UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE RIBEIRÃO PRETO

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Influência dos níveis de energia do laser Er:YAG na capacidade de ablação, microdureza e morfologia da dentina superficial e profunda

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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, opção Dentística.
Orientadora: Prof.ª Dr.ª Regina Guenka Palma Dibb

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RESUMO

CHINELATTI, M. A. **Influência dos níveis de energia do laser Er:YAG na capacidade de ablação, microdureza e morfologia da dentina superficial e profunda.** 2008. 76p. Tese (Doutorado) – Faculdade de Odontologia de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, 2008.

O objetivo do presente estudo foi avaliar *in vitro* a influência dos níveis de energia do laser de Er:YAG para preparo cavitário na capacidade de ablação, microdureza e morfologia da dentina superficial e profunda. Foram selecionados 72 terceiros molares humanos hígidos, que tiveram as raízes removidas e as coroas seccionadas no sentido vestibulo-lingual, obtendo-se 144 fragmentos. Os fragmentos foram alocados aleatoriamente em 2 grupos: dentina superficial (as faces oclusais foram lixadas até 1 mm abaixo da junção amelo-dentinaria - JAD) e dentina profunda (2 mm abaixo da JAD). Posteriormente, cada grupo foi subdividido em 6 subgrupos (n=12) de acordo com as energias do laser de Er:YAG utilizadas (160, 200, 260, 300 ou 360 mJ, ou controle- sem irradiação). Para avaliar a capacidade de ablação, a massa (mg) de cada fragmento foi obtida antes e depois da irradiação. Para a realização do teste de microdureza Knoop, após a irradiação as cavidades foram incluídas em resina acrílica e seccionadas longitudinalmente. As marcações (10 g; 20 s) foram localizadas a 20, 40, 60, 80, 100 e 200 μm abaixo da parede de fundo do preparo ou da margem superior dos espécimes do grupo controle. A análise morfológica foi realizada em MEV. Os valores de perda de massa e microdureza foram analisados individualmente pelos testes de ANOVA e Fisher (α=5%). Os resultados revelaram um aumento gradativo e significante na perda de massa quando se aumentou a energia do laser utilizada, independente do tipo de dentina irradiada, sendo que a energia de 360 mJ apresentou maiores valores e foi estatisticamente diferente das demais energias estudadas. Em relação a profundidade da dentina não observou-se diferença significante entre elas. A análise morfológica evidenciou que a ablação foi mais seletiva na dentina profunda em que ocorreu mais intensamente a ablação da dentina intertubular, apresentando a protrusão dos túbulos dentinários, contudo, a energia de 360 mJ o aspecto morfológico da dentina profunda foi semelhante a superficial. Podendo assim concluir-se que o aumento da energia do laser proporcionou maior perda de massa, independente da profundidade da dentina, contudo, morfologicamente pôde-se observar que a dentina profunda promoveu uma ablação seletiva, com menor remoção da dentina peritubular. Em relação à microdureza, as médias da dentina superficial foram significantemente superiores às da dentina profunda. As energias de 160 e 360 mJ foram diferentes entre si e das demais. Houve diferença entre todos os pontos medidos. Não foram observadas alterações morfológicas marcantes. Concluiu-se que a capacidade de ablação não depende da profundidade dentinária; a microdureza diminuiu com o aumento da energia, porém aumentou em relação às profundidades. A energia de 160 mJ promoveu um aumento nos valores de microdureza da dentina superficial na região mais próxima do preparo; a microdureza da dentina profunda não foi alterada quando foram utilizadas as energias de 160 e 200 mJ, enquanto os níveis de energia superiores promoveram uma diminuição na microdureza das dentinas superficial e profunda.

Palavras-chave: laser Er:YAG; ablação; dentina; preparo cavitário; microdureza dentinária; morfologia dentinária.

ABSTRACT

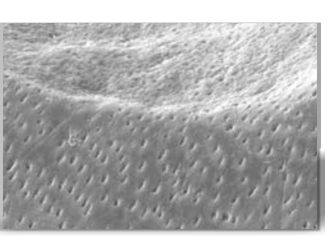
CHINELATTI, M. A. **Influence of Er:YAG laser energy levels on ablation rate, microhardness and morphology of superficial and deep dentin.** 2008. 76 p. Thesis (Doctorate) – School of Dentistry of Ribeirão Preto, University of São Paulo, Ribeirão Preto, 2008.

This study evaluated the ablation rate and the subsurface microhardness of superficial and deep dentin irradiated with different Er:YAG laser energy levels, and observed the micromorphological aspects of the lased substrates by means of Scanning Electron Microscopy (SEM). Seventy-two molar crowns were bisected, providing 144 specimens, which were randomly assigned into two groups (superficial or deep dentin) and later into six subgroups (160, 200, 260, 300, or 360mJ, or control- not irradiated). Initial masses of the specimens were obtained before irradiation. After laser irradiation, final masses were obtained and cavities were longitudinally bisected in half, being one hemi-section destined to SEM analysis and the other to the Knoop microhardness test. Microhardness measurements were performed at six points (20, 40, 60, 80, 100, and 200 µm) under the middle of the cavity floor. Data were submitted to ANOVA and Fisher's LSD Multiple-Comparison Tests (α=0.05). There was no difference between ablation rate of superficial and deep dentin; a significant and gradual increase in the mass-loss values was reached when energies were raised, regardless the dentin depth; the energy level of 360 mJ showed the highest ablation rate and was statistically different to the other energies. SEM images showed that deep dentin was more selectively ablated, especially intertubular dentin, promoting tubule protrusion; at 360 mJ, the micromorphological aspect was similar for both dentin depths. Superficial dentin presented higher microhardness than deep dentin; 160 mJ resulted in the highest microhardness values and 360 mJ the lowest ones; 200 mJ was lower than 160 mJ and the control, but it was higher than 260 and 300 mJ, which presented similar means. Values at all points were different, exhibiting increasing microhardness throughout; superficial dentin microhardness was the highest at 20 µm with 160 mJ, while the other energies demonstrated lower values than the control at all points; on deep dentin, 160 and 200 mJ were similar to the control, and other energies promoted significantly lower values. It may be concluded that the ablation rate did not depend on the depth of the dentin; an energy level lower than 360 mJ is recommended to ablate either superficial or deep dentin effectively without causing tissue damage. The lowest energy level increased superficial dentin microhardness at the closest extension under the cavity preparation; deep dentin subsurface microhardness was not altered by 160 and 200 mJ, while higher energy levels reduced subsurface microhardness of both superficial and deep dentin.

Key words: Er:YAG laser; ablation; dentin; cavity preparation; dentin microhardness; dentin morphology.

SUMÁRIO

Introdução



1. INTRODUÇÃO

A realização do preparo cavitário, por meio do corte ou desgaste da estrutura dental utilizando instrumentos rotatórios adaptados em turbinas de baixa e alta-rotação, é baseada em princípios mecânicos e biológicos. Entretanto, estes instrumentos quase sempre causam desconforto e estresse ao paciente, por proporcionarem vibração, pressão e ruído, que frequentemente são acompanhados de dor.

Pesquisas com novas tecnologias para a realização de preparos cavitários vem sendo desenvolvidas com o objetivo de diminuir esse estresse, minimizar o desgaste de estrutura dental sadia e proporcionar uma superfície mais adequada para a adesão dos materiais restauradores (VISURI et al., 1996; KATAUMI et al., 1998; MARTINEZ-INSUA et al., 2000; TAKAMORI et al., 2000; CORONA et al., 2001; PALMA-DIBB et al., 2003; RAMOS et al., 2002; MONGHINI et al., 2004; SOUZA et al., 2004). O laser é uma destas tecnologias emergentes para o preparo cavitário (DOSTALOVA et al., 1998; HIBST, 2002; TAKAMORI et al., 2003; ATTRILL et al., 2004; CHINELATTI, 2003; CHINELATTI et al., 2003, 2004), com características favoráveis no que se refere ao conforto do paciente, podendo, em muitos casos, eliminar a necessidade de anestesia (KELLER ; HIBST, 1995; COZEAN et al., 1997).

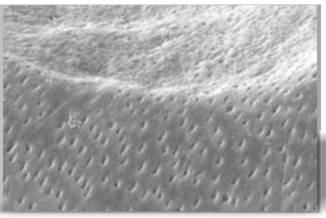
O laser Er:YAG possui como meio ativo um cristal de ítrio-alumínio (Ytrium-Aluminum-Garnet) dopado com íons érbio, que uma vez estimulado por uma lâmpada de *flash* dentro de um ressonador, emite um comprimento de onda de 2,94 μm, que coincide com o pico máximo de absorção da água e dos radicais hidroxila presentes nos tecidos dentais, podendo ser aplicado em tecidos biológicos (KUMAZAKI et al., 1998; GIMBEL, 2000). Desta forma, ocorre a vaporização da água e dos componentes hidratados dos tecidos, causando um rápido aquecimento seguido por microexplosões resultantes do aumento da pressão interna das moléculas teciduais (HIBST; KELLER, 1989), que leva a ejeção do substrato em forma de partículas microscópicas, gerando um efeito fotomecanico (KELLER; HIBST, 1995; MATSUMOTO et al., 1996). Esse processo de ablação consome a maior parte da energia irradiada, sendo liberada apenas uma pequena fração a estrutura dental remanescente (MATSUMOTO et al., 1996; HOSSAIN et al., 1999). Assim, desde que usado sob refrigeração ideal, o laser Er:YAG não causa danos térmicos a polpa dental (AOKI et al., 1998; HIBST, 2002; KIM et al., 2003; ATTRILL et al., 2004). Devido ao maior conteúdo de água na dentina, sua ablação e mais intensa do que a do esmalte quando se utiliza a mesma densidade de energia (HIBST; KELLER, 1989; SAKAKIBARA et al., 1994; JELINKOVA et al., 1996; ARMENGOL et al., 1999; MERCER et al., 2003). Da mesma forma, a ablação da dentina intertubular é maior do que a peritubular, pois apresenta maior quantidade de água (SAKAKIBARA et al., 1994; VISURI et al., 1996; ARMENGOL et al., 1999; SULEWSKI, 2000). Ainda, alguns estudos (HIBST; KELLER, 1989; AOKI et al., 1998) observaram maior ablação da dentina cariada, uma vez que esta apresenta alta permeabilidade e consequentemente e mais úmida do que a dentina hígida. Contudo, pouco se sabe sobre a atuação do laser Er:YAG em dentina profunda (SOUZA et al., 2004; FUENTES et al., 2004).

Em relação aos parâmetros do laser Er:YAG empregados para preparos cavitários, a energia merece destaque por estar diretamente relacionada a capacidade de ablação do substrato dental (MERCER et al., 2003; CORONA, 2003; MONGHINI et al., 2004) e a resistência adesiva dos materiais restauradores (RAMOS et al., 2002; MARTINEZ-INSUA et al., 2000; CHIMELLO et al., 2001; CEBALLOS et al., 2002). Alguns estudos (LI et al., 1992; JELINKOVA et al., 1996; SAKAKIBARA et al., 1994; MEHL et al., 1997; KATAUMI et al., 1998; SHIGETANI et al., 2002; CORONA, 2003) foram realizados na tentativa de padronizar os valores para que se obtenha uma ablação ideal e segura dos tecidos dentais mineralizados. No entanto, tais pesquisas ainda não resultaram em valores de aceitação comprovada. Apel et al. (2002) demonstraram que o laser Er: YAG proporciona uma diminuição na solubilidade dos íons cálcio do esmalte dental, deixando a superfície ligeiramente desmineralizada. No entanto, alguns pesquisadores (ARIAMOTO et al., 1999; KATAUMI et al., 1998; ARMENGOL et al., 2000; CEBALLOS et al., 2002) observaram a presença de áreas de fusão e recristalização, tornando a superfície hipermineralizada e acido-resistente. Isso reduziria a permeabilidade do substrato dental e impediria a penetração de microrganismos cariogenicos, podendo diminuir a recorrência de cáries secundárias (CEBALLOS et al., 2001). Por outro lado, a pouca difusão de monômeros resinosos prejudicaria a adesão de materiais restauradores estéticos (KATAUMI et al., 1998, KAMEYAMA et al., 2000; HOSSAIN et al., 2000).

Uma maneira seguramente comprovada (ARENDS et al., 1980; KOULOURIDES ; HOUSCH, 1983) de avaliar as mudanças superficiais na densidade mineral e a realização de ensaios de microdureza dos tecidos dentais duros, nos quais um penetrador de diamante produz uma deformação característica de acordo com o tipo de ponta, cuja medida do comprimento define a profundidade de penetração. Assim, é possível estabelecer uma relação direta entre o grau de dureza de uma região e a profundidade de penetração da ponta ativa do aparelho (KIELBASSA et al. 1999). Contudo, os estudos de capacidade de ablação e microdureza empregando laser Er:YAG são escassos e as metodologias e parâmetros são diferentes, tornando-os insuficientes para que se possa chegar a uma conclusão a respeito da consistência das cavidades produzidas por esse tipo de laser. Do mesmo modo, os estudos ainda não são conclusivos com relação às alterações morfológicas superficiais do substrato dental após a ablação com laser Er:YAG. Através de analise em microscopia eletrônica de varredura pode-se verificar que a superfície dentinária apresenta irregularidades (VISURI et al., 1996; TANJI, 1998; MARTINEZ-INSUA et al., 2000) e exposição dos túbulos (VISURI et al., 1996; TANJI, 1998; AOKI et al., 1998). Aliás, diferentemente dos ácidos, o laser não desmineraliza a dentina, nem amplia a embocadura dos túbulos (MARTINEZ-INSUA et al., 2000, CEBALLOS et al., 2001), podendo fusionar a rede de fibras colágenas da região basal da superfície irradiada, o que a torna destituída de espaços interfibrilares (CEBALLOS et al., 2002).

Diante das vantagens proporcionadas pelo laser Er:YAG em relação aos métodos convencionais para preparos cavitários, como diminuição de ruídos, vibrações e dor, sem causar danos ao tecido pulpar, são necessários estudos que avaliem as alterações da dentina superficial e profunda apos a irradiação em diferentes energias do laser Er:YAG, visando a determinação de parâmetros adequados para a utilização deste equipamento. Daí a importância de avaliar a capacidade de ablação, a microdureza e a morfologia do substrato dentinário em diferentes profundidades na tentativa de buscar um método alternativo viável para realização de preparos cavitários.

Proposição



2. PROPOSIÇÃO

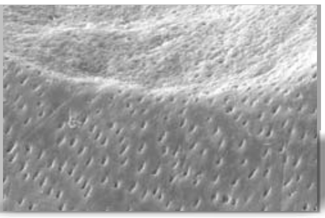
O presente estudo *in vitro*, composto por dois artigos científicos, teve como objetivos avaliar a influência da irradiação do laser Er:YAG utilizando diferentes níveis de energia indicados para a realização de preparos cavitários na:

· capacidade de ablação da dentina em diferentes profundidades (superficial e profunda);

· microdureza subsuperficial das paredes de fundo de cavidades realizadas em dentina superficial e profunda;

· morfologia das superfícies e subsuperfícies irradiadas.

Capítulo 1



3. CAPÍTULO 1

3.1. Ablation rate and morphology of superficial and deep dentin irradiated with different Er:YAG laser energy levels

Artigo científico enviado e considerado para publicaçaopublicação no periodicoperiódico *Photomedicine and Laser Surgery* (Anexo 2).

ABSTRACT

Objective: This study evaluated the ablation rate of superficial and deep dentin irradiated with different Er:YAG laser energy levels, and observed the micromorphological aspects of the lased substrates by means of Scanning Electron Microscopy (SEM). ***Background Data:*** Little is known about the effect of Er:YAG laser irradiation on different dentin depths. ***Methods:*** Sixty molar crowns were bisected, providing 120 specimens, which were randomly assigned into two groups (superficial or deep dentin) and later into five subgroups (160, 200, 260, 300, or 360mJ). Initial masses of the specimens were obtained. After laser irradiation, final masses were obtained and mass losses were calculated followed by the preparation of specimens for SEM. Mass-loss values were submitted to two-way ANOVA and Scheffé and Fisher's LSD Multiple-Comparison Tests (α=5%). ***Results:*** There was no difference between superficial and deep dentin; a significant and gradual increase in the mass-loss values was reached when energies were raised, regardless the dentin depth; the energy level of 360 mJ showed the highest values and was statistically different to the other energies. SEM images showed that deep dentin was more selectively ablated, especially intertubular dentin, promoting tubule protrusion; at 360 mJ, the micromorphological aspect was similar for both dentin depths. ***Conclusion:*** the ablation rate did not depend on the depth of the dentin; an energy level lower

than 360 mJ is recommended to ablate either superficial or deep dentin effectively without causing tissue damage.

INTRODUCTION

Er:YAG laser has increasingly been used in operative dentistry[1-6], becoming a more comfortable method for patients during cavity preparations, as conventional cavity drilling may cause noise and pain.[7-9] However, the time required for preparing cavities with the laser device is several times longer than the high-speed bur.[10,11] In the clinic, this time may be reduced by increasing the energy level of laser irradiation for removing hard dental tissue, but the risk of thermal damage in the tissues may be increased.

Er:YAG laser irradiation removes both enamel and dentin due to its wavelength of 2.94 µm, which matches the absorption peak of water and is absorbed by hydroxyapatite, limiting the laser effect on these tissues to a superficial layer of a few micrometers, while sparing the surrounding tissues.[12-16] This superficial layer can be heated up rapidly so that the pressure within the irradiated tissue abruptly increases until the strength of the substrate is surpassed. The overheated water is evaporated, resulting in a high steam pressure that causes microexplosions of tooth tissue, characterizing the thermomechanical ablation process.[7,12,13] Since Er:YAG laser energy is well absorbed by water, the higher content of water in dentin facilitates the action of the laser, and the relatively predominant organic composition of dentin, became this tissue less resistant to laser ablation than enamel.[12,17-21]

Basically, dentine consists of an organic matrix made up primarily of a hydrated type I collagen and an inorganic phase made up of a nanocrystalline-carbonated apatite[,22]. Its characteristic microstructure consists of oriented tubules of 1-2 µm in diameter surrounded by highly mineralized (approx. 95 Vol% mineral phase) peritubular dentin embedded within a partially mineralized (approx. 30 Vol % mineral phase) collagen matrix (intertubular dentin).[24-26] In the arrangement of the dentin microstructure, the tubules run continuously between the enamel and the pulp and vary in density from about 15,000/mm^2 at the dentine-enamel junction (superficial dentin) to 65,000/mm^2 at the pulp (deeper dentine).[27] These marked differences in the various regions of a tooth, such as tubule density and peritubular and intertubular areas, distinguish superficial from deep dentin.[24,27,28]

The apatite phase contributes to most of the compressive strength that might directly affect hardness, while the collagen phase provides elasticity[23]. Changes in these two phases might contribute to changes in the physical properties, hardness and elasticity modulus of these biological composites. Since during irradiation with Er:YAG laser there is a non-uniform destruction of tooth structure and the ejection of both organic and inorganic tissue particles[7,12,13], it is necessary to evaluate Studying the mechanical properties of dental tissues after cavity preparations using Er:YAG laser, is important to verify whether or not occur alterations in their properties, such as microhardness, giving us an idea on how the mechanical behavior of the irradiated tissue under clinical loading conditions could be. Microhardness is defined as the resistance to local deformation based on the induced permanent surface deformation that remains after removing the load, being considered a supported method to evaluate superficial changes in the mineral density of hard dental tissues.

In this particular field of application, the literature available on the Er:YAG laser still presents varying parameters of energy settings, and, besides the fact that little is known about the effect of Er:YAG laser irradiation on different dentin depths,[31,32] there are divergent results as well. Consequently, an optimal irradiation energy level should be determined to optimize the efficiency of Er:YAG laser for removing both superficial and deep dentin without affecting the microstructure of these substrates. Thus, the objective of this study was to evaluate the ablation rate of dentin as a function of Er:YAG laser energy level and substrate depth, and to observe the micromorphological aspects of the irradiated surfaces.

MATERIALS AND METHODS

Initially, this study was submitted to the Ethics Committee of the School of Dentistry of Ribeirão Preto, University of São Paulo, and initiated after being approved (process # 2005.1.656.58.1) (Anexo 1)..

- Experimental design

This study was composed of a randomized complete block design with 12 experimental specimens per *group / subgroup*. The variation factors examined were *dentin* at two levels (superficial and deep) and *energy* at five levels (160, 200, 260, 300 and 360 mJ). The response variable was *mass loss* numeric numbers in mg.

- Specimen obtainment

Extracted sound human third molars, coming from the Tooth Bank of the School of Dentistry of Ribeirão Preto, University of São Paulo, were used in this study. These teeth, kept in distilled water at 4°C for no longer than six months, were cleaned with scalpels and water/pumice slurry with dental prophylactic cups and examined under a 20X magnifier to discard those with structural defects. Then, sixty teeth were selected and their crowns and roots were separated by a cut 2 mm below the cementoenamel junction with a water-cooled diamond saw in a precision cutting machine (Isomet 4000, Buehler GmbH, 40599 Dusseldorf, Germany).

The crown of each tooth was fixed with wax in an acrylic plate and sectioned longitudinally into two pieces in a bucco-lingual direction using a low-speed double-faced diamond disk (#7015, KG Sorensen, Barueri, 06454-920, Brazil). To reduce experimental error caused by the specimen condition,[33] half of each sectioned tooth crown was used as superficial dentin and the other half as deep dentin. Dentin discs were obtained by transversal sectioning of the hemi-crowns with a low-speed diamond saw in a precision cutting machine under water irrigation. Superficial dentin was considered dentin within 0.5 mm of the enamel in the central occlusal groove, and deep dentin was defined as the dentin surface within 1.5 mm of the highest pulp horn. The method of obtaining both dentins was based in previous studies,[29,30] but, according to our pilot study, a modification in the location of deep dentin was necessary to avoid pulp exposure during irradiation.

- Ablation rate assessment

The specimens of both superficial dentin and deep dentin groups were randomly assigned to five subgroups according to the irradiation energy used for preparing cavities: 160 mJ, 200 mJ, 260 mJ, 300 mJ and 360 mJ. The specimens were identified and individually stored in plastic containers with distilled water at 4ºC for 24 hours, with the purpose of re-humidifying the substrate. After this period, the containers were kept at 37^{o}C for 2 hours, and subsequently specimens were removed from the water, dried with absorbing paper for 20 seconds, and individually weighed in a precision analytical balance (Mettler, H54, Switzerland) with six decimal places of accuracy to determine the initial mass (m_I) in milligrams. Once the initial masses were recorded, the specimens were hydrated for 1 hour at room temperature. Before performing the irradiation, each specimen was fixed on an acrylic plate and a 3-mm-diameter ablation site was delimited by attaching a piece of insulating tape with a central orifice made by a punch.

The Er:YAG laser system used was the Twin Light operating at a pulse repetition rate of 3 Hz in non-contact and focused mode (irradiation distance of 12 mm), irradiation time of 30 seconds, and with the pulse energy levels described above. The water supply system was used throughout the irradiation time at a 2.5 mL/min flow. Once the irradiation was performed, the insulating tape was removed; the specimens were removed from the plates, thoroughly cleaned and stored in distilled water for 1 hour. Then, the final masses (m_2) were recorded by individually weighing the specimens in the precision analytical balance following the same protocol described above. Dentin mass loss was calculated in milligrams by subtracting the final from the initial mass (m_1 - m_2). The amount of dentin removed during irradiation indicated the ablation rate under the experimental conditions tested.

-Statistical analysis

Data obtained from resulting amounts of dentin removed were analyzed by two-way ANOVA (factors: *dentin depth* and *energy*) and Fisher's LSD Multiple-Comparisons tests using a statistical software (NCSS/PASS Dawson edition, NCSS, Kaysville, Utah, 84037, USA) at a α=5% significance level.

- Micromorphological analysis

After being weighed, the specimens were submitted to the micromorphological analysis of their laser-ablated surfaces by means of scanning electron microscopy. The preparation of the specimens was performed according to the following protocol: immersion in 2.5% glutaraldehyde (Merck KGaA, Frankfurter Str. 250, D-64293 Darmstadt, Germany) in an 0.1M sodium cacodylate buffer solution (pH 7.4) for 12 hours at 4°C; after fixation, the specimens were rinsed with an 0.1M sodium cacodylate (Merck KGaA, Frankfurter Str. 250, D-64293 Darmstadt, Germany) buffer solution several times and sequentially dehydrated in ethanol (Labsynth Produtos para Laboratório Ltda., Diadema-SP, Brasil) solutions, as follows: 25% for 20 min, 50% for 20 min, 75% for 20 min, 90% for 30 min and 100% for 60 min, after which they were immersed in a hexamethyldisizilane (HMDS) solution (Merck KGaA, Frankfurter Str. 250, D-64293 Darmstadt, Germany) for 10 minutes, placed on absorbing paper inside glass plates and left drying in an exhaust system. Specimens were mounted on metallic stubs with their ablated surfaces turned up, sputter-coated with gold (SDC 050, Bal-Tec AG, Foehrenweg 16, FL-9496 Balzers, Liechtenstein) and examined in scanning electron microscope (Philips XL30 FEG-SEM, Philips Electron Optics, Eindhoven, Holland) operating at 10 kV. The entire ablated surface of each specimen was scanned and the most representative areas were recorded in different magnifications.

RESULTS

Table 1. Analysis of variance table

Source term	DF	F-Ratio	Prob level	Power (alpha=0.03)
A: Dentin	1	0.62	0.433986	0.083385
B: Energy	4	35.78	0.000000*	1.000000
AxB	4	0.35	0.846853	0.085361

* Term significant at alpha = 0.03

Table 2. Ablation amount related to the factor energy

Energy (mJ)	Mean of mass loss (mg)
160	4.00000 ^{d}
200	4.42917 ^{cd}
260	5.85000 ^{bc}
300	6.67083 ^{b}
360	8.90000 ^{a}

Same superscript letters indicate statistical similarity

Table 3. Mean values and standard deviations of mass loss (mg) according to the dentin depth and energy level

	160 *mJ*	200 *mJ*	260 *mJ*	300 *mJ*	360 *mJ*
Superficial dentin	3.575 $^{A\ a}$ (1.28)	4.491 $^{AB\ a}$ (0.96)	5.775 $^{BC\ b}$ (1.86)	6.516 $^{C\ ce}$ (1.32)	8.916 $^{D\ d}$ (1.76)
Deep dentin	4.425 $^{E\ a}$ (2.15)	4.366 $^{E\ a}$ (1.54)	5.925 $^{F\ be}$ (1.43)	6.825 $^{F\ ce}$ (1.53)	8.883 $^{G\ d}$ (1.86)

Same superscript letters indicate statistical similarity.
Capital letters: to compare rows; Lower case: to compare columns.

- Ablation rate

The analysis of variance (Table 1) revealed that there was no significant difference between superficial and deep dentin (Fig. 1), there was a difference among the energies, and the interaction of the two factors was not significant. Comparing the energy levels, regardless of the dentin depth, there was an increase in the mass-loss values with an increase in the laser energy level (Fig. 2); 360 mJ provided the highest mass loss, which was significantly different from all the others; there was no difference between 160 and 200 mJ, between 200 and 260 mJ, and between 260 and 300 mJ (Table 2). The interaction dentin depth x energy (Table 3) showed that significantly higher mass-loss values were obtained with 360 mJ laser irradiation in either superficial or deep dentin (Fig. 3).

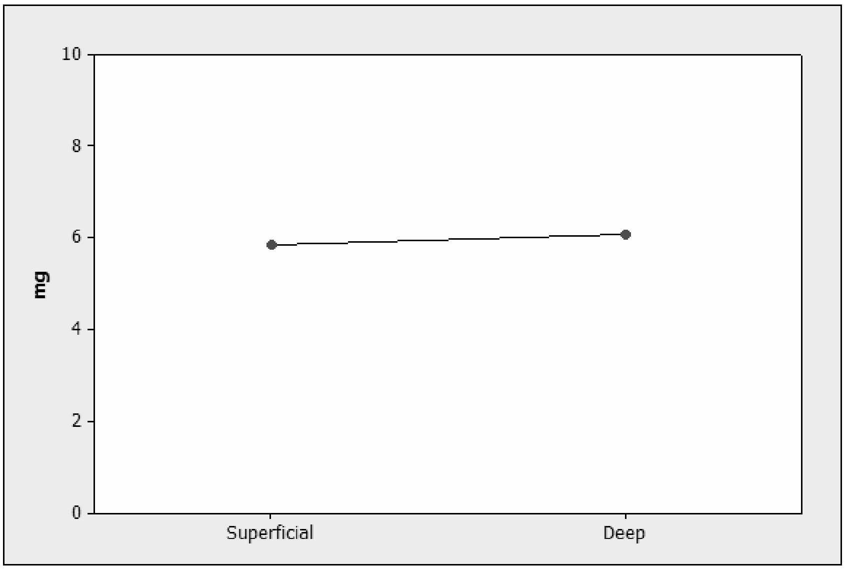


FIGURE 1. Amount (mg) of mass ablated as a function of the depth of dentin.

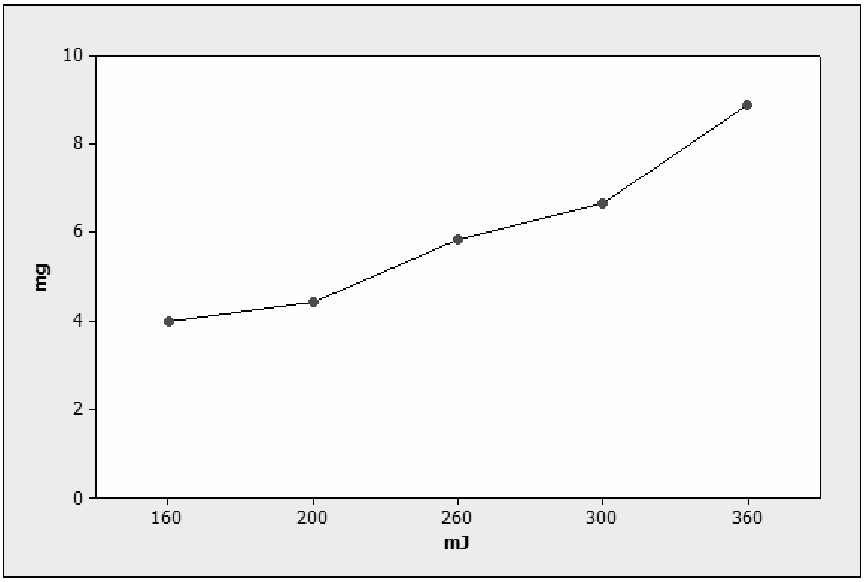


FIGURE 2. Amount (mg) of mass ablated as a function of the energy level (mJ), regardless of dentin depth.

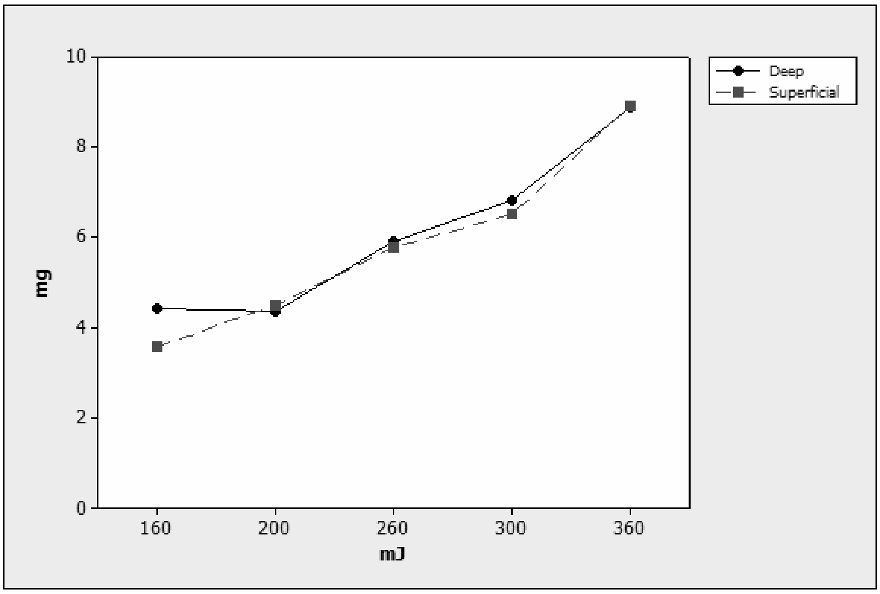
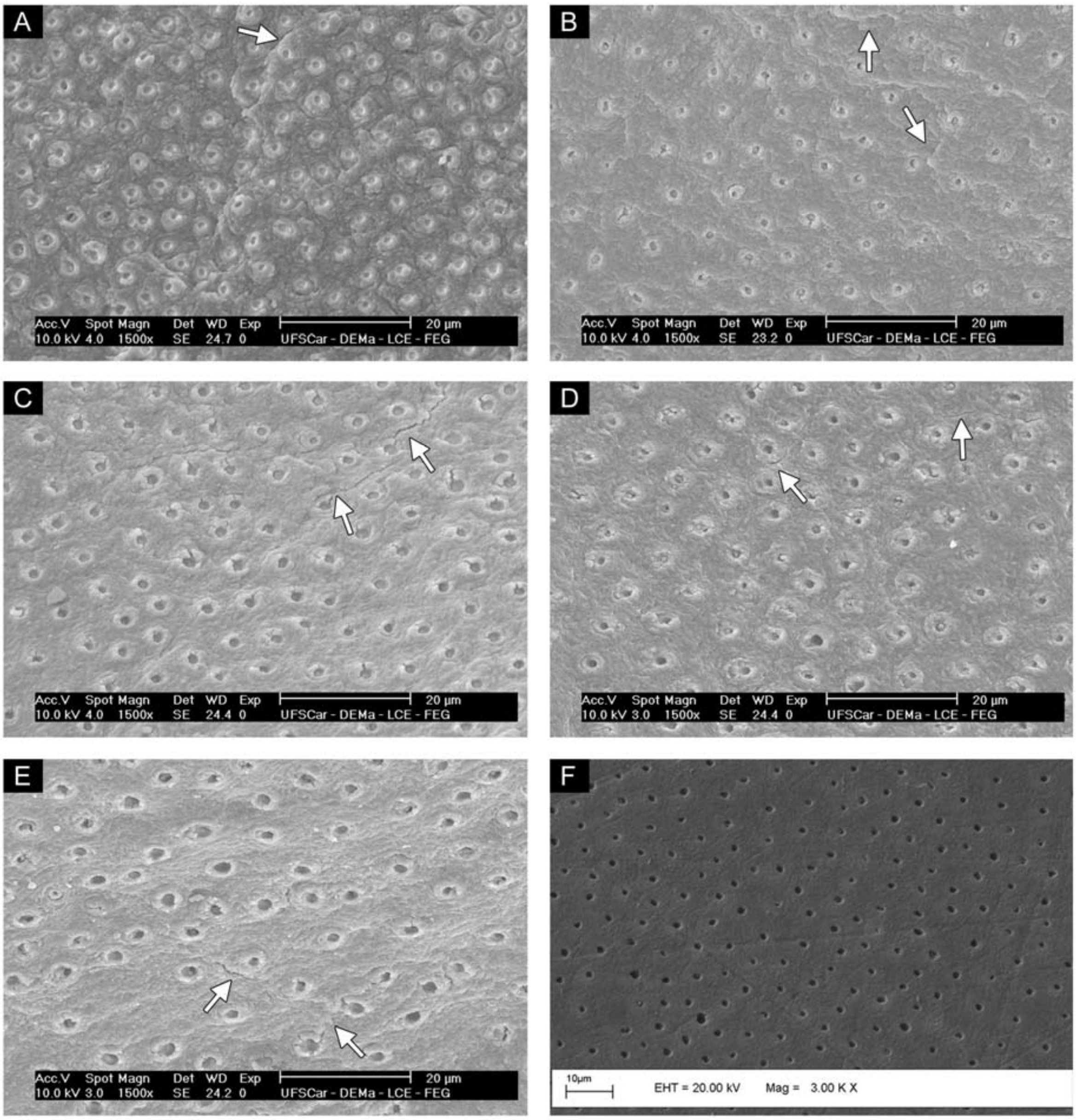


FIGURE 3. Amount (mg) of mass ablated as a function of the energy level (mJ) and dentin depth.

- Micromorphological analysis

In deep dentin, laser irradiation at 160, 200, 260, and 300 mJ energy levels (Fig. 5.A, 5.B, 5.C, and 5.D, respectively) resulted in irregular surfaces with microcracks and higher removal of intertubular dentin, leaving the peritubular dentin with a protrusion aspect (tubule protrusion). This aspect was not observed in superficial dentin, which exhibited flat surfaces, and similar ablation of both intertubular and peritubular dentin at the same energy levels (Fig. 4.A, 4.B, 4.C, and 4.D).

Both in superficial and deep dentin (Fig. 4.E and 5.E, respectively), the energy of 360 mJ promoted flat surfaces with microcracks, no melting, and similar ablation of intertubular or peritubular dentin.



FFIGURE 4. SEM images - micromorphological aspects of superficial dentin irradiated by Er:YAG laser according to the energy employed: **A- 160 mJ, B- 200 mJ, C- 260 mJ, D- 300 mJ,** and **E- 360 mJ:** flatter surfaces due to a non-selective ablation pattern, with microcracks in intertubular dentin (arrows), and no melting. **F-** control superficial dentin

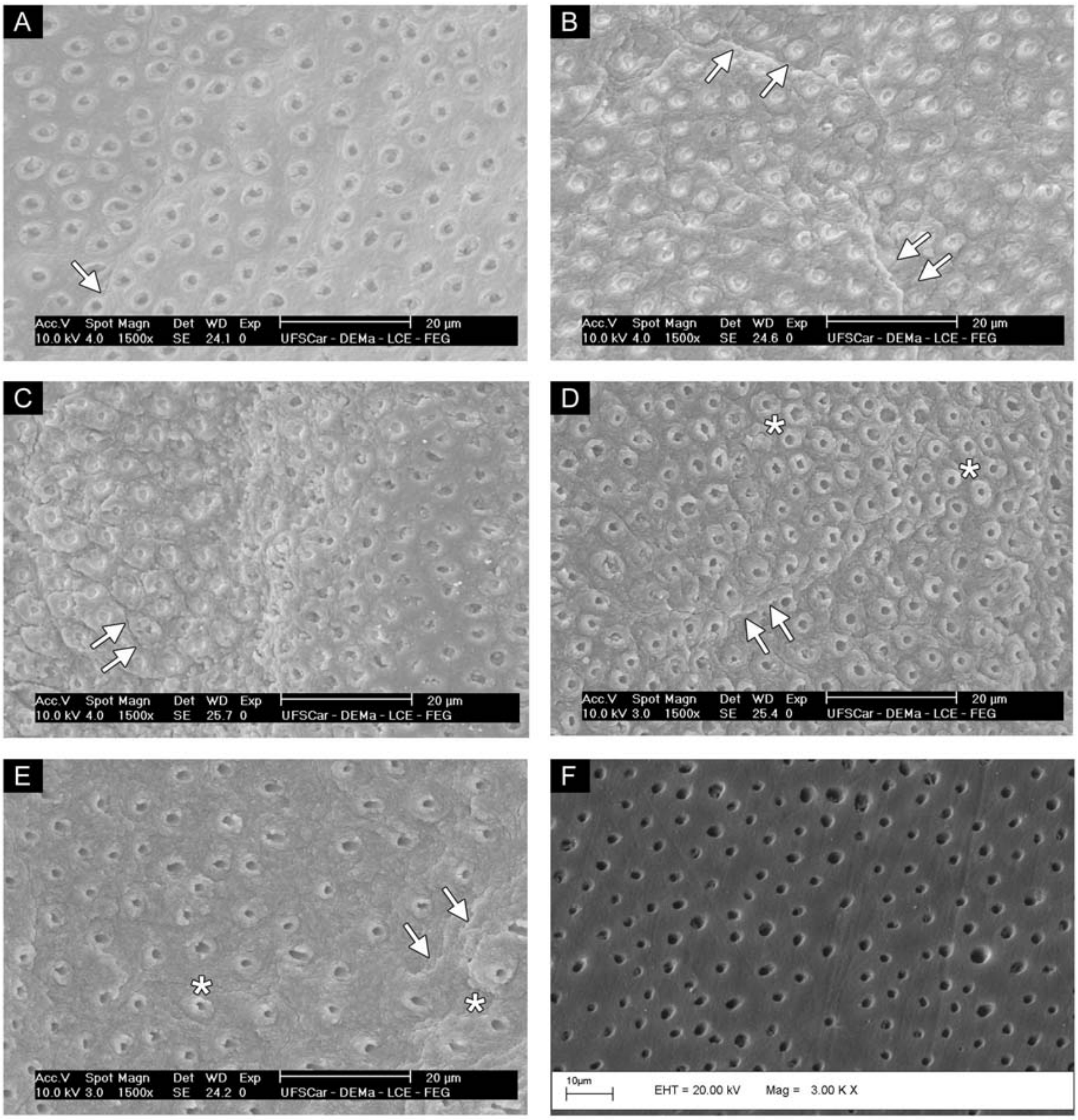


FIGURE 5. SEM images - micromorphological aspects of deep dentin irradiated by Er:YAG laser according to the energy employed: **A- 160 mJ, B- 200 mJ, C- 260 mJ,** and **D- 300 mJ**: irregular surfaces, with microcracks (arrows) in intertubular dentin, no melting, and tubule protrusion (asterisks) due to a selective ablation of intertubular dentin. **E- 360 mJ:** flatter surface without tubule protrusion- a non-selective ablation pattern. **F-** control deep dentin

DISCUSSION

The ablation rate can be evaluated in terms of the amount of dental tissue removed within a given time[33] and is correlated with such parameters of irradiation as the number of pulses per second, energy per pulse, and duration of the treatment.[34-37] The amount of tissue removed is thought to increase in proportion to the Er:YAG laser energy. However, with increased ablation efficiency, the dentin microstructure may be affected by the thermal effect and the resulting surface may vary from a white spot to charring, fusion, roughening, melting, recrystallization, cracking, and both de/remineralization and deproteinization.[8,10,13,38-41] Researches[34-36,42] indicate that adjustments to variable parameters, including irradiation time, water spray, pulse energy, and pulse repetition rate, should be made to improve the ablation ability of the laser. Moreover, different depths of dentin should be considered as additional variables affecting the ablation rate.

In this study, the ablation rate was not significantly influenced by dentin depth, and both superficial and deep dentin had a direct relationship with the laser energy resulting in higher tissue removal as the energy level was raised. In other words, if the energy levels are maintained constant, changing the dentin depth will not influence the amount of tissue removed. It can be understood that only one of these factors may influence the amount of dentin removed, that is, the energy level. This study supports previous statements[36,40,42] that the ablation rate generally increases as a function of the energy level and adds new information concerning the dentin location. The explanation is based on the principle that the higher the energy per pulse on tooth tissue, the higher the energy density per pulse repetition, and, consequently, a higher inertial-confined heating of water creates enormous subsurface pressures that lead to the microexplosive removal of the surrounding mineral matrix and consequently more ablation of the tissue.[35,43]

On the other hand, small increases in the energy levels did not result in significant amounts of dentin removed. These results suggest that, regardless of the dentin depth, there is a similar amount of tissue removed when the energy level is not highly increased. This behavior was previously described as saturation-type and shows the occurrence of a shielding resulting from ablation products not completely removed and deposited at the irradiated tissue.[35,44] Diverging results were found by Shigetani et al.[42] who obtained differences between 50 mJ and 100 mJ, and between 150 mJ and 200 mJ. This disagreement may be attributed to differences in laser devices, pulse repetition rates, and types of substrate employed.

Concerning the variation in the depth of the substrate, there was no difference between the mass losses of either superficial or deep dentin. Thus, variations in the water and mineral contents in dentin were not sufficiently significant to affect the gross amount of tissue removal. This fact probably is due to the absence of a significant influence of the mineralization degrees of superficial and deep dentin, therefore variations in microhardness were related to the decreased hardness near the pulp by a decrease in the hardness of the intertubular dentin matrix, which may be less mineralized.[45]

However, the micromorphological analysis revealed that deep dentin was more selectively ablated than superficial dentin, exhibiting greater intertubular dentin removal, which characterizes the protruded aspect of the tubules indicating that peritubular dentin is more resistant to laser energy. This pattern can be explained by the fact that peritubular dentin has a high mineral content and lacks collagen as an organic matrix, different from the intertubular dentin, it makes up 92% of the collagen matrix.[46,47] Moreover, such features are present mainly in the intertubular dentin located next the pulp.[24,28,45] Another interesting micromorphological observation was made for both superficial and deep dentin ablated using the energy of 360 mJ, showing a flat surface resulting from similar ablation of both intertubular and peritubular dentin, which means that this energy level promoted a non-selective ablation pattern.

The comparison of the results obtained in this study is difficult due to the scarceness of published studies about Er:YAG laser irradiation on superficial and deep dentin. Therefore, more studies need to be performed on this issue in an attempt to standardize the optimal parameters for tooth tissue ablation without compromising their intactness as well as their mechanical properties.

CONCLUSION

Based on the results of this study, it can be concluded that the ablation rate was not dependent on the dentin depth and was influenced only by the energy, which may be recommended at a level lower than 360 mJ to ablate both superficial and deep dentin effectively without damaging the integrity of the substrates; although variations in the depth of the substrate did not change the ablation rate, they influenced the selectiveness of the ablation, as deep dentin was more selectively ablated with minor removal of peritubular dentin.

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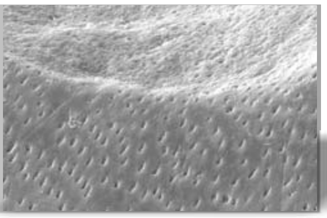
Capítulo 2



4. CAPÍTULO 2

4.1. Effect of Er:YAG laser energies on superficial and deep dentin microhardness

Artigo científico submetido para publicação ao periódico *Lasers in Medical Science* (Anexo 3)

ABSTRACT

This study evaluated the microhardness of superficial and deep dentin irradiated with different Er:YAG laser energies. Seventy-two molars were bisected and randomly assigned into two groups (superficial or deep dentin) and into six subgroups (160, 200, 260, 300, 360mJ, control). After irradiation, cavities were longitudinally bisected. Microhardness were performed at six points (20, 40, 60, 80, 100, 200µm) under the cavity floor. Data were submitted to ANOVA and Fisher's Tests (α=0.05). Superficial dentin presented higher microhardness than deep dentin; 160mJ resulted in the highest microhardness and 360mJ the lowest ones. Values at all points were different, exhibiting increasing microhardness throughout; superficial dentin microhardness was the highest at 20µm with 160mJ; on deep dentin, 160 and 200mJ were similar to the control. The lowest energy increased superficial dentin microhardness at the closest extension under the cavity; deep dentin microhardness was not altered by 160 and 200mJ.

INTRODUCTION

Er:YAG laser has increasingly been used in operative dentistry [1-6], becoming a more comfortable method for patients during cavity preparations, as conventional cavity drilling may cause noise and pain [7-9]. Er:YAG laser irradiation removes both enamel and dentin due to its wavelength of 2.94 µm, which matches the absorption peak of water and is absorbed by hydroxyapatite, limiting the laser effect on these tissues to a superficial layer of a few micrometers, while sparing the surrounding tissues [10-14]. This superficial layer can be heated up rapidly so that the pressure within the irradiated tissue abruptly increases until the strength of the substrate is surpassed. The overheated water is evaporated, resulting in a high steam pressure that causes microexplosions of tooth tissue, characterizing the thermomechanical ablation process [7, 10, 11]. Since Er:YAG laser energy is well absorbed by water, the higher content of water in dentin facilitates the action of the laser, and the relatively predominant organic composition of dentin, became this tissue less resistant to laser ablation than enamel [10, 15-19].

Basically, dentine consists of an organic matrix made up primarily of a hydrated type I collagen and an inorganic phase made up of a nanocrystalline-carbonated apatite [20]. Its characteristic microstructure consists of oriented tubules of 1-2 µm in diameter surrounded by highly mineralized (approx. 95 Vol% mineral phase) peritubular dentin embedded within a partially mineralized (approx. 30 Vol % mineral phase) collagen matrix (intertubular dentin) [21-23]. In the arrangement of the dentin microstructure, the tubules run continuously between the enamel and the pulp and vary in density from about 15,000/mm^2 at the dentine-enamel junction (superficial dentin) to 65,000/mm^2 at the pulp (deeper dentine) [24]. These marked differences in the various regions of a tooth, such as tubule density and peritubular and intertubular areas, distinguish superficial from deep dentin [21, 24, 25].

The apatite phase contributes to most of the compressive strength that might directly affect hardness, while the collagen phase provides elasticity [26]. Changes in these two phases might contribute to changes in the physical properties, hardness and elasticity modulus of these biological composites [27]. Since during irradiation with Er:YAG laser there is a non-uniform destruction of tooth structure and the ejection of both organic and inorganic tissue particles [7, 10, 11], it is necessary to evaluate the mechanical properties of dental tissues after cavity preparations using Er:YAG laser, such as microhardness, giving us an idea on how the mechanical behavior of the irradiated tissue under clinical loading conditions could be. Microhardness is defined as the resistance to local deformation, based on the induced permanent surface deformation that remains after removing the load [28, 29]. It is considered a supported method to evaluate superficial changes in the mineral density of dental hard tissues [30, 31], as well as plays an important role in stress distribution when mastication forces are applied on a restored tooth [32].

The literature available on the Er:YAG laser still presents varying parameters of energy settings, and, besides the fact that little is known about the effect of Er:YAG laser irradiation on different dentin depths [33, 34], there are divergent results as well. Consequently, an optimal irradiation energy level should be determined to optimize the efficiency of Er:YAG laser for removing both superficial and deep dentin without damaging their mechanical properties. Thus, the objective of this study was to evaluate the ablation rate of dentin as a function of Er:YAG laser energy level and substrate depth, and to observe the micromorphological aspects of the irradiated surfaces. Thus, this study aimed to evaluate possible alterations in the microhardness of superficial and deep dentin after cavity preparations by Er:YAG laser with different irradiation energy levels.

MATERIAL AND METHODS

Initially, this study was submitted to the Ethics Committee of the School of Dentistry of Ribeirão Preto, University of São Paulo, and initiated after being approved (process # 2005.1.656.58.1) (Anexo 1).

Experimental design

This study was composed of a randomized complete block design with 12 experimental specimens per *group / subgroup*. The variation factors examined were *dentin* at two levels (superficial and deep), *energy* at six levels (160, 200, 260, 300 and 360 mJ, and control- not irradiated), and *subsurface point* at six levels (20, 40, 60, 80, 100 and 200 µm). The response variable was *Knoop microhardness* numeric numbers in kgf.

Specimen obtainment

Extracted sound human third molars, coming from the Tooth Bank of the School of Dentistry of Ribeirão Preto, University of São Paulo, were used in this study. These teeth, kept in distilled water at 4°C for no longer than six months, were cleaned with scalpels and water/pumice slurry with dental prophylactic cups and examined under a 20X magnifier to discard those with structural defects. Then, seventy-two teeth were selected and their crowns and roots were separated by a cut 2 mm below the cementoenamel junction with a water-cooled diamond saw in a precision cutting machine (Isomet 4000, Buehler GmbH, 40599 Dusseldorf, Germany). The crown of each tooth was fixed with wax in an acrylic plate and sectioned longitudinally into two pieces in a bucco-lingual direction using a low-speed double-faced diamond disk (#7015, KG Sorensen, Barueri, 06454-920, Brazil). To reduce experimental error caused by the specimen condition [35], half of each sectioned tooth crown was used as superficial dentin and the other half as deep dentin. Dentin discs were obtained by transversal sectioning of the hemi-crowns with a low-speed diamond saw in a precision cutting machine under water irrigation. Superficial dentin was considered dentin within 0.5 mm of the enamel in the central occlusal groove, and deep dentin was defined as the dentin surface within 1.5 mm of the highest pulp horn. The method of obtaining both dentins was based in previous studies [32, 36], but, according to our pilot study, a modification in the location of deep dentin was necessary to avoid pulp exposure during irradiation.

The specimens of both superficial dentin and deep dentin groups were randomly assigned into six subgroups, being five according to the irradiation energy used for preparing cavities (160 mJ, 200 mJ, 260 mJ, 300 mJ and 360 mJ) and one the control (without irradiation). The specimens were identified and individually stored in plastic containers with distilled water at 4ºC for 24 hours, with the purpose of re-humidifying the substrate. Before performing the irradiation, each specimen was fixed on an acrylic plate and a 2-mm-diameter ablation site was delimited by attaching a piece of insulating tape with a central orifice made by a punch.

The Er:YAG laser system used was the Twin Light (Fotona Medical Lasers, Slovenia, Ljubjana) operating at a pulse repetition rate of 3 Hz in non-contact and focused mode (irradiation distance of 12 mm), irradiation time of 30 seconds, and with the pulse energy levels described above. The water supply system was used throughout the irradiation time at a 2.5 mL/min flow. Once the irradiation was performed, the insulating tape was removed; the specimens were removed from the plates and thoroughly cleaned. Specimens were individually placed in acrylic resin blocks to facilitate the bisection of cavities in their long axis, being one half destined to microhardness test and the other half to SEM analysis. Specimens of control subgroup did not receive laser irradiation; they were included and longitudinally bisected.

Microhardness test

To provide a more uniform surface for the reading and to improve the precision of the penetrations, the section-face of each hemi-specimen was ground with #1000- and #2000-grit SiC papers and polished with 0.3- and 0.05-µm alumina suspensions. The polished hemisections were cleansed with distilled water in an ultrasonic apparatus to remove any debris. Before testing microhardness, each specimen was placed on a plate and fixed with plastic wax using a parallelometer (ELQuip, São Carlos, SP, Brazil) to ensure that its surface was kept parallel to the horizontal plane. Knoop hardness was assessed using a microhardness tester (Shimadzu HMV-2000, Shimadzu Corporation, Kyoto, Japan). Settings for load and penetration were 10 g and 20 s. Penetrations were performed under cavity preparations at distances of 20, 40, 60, 80, 100, and 200 µm apart from the middle of the cavity floor or apart from the superior edge of control specimens. At each distance, three horizontally 100-µm-spaced measurements were performed and their mean was calculated.

Micromorphological analysis

The hemi-specimens destined to SEM analysis were prepared according to the following protocol: immersion in 2.5% glutaraldehyde in an 0.1M sodium cacodylate buffer solution (pH 7.4) for 12 hours at 4°C; after fixation, the specimens were rinsed with an 0.1M sodium cacodylate buffer solution several times and sequentially dehydrated in ethanol solutions, as follows: 25% for 20 min, 50% for 20 min, 75% for 20 min, 90% for 30 min and 100% for 60 min, after which they were immersed in a hexamethyldisizilane (HMDS) solution for 10 minutes, placed on absorbing paper inside glass plates and left drying in an exhaust system. Specimens were mounted on metallic stubs with their longitudinal surfaces turned up, sputter-coated with gold and examined in scanning electron microscope operating at 20 kV. The region underneath the cavity floor (subsurface) was scanned and the most representative areas were recorded in different magnifications.

Statistical analysis

Data of dentin microhardness were analyzed by three-way ANOVA (factors: *dentin, energy* and *subsurface*) and Fisher's LSD Multiple-Comparisons tests using a statistical software (NCSS/PASS Dawson edition, NCSS, USA) at α=5% significance level.

RESULTS

The variance analysis revealed that there was a significant difference between dentin depths, energies, subsurface points, and in the factor interactions. Fisher's LSD Multiple-Comparison Test showed that microhardness of superficial dentin was significantly higher than deep dentin (Fig. 1). Comparing the energy levels (Fig. 2), regardless of the dentin depth, the energy of 160 mJ resulted in the highest microhardness values, and 360 mJ the lowest ones; microhardness values of both dentins irradiated with 200 mJ were lower than 160 mJ and the control, and higher than those lased with 260 and 300 mJ, which presented similar means. Values at all subsurface points were different and exhibited increasing microhardness throughout (Fig. 3).

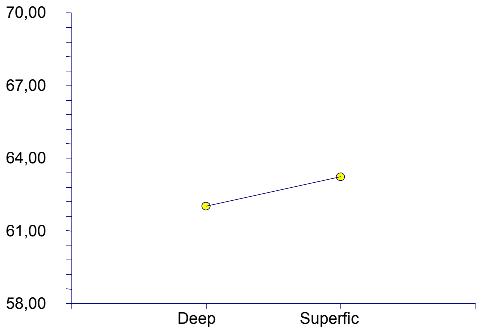


Figure 1. Overall means of subsurface microhardness according with the dentin depth.

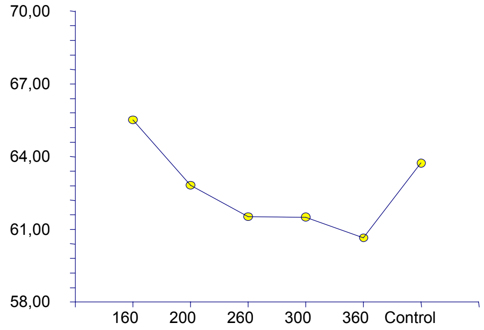


Figure 2. Means of subsurface microhardness as a function of the energy level, regardless of the dentin depth.

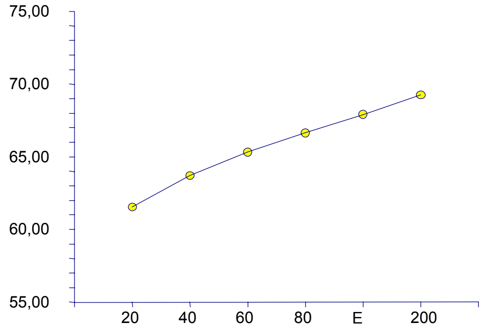


Figure 3. Means of subsurface microhardness of both dentin depths as a function of the subsurface points.

The interaction dentin depth x energy (Fig. 4) showed that the significantly lowest microhardness values of either superficial or deep dentin were obtained with a laser irradiation of 360 mJ; microhardness of superficial dentin ablated with 160 mJ was significantly higher than both the control and that irradiated with the other energies; microhardness of deep dentin lased with 160 and 200 mJ was similar to the control, and that with 260 and 300 mJ was lower in comparison to the control deep dentin; superficial and deep dentin presented similar microhardness when both were irradiated either with 260 or 300 mJ.

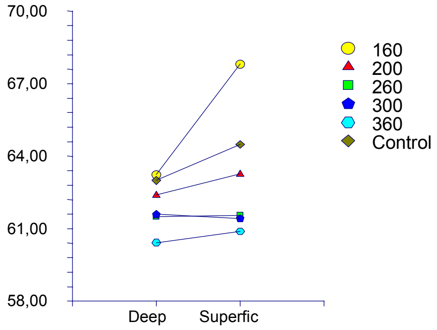


Figure 4. Means of subsurface microhardness as a function of the dentin depth and the energy level

Table 1. Knoop microhardness means values of superficial dentin and standard deviations according to the energy levels and depths

Subsurface/ Energy	***20 µm***	***40 µm***	***60 µm***	***80 µm***	***100 µm***	***200 µm***
160 mJ	70.6 ±1.2	64.9^a ±1.7	66.2^b ±1.7	67.7^c ±1.9	69.1^d ±1.5	70.1^e ±1.4
200 mJ	61.9 ±1	64.5 ±0.6	65.5 ±1	66.1 ±1.2	67.1 ±1.3	67.3 ±1.2
260 mJ	59.4 ±0.7	63.3 ±0.8	64.6 ±0.9	65.4 ±0.8	66.2 ±0.9	67.7 ±1.3
300 mJ	59.4 ±1.1	63.7 ±0.7	64.8 ±1	66.1 ±1	66.3 ±0.8	67.8 ±0.6
360 mJ	58.3 ±0.9	63.4 ±0.9	64.8 ±0.9	65.9 ±0.8	66.8 ±0.6	67.6 ±0.6
control	63.8 ±0.8	65.1^a ±0.7	66.2^b ±0.7	67.4^c ±0.6	68.6^d ±0.8	69.6^e ±0.5

Same superscript letters indicate statistical similarity

Tables 1 and 2 display the microhardness of superficial and deep dentin, respectively, according to each subsurface point and energy. The interaction of all factors revealed that for superficial dentin irradiated with 160 mJ, the subsurface microhardness was higher than the control at 20 μm and similar at the remaining points; for deep dentin with 160 mJ, the subsurface microhardness was similar to the control at all points; the other energies resulted in lower values than control at all subsurface points of both superficial and deep dentin.

Subsurface/ Energy	***20 µm***	***40 µm***	***60 µm***	***80 µm***	***100 µm***	***200 µm***
160 mJ	62.5^{a} ±1.1	63.9^{b} ±0.8	65.8^{c} ±0.8	67.2^{d} ±0.8	68.2^{e} ±0.8	69.7^{e} ±0.9
200 mJ	62.3^{a,f} ±1.2	62.8^{a,f} ±1.2	64.6 ±1.5	65.8 ±1.1	67.1 ±1.2	69.4 ±1.1
260 mJ	60.5 ±0.6	61.2 ±0.8	63.8 ±0.7	67.6 ±0.8	69.2 ±0.9	70.4 ±0.8
300 mJ	60.1 ±1.1	62.8 ±0.9	65.2 ±0.6	67.1 ±0.8	68.3 ±0.7	70.2 ±0.8
360 mJ	59.2 ±0.7	61.5 ±0.7	63.4 ±0.7	65.4 ±1.2	67.6 ±1.2	69.1 ±0.5
control	62.7^{a,f} ±1.	63.5^{b,f} ±1.5	65.8^{c} ±1.4	66.1^{d} ±1.3	68.6^{e} ±1.3	70.2^{e} ±1.2

Table 2. Knoop microhardness means values of deep dentin and standard deviations according to the energy levels and depths

Same superscript letters indicate statistical similarity

SEM micrographs of subsurface of both superficial and deep dentin irradiated with 160, 200, and 260 mJ (Figs. 5, 6, 7, 8, 9, and 10, respectively) showed a corrugated and wave appearance with opened tubules and the absence of smear layer, and a crystalline-like aspect along the interfaces. The SEM micrographs of both dentin depths irradiated with 300 and 360 mJ (Figs. 11, 12, 13, and 14, respectively) showed flatter surfaces with opened tubules and the absence of smear layer.

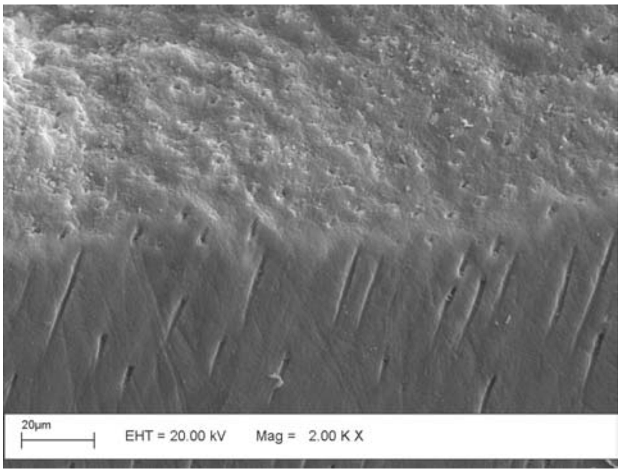


Figure 5. SEM micrograph of superficial dentin subsurface irradiated with 160 mJ

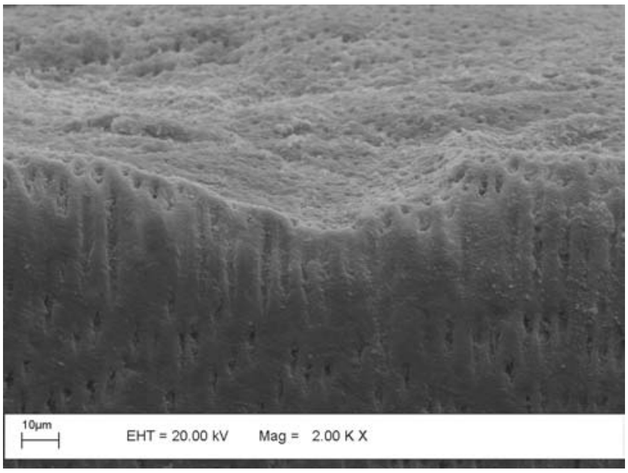


Figure 6. SEM micrograph of deep dentin subsurface irradiated with 160 mJ

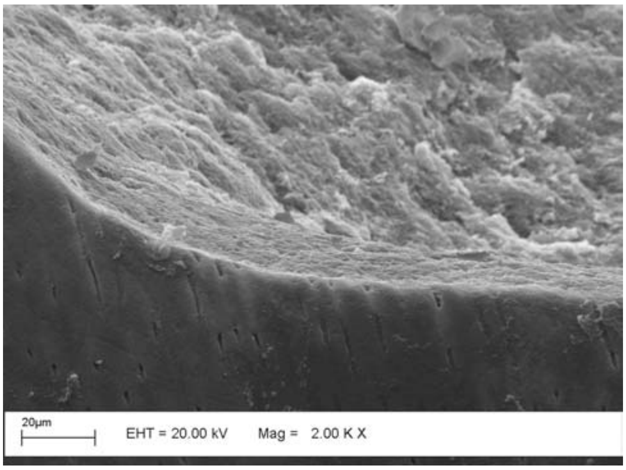


Figure 7. SEM micrograph of superficial dentin subsurface irradiated with 200 mJ

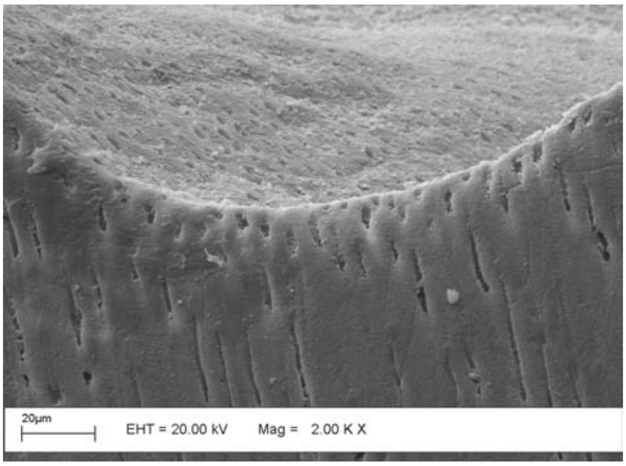


Figure 8. SEM micrograph of deep dentin subsurface irradiated with 200 mJ

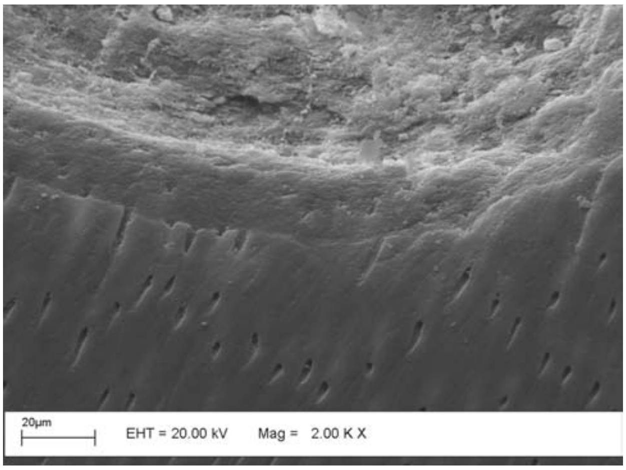


Figure 9. SEM micrograph of superficial dentin subsurface irradiated with 260 mJ

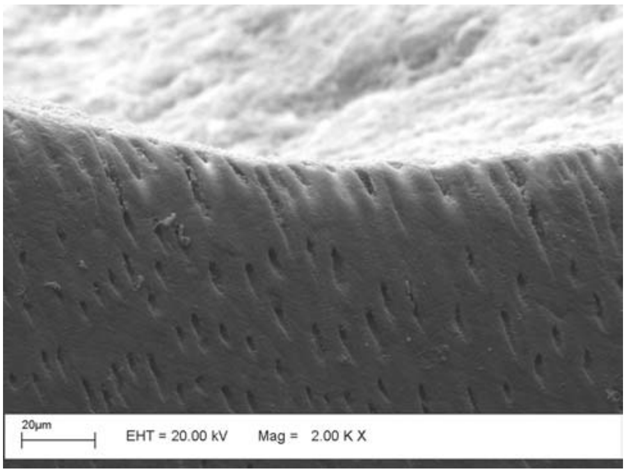


Figure 10. SEM micrograph of deep dentin subsurface irradiated with 260 mJ

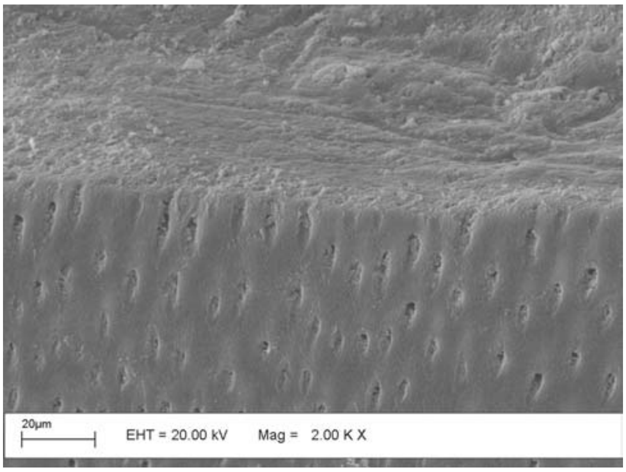


Figure 11. SEM micrograph of superficial dentin subsurface irradiated with 300 mJ

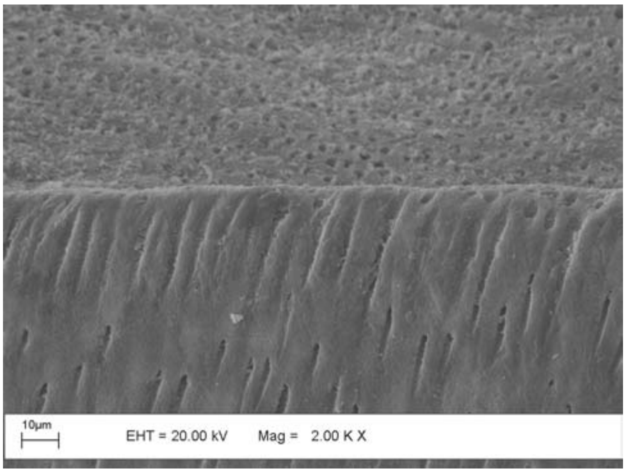


Figure 12. SEM micrograph of deep dentin subsurface irradiated with 300 mJ

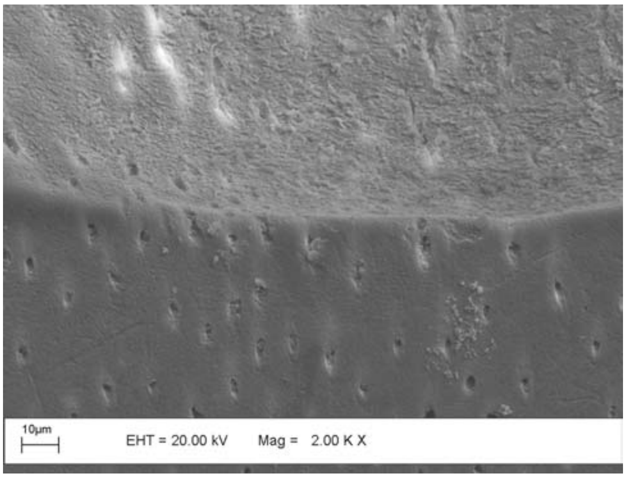


Figure 13. SEM micrograph of superficial dentin subsurface irradiated with 360 mJ

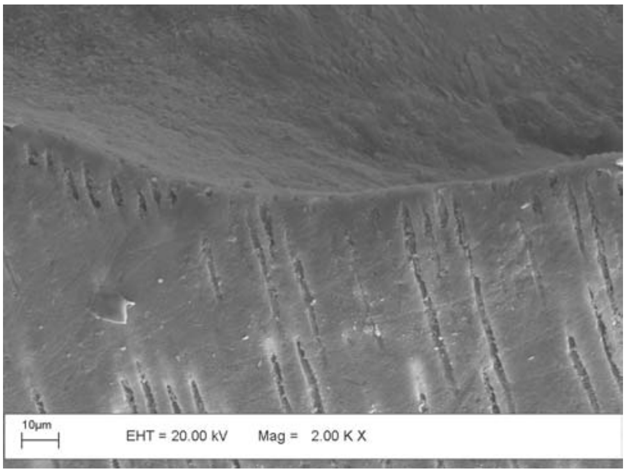


Figure 14. SEM micrograph of deep dentin subsurface irradiated with 360 mJ

DISCUSSION

The results obtained in this study indicated a decrease in the microhardness values of both superficial and deep dentin, in the tissues underneath the floor of the cavities prepared by Er:YAG laser using higher energy levels (mainly 260 mJ, 300 mJ and 360 mJ). In the face of such situation, it is quite possible to consider that the three highest energies were the most influent in the response of the microhardness. This finding reinforces previous statements [2, 9] that, although the use of high energy levels in dentin accelerates the ablation process, it reduces the mechanical properties of the irradiated substrates.

It is already known that the interaction of lasers with tissue components determines their main effect in biologic tissues. In human dentin, basically composed of water, hydroxyapatite and organic matrix [21], the Er:YAG laser provides efficient ablation rates since the incident energy is highly absorbed by both water and hydroxyapatite [18, 37]. Thus, the absorbed energy is transformed into mounting pressure inside the tissue, occurring successive microexplosions, which causes the removal of tissue components [38]. However, the elimination of the mineral phase from dentin surfaces modifies their morphology and mechanical properties [28, 32, 36], having a positive correlation between hardness and mineral content [39]. Moreover, changes in hardness and tissue composition produced by high Er:YAG laser energy levels may be related to the denaturation of the dentin organic matrix, with a strong modification of the collagen chain [40].

Additionally, it has been reported [41] that Er:YAG laser radiation modifies the calcium-to-phosphorus ratio. Besides, increased energies produce surface irregularities with greater ablation of intertubular dentin, which resulted in a protruded appearance of peritubular dentin and the opening of dentinal tubules [37]. Since peritubular dentin is highly mineralized [26], the thermal effects, the changes in size and the ultrastructure of apatite crystals probably corroborate the microhardness changes [42]. This way, the intense removal and/or modification of both inorganic and organic structures would become the substrate less resistant to local deformations under load conditions, which may explain the reduction in the dentin microhardness values after being irradiated with the high energy levels used in this study.

A significant increase in microhardness values was found only in the closest point (until 20 µm) under the floor of cavities prepared in superficial dentin using the lowest energy level (160 mJ). As already described above, the higher the energy, the higher the ablation rate, but causing evident alterations in the organic structures. Then, it may be considered that 160 mJ promoted lower tissue removal with unremarkable damages in the organic matrix, which could have increased the proportion of the mineral content. Additionally, this most superficial layer has a natural increased stiffness because its abundant intertubular dentin presents a more homogeneous distribution of the mineral phase within the collagen matrix [43].

In this study, the subsurface microhardness obtained at each point under the cavity floor was increasing throughout the full extent. This shows that during Er:YAG laser irradiation, almost all incident energy is consumed in the ablation process, which limits the deleterious thermal side effects in the tissues under the irradiated surface, especially under water [10, 11, 13, 14].

Taking into account the dentin depths, the superficial dentin microhardness was higher than deep dentin, thus accepting the hypothesis that, as in sound dentin [43], the decreased hardness near the pulp could be explained by a decrease in the hardness of the intertubular dentin matrix. Thus, it is likely that the intertubular dentin near the pulp is less mineralized [26] and influence the resistance to deformation of these substrates after being submitted to the ablation process by Er:YAG laser.

The clinical application of the knowledge on the mechanical properties of dentin under normal and altered conditions would help the adhesive restorative treatment [37, 44], since there is a strong relationship between dentin microhardness and bond strength [39]. Adhesive restorations are more retained by hard dentin due to their better mechanical stability, which indicates that microhardness may provide a first step towards predicting the behavior of dentin/restorations interfaces [24, 36], besides being a quantitative method to predict variations in the mineral content of dentin irradiated by Er:YAG laser [45].

CONCLUSIONS

Based on the results obtained, it may be concluded that the lowest energy level increased superficial dentin microhardness until the closest point under cavity preparation; deep dentin subsurface microhardness was not altered by 160 and 200 mJ, while higher energy levels reduced subsurface microhardness of both superficial and deep dentin.

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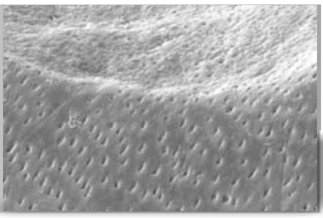
Conclusões



5. CONCLUSÕES GERAIS

Com base nos resultados obtidos neste estudo, pode-se concluir que:

- a capacidade de ablação da dentina foi diretamente relacionada com o nível de energia do laser Er:YAG utilizado para a realização de preparos cavitários e não dependeu da profundidade do substrato;
- morfologicamente pôde-se observar que na dentina profunda ocorreu uma ablação mais seletiva, com menor remoção da dentina peritubular;
- a microdureza da dentina foi influenciada pela profundidade do substrato e pelo nível de energia de irradiação do laser Er:YAG;
- na dentina superficial, a energia de 160 mJ promoveu um aumento na microdureza da região mais próxima ao preparo;
- a microdureza da dentina profunda não foi alterada quando foram utilizadas as energias de 160 e 200 mJ, enquanto os níveis de energia superiores promoveram uma diminuição na microdureza de ambas as dentinas.

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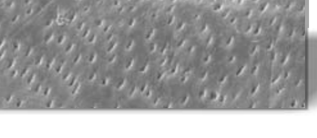

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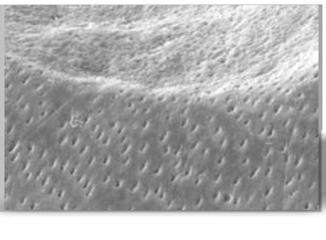
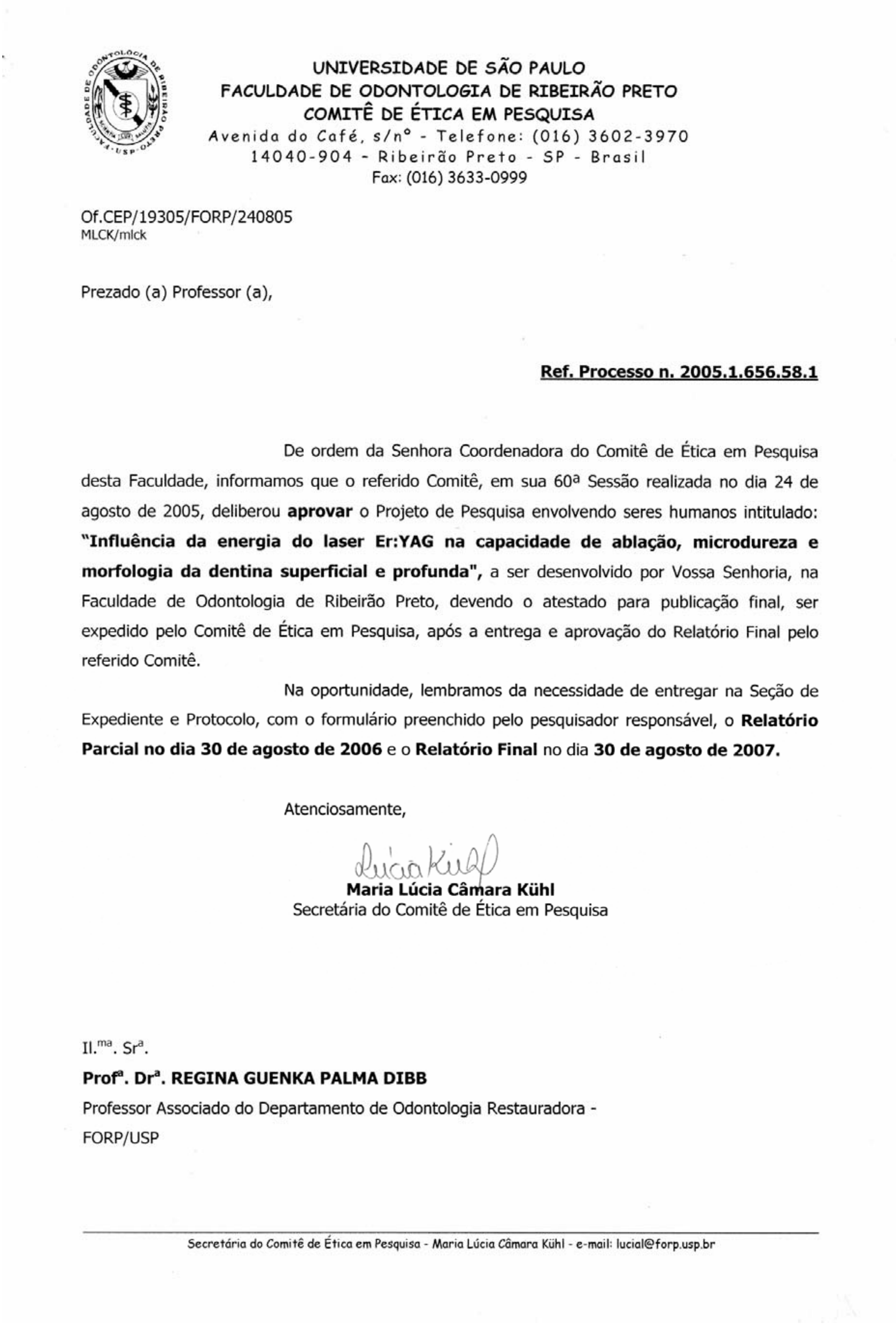
Anexos



ANEXO 1



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Ref. Processo n. 2005.1.656.58.1

De ordem da Senhora Coordenadora do Comitê de Ética em Pesquisa desta Faculdade, informamos que o referido Comitê, em sua 60ª Sessão realizada no dia 24 de agosto de 2005, deliberou **aprovar** o Projeto de Pesquisa envolvendo seres humanos intitulado: **"Influência da energia do laser Er:YAG na capacidade de ablação, microdureza e morfologia da dentina superficial e profunda",** a ser 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 entregar na Seção de Expediente e Protocolo, com o formulário preenchido pelo pesquisador responsável, o **Relatório Parcial no dia 30 de agosto de 2006** e o **Relatório Final** no dia **30 de agosto de 2007.**

Atenciosamente,

Lúcia Kühl
Maria Lúcia Câmara Kühl
Secretária do Comitê de Ética em Pesquisa

Il.ª. Srª.
Profª. Drª. REGINA GUENKA PALMA DIBB
Professor Associado do Departamento de Odontologia Restauradora -
FORP/USP

Secretária do Comitê de Ética em Pesquisa - Maria Lúcia Câmara Kühl - e-mail: lucial@forp.usp.br

ANEXO 2

Mensagem de Impressão do Windows Live Hotmail Página 1 de 3

Fwd: [Fwd: Photomedicine and Laser Surgery - Decision on Manuscrip
ID PHO-2007-2201]

De: **Michelle Chinelatti** (michinelatti@gmail.com)
Enviada: quinta-feira, 3 de janeiro de 2008 17:38:54
Para: Michelle Chinelatti (michinelatti@hotmail.com)

---------- Forwarded message ----------
From: **Regina Guenka Palma Dibb** <rgpalma@forp.usp.br>
Date: 20/12/2007 14:09
Subject: [Fwd: Photomedicine and Laser Surgery - Decision on Manuscript ID PHO-2007-2201]
To: michinelatti@gmail.com

19-Dec-2007

Dear Dr. Palma-Dibb:

Manuscript ID PHO-2007-2201 entitled "Ablation rate and morphology of superficial and deep dentin irradiated with different energies of Er:YAG laser" which you submitted to Photomedicine and Laser Surgery, has been reviewed. The comments of the reviewer(s) are included at the bottom of this letter.

The reviewer(s) have recommended revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript.

To revise your manuscript, log into http://mc.manuscriptcentral.com/photomedicine and enter your Author Center, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision.

You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript using a word processing program and save it on your computer. Please also highlight the changes to your manuscript within the document by using the track changes mode in MS Word or by using bold or colored text.Once the revised manuscript is prepared, you can upload it and submit it through your Author Center.

When submitting your revised manuscript, you will be able to respond to the comments made by the reviewer(s) in the space provided. You can use this space to document any changes you make to the original manuscript. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response to the reviewer(s).

As our readership is largely English-speaking we ask that all manuscripts be reviewed carefully for English grammar and syntax.

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Once again, thank you for submitting your manuscript to Photomedicine and Laser Surgery and I look forward to receiving your revision.

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ANEXO 3

Submissions Being Processed for Author Michelle Chinelatti

Página 1 de 1

Submissions Being Processed for Author Michelle Chinelatti

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		Effect of Er:YAG laser energies on superficial and deep dentin microhardness	Jan 29, 2008	Jan 29, 2008	Submitted

Page: 1 of 1 (1 total submissions)

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