professora Associada do Departamento de Odontologia Restauradora -
FORP - USP

UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE RIBEIRÃO PRETO
COMITÊ DE ÉTICA EM PESQUISA
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14040-904 – Ribeirão Preto - SP - Brasil
Fax: (016) 3633-0999

OF.CEP/285/FORP/29082006

Prezado(a) Professor(a),

Ref.: Processo n. 2004.1.945.58.2

De ordem da Senhora Coordenadora do Comitê de Ética em Pesquisa desta Faculdade, informamos que o referido Comitê, em sua 70ª Sessão realizada no dia 23 de agosto de 2006, deliberou **aprovar:**

1) Alteração do Título do Projeto "Avaliação da desmineralização ao redor de cavidades preparadas com laser de Er:YAG – Estudo *in situ*" para **"Influência do preparo cavitário com laser de Er:YAG nas alterações do esmalte adjacente à restaurações submetidas a desafio cariogênico *in situ*".**

2) Alterações no Projeto de Pesquisa conforme solicitado por Vossa Senhoria.

3) Prorrogação da entrega do Relatório Final para 30 de dezembro de 2008, no Projeto de Pesquisa envolvendo seres humanos intitulado: **"Influência do preparo cavitário com laser de Er:YAG nas alterações do esmalte adjacente à restaurações submetidas a desafio cariogênico *in situ*",** que está sendo desenvolvido por Vossa Senhoria, na Faculdade de Odontologia de Ribeirão Preto.

Na oportunidade, lembramos da necessidade de entregar na Secretaria do Comitê, com o formulário preenchido pelo pesquisador responsável, o **Relatório Parcial** até o dia **30 de julho de 2007** e o **Relatório Final** até o dia **30 de dezembro de 2008.**

Atenciosamente,

Glauce Della Rosa
Secretária do Comitê de Ética em Pesquisa

Ilma. Sra.
Profa. Dra. SILMARA APARECIDA MILORI CORONA
Professora Associada do Departamento de Odontologia Restauradora – FORP/USP

GDR/bgcp

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Daniela Thomazatti Chimello de Sousa