Universidade Estadual de Campinas
Faculdade de Odontologia de Piracicaba

Lidiany Karla Azevêdo Rodrigues

Cirurgiã-dentista

“O USO DO LASER DE CO_2 NA PREVENÇÃO DA CÁRIE DENTÁRIA”

Tese apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas para a obtenção do título de Doutor em Odontologia, Área de Cariologia.

Piracicaba 2005

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Orientador: Profa. Dra. Marinês Nobre dos Santos Uchôa

Banca examinadora:
Prof. Dr. Alberto Carlos Botazzo Delbem
Prof. Dr. Carlos Augusto de Oliveira Fernandes
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Piracicaba 2005

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Aos meus pais, Paiva e Socorro

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[Albert Einstein]

SUMÁRIO

RESUMO 1

ABSTRACT 3

I- INTRODUÇÃO GERAL 5

II – PROPOSIÇÃO 8

III – CAPÍTULOS 9

CAPÍTULO 1 10

Carbon dioxide laser in dental caries prevention 10

CAPÍTULO 2 21

Caries inhibition around composite restorations by pulsed carbon dioxide laser application 21

CAPÍTULO 3 28

In Situ Evaluation of the Effects of CO_2 Laser and Fluoride Dentifrice on Caries Development in Human Enamel 28

IV – DISCUSSAO GERAL 47

V – CONCLUSÃO GERAL 53

VI – REFERÊNCIAS 54

ANEXO 60

RESUMO

A irradiação do esmalte dental com laser de CO_2, especialmente se associada ao flúor, aumenta a resistência deste substrato ao desafio ácido. Deste modo, esta tese, constituída por 3 artigos, teve por objetivos: (1) descrever as características do laser de CO_2 e revisar a literatura disponível enfocando seus efeitos na prevenção de cárie em esmalte e dentina, bem como discutir os efeitos deste mesmo laser quando associado ao flúor; (2) investigar, *in vitro,* o efeito do laser de CO_2 (λ = 10,6 μm), com duas densidades de energia, na inibição da desmineralização ao redor de restaurações de resina composta; (3) avaliar *in situ* os efeitos combinados de um TEA (Transversely Excited Atmospheric-pressure) laser de CO_2 (λ = 9,6 μm) e do dentifrício fluoretado na desmineralização do esmalte dental humano. No estudo 1, a literatura científica pertinente ao assunto foi pesquisada usando a base de dados *medline* e busca manual de referências citadas em artigos científicos. No estudo 2, preparos cavitários realizados com ponta diamantada em esmalte hígido tiveram seu ângulo cavo-superficial irradiado com laser de CO_2 com 8 ou 16 J/cm^2. Através de microdureza em corte longitudinal, avaliou-se a perda mineral *in vitro* dos grupos experimentais e controle no esmalte ao redor da restauração. No estudo 3, foi testado *in situ* o efeito do laser de CO_2 com 1,5 J/cm^2 associado ou não à utilização de dentifrício fluoretado na prevenção de cárie dentária. Avaliou-se a perda mineral do esmalte dental humano nos grupos experimentais e controle. Os resultados dos estudos 2 e 3 foram analisados estatisticamente pelos testes ANOVA e Tukey com nível de significância fixado em 5%. A análise da literatura apresentada no artigo 1 mostrou que pode haver um futuro promissor para o laser de CO_2 na prevenção de cárie dentária tendo seu efeito preventivo

potencializado quando utilizado em associação a compostos fluoretados. Os resultados do artigo 2 demonstraram que o laser utilizado foi efetivo na inibição da desmineralização do esmalte ao redor de restaurações de resina composta ($p < 0,05$) e que o aumento da energia não potencializou o efeito do laser. No terceiro estudo, observou-se que os tratamentos com laser e/ou dentifrício fluoretado foram capazes de inibir a desmineralização do esmalte *in situ*, tendo sido observado o melhor resultado de inibição da desmineralização quando o laser foi associado à utilização de dentifrício fluoretado. Em conclusão, os resultados desses estudos indicam que o laser de CO_2 é capaz de inibir a desmineralização do esmalte dental humano em situações de alto desafio cariogênico *in vitro* e *in situ*, apresentando efeito sinérgico quando associado ao flúor.

ABSTRACT

The irradiation of dental enamel by CO_2 laser, especially if combined with fluoride, increases the enamel acid resistance. Thus, this thesis, comprised by 3 manuscripts, aimed: (1) to describe the characteristics of the CO_2 laser and to review the literature with regard to its effects on caries inhibition in enamel and dentin. Another aim of this review is to discuss the effects of the CO_2 laser in combination with fluoride; (2) to investigate, *in vitro*, the effect of a carbon dioxide laser (λ = 10.6 µm), with two energy densities, on the enamel inhibition of demineralization around composite restorations; (3) to assess *in situ* the combined effects of a 9.6 µm TEA (Transversely Excited Atmospheric-pressure) CO_2 laser and fluoride dentifrice on the demineralization of human dental enamel. In study 1, the scientific literature related to the issue was searched using medline and manual tracing of references cited scientific papers. In study 2, cavity preparations performed with diamond bur on sound enamel had their cavo surface angle irradiated with CO_2 laser using 8 or 16 J/cm^2. *In vitro* mineral loss, in experimental and control groups, was evaluated in the enamel around the restoration. In manuscript 3, the *in situ* caries preventive effect of the CO_2 laser, with 1.5 J/cm^2, associated or not to fluoridated dentifrice, was tested. In the human dental enamel, mineral loss was evaluated, by cross-sectional microhardness, in experimental and control groups. The results of studies 2 and 3 were analyzed by ANOVA and Tukey test. The literature analysis presented in study 1 showed that there can be a promising future for CO_2 laser in caries prevention and its preventive effect is improved when associated to fluoride products. The results of study 2 demonstrated that the laser used was effective in inhibiting enamel demineralization around the composite restorations ($p < 0.05$). In the third manuscript, it was observed that the treatments with laser and/or

fluoridated dentifrice were able to inhibit the *in situ* enamel demineralization and the best demineralization inhibition result was observed when laser was combined with fluoridated dentifrice use. In conclusion, the results of these studies suggest that CO_2 laser is able of inhibiting enamel demineralization, in *in vitro* and *in situ* high cariogenic challenge situations, showing synergic effect with fluoride.

I- INTRODUÇÃO GERAL

O declínio da doença cárie ocorrido no mundo nas últimas décadas é atribuído ao amplo uso de compostos fluoretados (Clarkson & McLoughlin, 2000, Marthaler, 2004). Entre os métodos tópicos de uso de flúor, o dentifrício fluoretado e as aplicações tópicas profissionais com vernizes fluoretados são os mais comumente empregados. No entanto, o dentifrício fluoretado é considerado o mais relevante na acentuada redução da prevalência de cárie (Bratthall, 1996, Marthaler, 2003). Já foi demonstrado que o flúor do dentifrício é capaz de reduzir a perda de mineral do esmalte de dente íntegro, ou ativar a reposição de mineral do dente com lesão de cárie (Lynch *et al*., 2004), aumentando em 2 vezes a capacidade da saliva de repor mineral na superfície do esmalte desmineralizado (Cury, 2002).

Em conseqüência do seu declínio, a doença cárie tornou-se fortemente polarizada (Seppä, 2001), entretanto, grupos de crianças continuam apresentando alta atividade da doença (Silva Bastos *et al*., 2005). Adicionalmente, estudos clínicos têm demonstrado que a cárie adjacente é uma das principais razões para a troca de restaurações (Foster, 1994, Jokstad *et al*., 1994, Mjör & Qvist, 1997). Tais fatos enfatizam a necessidade do aperfeiçoamento de métodos preventivos já existentes, com a introdução de técnicas inovadoras que possam agir como coadjuvantes na prevenção e controle da cárie dentária neste segmento da população bem como em situações de maior susceptibilidade ao aparecimento de lesões, como no caso de cáries adjacentes a restaurações.

Neste contexto, desde o desenvolvimento do laser de rubi por Maiman em 1960, diferentes tipos de lasers, tais como Nd:YAG, Argônio, Er:YAG e CO_2 têm sido estudados para uso em Odontologia com o objetivo de prevenir a cárie dentária. No entanto, o comprimento de onda (λ) dos lasers de argônio (λ = 488-514 nm) e Nd:YAG (λ = 1.064 nm) parecem não ser tão efetivamente absorvidos pelo esmalte dental (Gonzalez *et al*., 1996). Além do mais, preparos cavitários confeccionados com lasers de Er:YAG (λ = 2.940 nm) e Er,Cr:YSGG (λ = 2.780 nm) não foram capazes de diminuir a susceptibilidade do esmalte adjacente a restaurações de resina, ao desenvolvimento subseqüente de lesões de cárie, *in vitro* (Apel *et al*., 2003). Durante os últimos 20 anos, vários estudos avaliaram os efeitos do laser de dióxido de carbono (CO_2) no potencial de prevenção da cárie e demonstraram que a irradiação do esmalte dental com laser de CO_2 promove uma redução significativa reatividade ácida deste substrato (Nelson *et al*., 1986, 1987, Featherstone *et al*., 1998; Kantorowitz *et al*., 1998; Tange *et al*., 2000, Hsu *et al*., 2000) e, quando associada a agentes fluoretados, o efeito de inibição de desmineralização pode ser potencializado (Featherstone *et al*., 1991; Nobre dos Santos *et al*., 2001, Hsu *et al*., 2001, Nobre dos Santos *et al*., 2002, Tepper *et al*., 2004).

Os comprimentos de onda obtidos com os lasers de CO_2 (λ= 9,3, 9,6, 10,3 e 10,6 μm) são mais apropriados para a utilização em esmalte dental, pois produzem radiação na região do infra-vermelho que coincide com algumas bandas de absorção da hidroxiapatita, principalmente os grupamentos fosfato e carbonato (Featherstone *et al*., 1998, Kantorowitz *et al*., 1998). Desta forma, maior efetividade na prevenção de cárie pode ser obtida com menor ocorrência de efeitos deletérios aos tecidos dentários (Zuerlein *et al*., 1999), proporcionando uma menor dissipação de raios incidentes, com maior rapidez e eficácia do laser. Com este laser, a maior parte da luz é absorvida nos poucos micrômetros externos da superfície do esmalte e convertida em calor, causando perda de carbonato do mineral e fusão dos cristais de hidroxiapatita, tendo como conseqüência uma diminuição na dissolução ácida desta estrutura (Hsu *et al*., 1994; Featherstone & Nelson, 1987).

A utilização da tecnologia laser associada ao flúor em indivíduos com alto risco de cárie ou lesões incipientes poderia ser um recurso preventivo efetivo, com as vantagens de uma única aplicação ser suficiente para o controle do desenvolvimento e progressão da doença, além de ser um método indolor e não invasivo.

No entanto, há ainda uma escassez de trabalhos científicos que tenham explorado os efeitos benéficos destes lasers na prevenção de cárie, principalmente em situações peculiares como ao redor de restaurações de resina composta, associado a compostos fluoretados bem como em estudos *in situ* e *in vivo*. Consequentemente, para que esta tecnologia possa ser empregada clinicamente com segurança torna-se necessária a realização de estudos que comprovem sua eficácia em situações de alto desafio cariogênico.

II – PROPOSIÇÃO

Os objetivos desse estudo foram:

1. Descrever as características do laser e CO_2 e revisar a literatura disponível enfocando seus efeitos na prevenção de cárie em esmalte e dentina, bem como discutir os efeitos do mesmo laser quando associado ao flúor.

2. Investigar, *in vitro,* o efeito de laser de CO_2 (λ = 10,6 µm), com duas densidades de energia, na inibição da desmineralização ao redor de restaurações de resina composta.

3. Avaliar *in situ* os efeitos combinados de um TEA laser de CO_2 (λ = 9,6 µm) e do dentifrício fluoretado na desmineralização do esmalte dental humano.

III – CAPÍTULOS

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

✓ Capítulo 1

"Carbon Dioxide Laser in Dental Caries Prevention." Rodrigues LKA, Nobre dos Santos, M, Pereira, D, Assaf, AV, Pardi, V. ***Journal of Dentistry***, v. 32, p. 531-540, 2004.

✓ Capítulo 2

"Caries inhibition around composite restorations by pulsed carbon dioxide laser application." Klein ALL, Rodrigues LKA, Eduardo CP, Nobre dos Santos M, Cury JA. ***European Journal of Oral Sciences***, v. 113, p. 239-244, 2005.

✓ Capítulo 3

"*In Situ* Evaluation of the Effects of CO_2 Laser and Fluoride Dentifrice on Caries Development in Human Enamel." Rodrigues LKA, Nobre dos Santos M, Fried D, Featherstone JDB. Este artigo será submetido à publicação no periódico ***Journal of Dental Research*.**

CAPÍTULO 1

Carbon dioxide laser in dental caries prevention

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Carbon dioxide laser in dental caries prevention

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KEYWORDS
CO_2 laser; Caries prevention; Enamel; Dentin; Fluoride

Summary *Objectives*. To describe CO_2 laser characteristics and to review the literature regarding its effects on caries inhibition in enamel and dentin. Another aim of this review is to discuss the effects of CO_2 laser in combination with fluoride.

Data and sources. The literature was searched for review and original research papers relating CO_2 laser characteristics, CO_2 laser effects on enamel and dentin, use of CO_2 laser in dental caries prevention and the effects of CO_2 laser in combination with fluoride. The articles have been selected using Medline and manual tracing of references cited in key papers otherwise not elicited.

Study selection. Dental studies pertinent to key aspects of review, and those that focus on CO_2 laser.

Conclusions. Irradiation of dental enamel by specific wavelengths and fluencies of CO_2 laser alters the hydroxyapatite crystals reducing the acid reactivity of the mineral; CO_2 laser irradiation in combination with fluoride treatment is more effective in inhibiting caries-like lesions than CO_2 laser irradiation or fluoride alone; When laser and fluoride are combined, it is possible to reduce laser energy density and fluoride levels; If this laser technology becomes available at a reasonable cost and the results can be applied in clinical practice, there will be a promising future for this laser in caries prevention.

Introduction

A significant decline in dental caries has been observed over the last few decades, not only in industrialized countries,[1] but also in developing ones.[2] However, the manifestation of this disease is still high in some individuals. Epidemiological studies have shown that a low percentage of

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children have a high number of dental caries.[3,4] In the high caries risk situations cited above, the development of new methods to prevent dental caries is extremely important to control the disease completely.

Since the development of a ruby crystal laser in 1960 by Maiman,[5] different lasers have been studied for use in dentistry. Many studies were performed to examine the effects of lasers on hard dental substrates with several different applications. Stern and Sognnaes[6] carried out the first study, which demonstrated that dental enamel exposure to ruby laser irradiation increased its acid resistance. Thus, initially, this kind of laser technology was used to remove carious enamel and dentin. Subsequently, lasers of various types were introduced, and the number of potential applications in dentistry increased.[7]

During the last 35 years, several studies, using different kinds of lasers, have demonstrated the potential of laser pre-treatment of enamel or tooth roots in inhibiting subsequent artificial caries-like lesions in the laboratory.[8–21] However, the wavelengths (λ) of the Argon lasers ($\lambda = 488$–514 nm) and Nd:YAG lasers ($\lambda = 1064$ nm) seem to fail to be effectively absorbed by enamel.[7] On the other hand, studies have been carried out to evaluate the effect of CO_2 laser on enamel and dentin structures, showing its absorption by dental tissues to be high.[22,23]

This laser was developed in 1964 by Patel et al.,[24] and it seems to be the most appropriate for preventing dental caries. Therefore, the aim of this article is to describe the characteristics of the CO_2 laser and to review the literature with regard to its effects on caries inhibition in enamel and dentin. Another aim of this review is to discuss the effects of the CO_2 laser in combination with fluoride.

Laser principles and definition of terms

The word 'laser' is an acronym for Light Amplification by Stimulated Emission of Radiation.[7] Lasers are devices that generate or amplify light and cover radiation at wavelengths ranging from infrared range to ultraviolet and even soft X-ray range. In general, a laser device consists of: (1) a laser medium like atoms, molecules, ions or semiconductor crystals; (2) a pumping process to excite these atoms (molecules, etc.) into higher energy levels; and (3) an optical resonator (laser cavity) that is composed of suitable optical feedback elements that allow the beam of radiation to pass through the laser medium.[7] In laser therapy several factors related to exposure need to be understood and considered. Thus the following definitions, frequently used in laser research publications, will help clarify the terminology used in this review.

- Wavelength is the distance between two successive wave crests (curved tops or ridges of an oscillating wave).
- Watt is a metric unit of measurement of the intensity of power that gives rise to the production of energy at the rate of 1 J/s.
- Joule is a measurement of energy that is equivalent to 0.239 cal.
- Energy density is the total amount of energy per unit surface area and is expressed in joules per square centimeter (J/cm^2).
- Hertz is a measure of frequency.

Laser characteristics

A carbon dioxide laser is one of the most popular and useful sources of coherent electromagnetic waves in the infrared spectrum. This is due to the several ways in which the laser operates (high voltage power supply in continuous wave (cw) or pulsed operation; emission of laser lines in regular, hot or sequential vibrational bands of CO_2 laser molecules) and the several orders of magnitude range of possible laser powers (from milliWatts to GigaWatts) that permit laser application in science, medicine and technology.[25] This laser uses a mixture of CO_2, N_2 and He, with CO_2 being the active laser medium (molecules that will collide with nitrogen molecules and will give out energy). Carbon dioxide lasers present over a hundred different emission laser lines with wavelengths ranging from 9 to 11 μm. The most powerful laser lines are centered at 9.3, 9.6, 10.3 and 10.6 μm, respectively. The 10.6 μm laser line is the strongest one, and most of the commercially available medical CO_2 lasers operate only at this wavelength. However, this kind of laser can be adapted to operate at the other wavelengths by various dispersive and non-dispersive methods and suppliers such as prisms, gratings (tool of choice when there is a need to separate light of different wavelengths with high resolution) and windows.[26]

For dentistry applications, all of the CO_2 lasers work in a non-contact mode and can be operated in cw, or pulsed beam.[7] The most important parameter in the way light affects the tissue is the laser line wavelength, but power and time exposure are also important.[27,28] The development of hollow waveguide technology with tubes of small diameter, a very short focal distance and tiny hand pieces, put an end to the problems of laser radiation transmission and access to difficult areas of the mouth, as well as preventing accidental irradiation of non-target tissues.[29]

The most efficient CO_2 laser for hard tissue is the TEA laser. The name TEA is an acronym for transversely excited atmospheric pressure laser. This CO_2 gas laser uses a transverse flow of gas and operates at higher pressures than other gas lasers, generally near atmospheric pressure. The laser is operated in a pulsed regime of a few Hz of repetition rate and pulses of 0.1–0.2 μs duration, resulting in peak powers in the GigaWatts range.[30]

Laser-tissue interactions

Laser energy (power × time) interacts with target substances according to the individual wavelengths. The different wavelengths have several degrees of relative absorption into the various components of hard and soft tissues. Laser-tissue interaction is also controlled by other irradiation parameters such as continuous or pulsed emission, repetition rate, pulse duration, pulse energy, beam size and delivery method, spatial and temporal characteristics of the laser beam, and optical properties of the substrate.[30,31] Earlier researches and new observations have provided parameters on which scientists and dentists can base the choice of appropriate wavelengths and other laser conditions to perform the desired tasks.[31,32] When delivered to the target tissue site, the laser light can be:[28,31]

- *Reflected:* It happens when the laser light reflects off of a surface in a direct or diffuse fashion.
- *Absorbed:* The laser energy interacts with the atoms in the target tissue and is generally converted to heat.
- *Transmitted:* The energy travels directly through tissue, causing no effect. It passes into underlying tissue.
- *Scattered:* The laser energy spreads out into a larger area. If the light is scattered, it is no longer a coherent beam and it is not delivered where needed.

To prevent dental caries, the laser light must alter the composition or solubility of the dental substrate and the energy must be strongly absorbed and efficiently converted to heat without damage to underlying or surrounding tissues.[32] Therefore, knowledge of the absorption (μ_a) and scattering (μ_s) coefficients (related to the energy absorbed per length unit) for diverse dental tissues is relevant. These coefficients have been determined and are given values with units of reciprocal centimeters (cm^{-1}). For materials with high absorption, $\mu_a > 100\ cm^{-1}$, the laser energy is absorbed within 100 μm of the surface and converted to heat.[30,31] Energy transport into the tissue is primarily due to heat conduction away from this surface, and light scattering is insignificant. This condition is representative of the interaction between dental hard substrates and CO_2 lasers.[30]

Dental enamel and dentin have weak absorption in the visible (400–700 nm) and near-infrared (1064 nm) spectrum.[30,33] Thus, the majority of the earliest studies that were carried out using near infrared or visible light lasers often applied very high irradiation intensities ($>10^7\ W/cm^2$) to generate the desired effects. The high energies used as well as the high transmission of these tissues in the visible and near infrared spectrum would be expected to result in subsurface damage to the pulp.[30]

On the other hand, regions and wavelengths where absorption is high correspond to specific components in the tissue. This condition is representative of the interaction between tooth substrates and CO_2 lasers.[30] Table 1 summarizes the absorption and scattering coefficients and reflectance percentage of human enamel and dentin for the CO_2 laser wavelengths.

Carbon dioxide laser energy weakens rapidly in most tissues because it is absorbed by water

Table 1 Approximate absorption and scattering coefficients and reflectance for dental enamel and dentin.

CO_2 wavelength (μm)	Absorption coefficient (cm^{-1})		Scattering coefficient (cm^{-1})		Reflectance (%)	
	Enamel	Dentin	Enamel	Dentin	Enamel	Dentin
9.3	5500	5000	Insignificant	Insignificant	37.7	8.6
9.6	8000	6500	Insignificant	Insignificant	49.4	16.7
10.3	1125	1200	Insignificant	Insignificant	15.8	10.3
10.6	825	800	Insignificant	Insignificant	13.2	8.8

Data from Refs. 22,23,31.

regardless of tissue color.[28,29] It is well absorbed by all biological tissues. This means that it is highly absorbed in oral mucosa, which is more than 90% water.[29] For dental enamel, the absorption coefficient is extremely high at 9.6 μm. This is due to the fact that the carbon dioxide laser produces radiation in the infrared region that coincides closely with some of the apatite absorption bands, mainly phosphate and carbonate group absorption bands.[16,17,32,33] Conventional CO_2 lasers used in medicine and dentistry emit light at 10.6 μm, which is also strongly absorbed by the mineral. However, the absorption of the wavelengths 9.3 and 9.6 μm is an order of magnitude higher than that for the conventional 10.6 μm CO_2 laser. The implications are that if there is an application that requires efficient and short heating of the mineral, (like for dental caries prevention using laser technology) 9.3 and 9.6 μm would be the preferred wavelengths.[16,22,23,32–34] To produce similar caries-inhibitory effects using a 9.6 and 10.6 μm, a 14-fold increase in the energy density is necessary when the second wavelength is used.[16]

In most studies using the cw CO_2 lasers, typical interaction times of 50 ms to 2 s were used. These interaction times are much longer than the thermal relaxation time of enamel (necessary time for cooling of the substrate), which is 100 μs.[30] The axial thermal relaxation time (T_r) of enamel was calculated to be approximately 60 μs for a 10 μm thermal gradient length and absorption coefficient of 1000 cm^{-1}. For those long interaction times, a large fraction of the absorbed laser energy is conducted away from the enamel surface into the interior of the tooth during the laser radiation, resulting in inefficient surface heating and possible pulp damage. In this way, the use of cw CO_2 laser irradiation for caries prevention is normally less effective and more dangerous than the use of pulsed CO_2 lasers.

Thus, pulsed lasers provide a way of increasing the peak power density while keeping the pulse energy density at low levels (hundreds of mJ/cm^2), thereby minimizing the cumulative energy deposition.[17,30] This means that changes such as fusion, melting, carbonate loss and re-crystallization of enamel crystals can be confined to a thin surface region without affecting the underlying dentin or pulp. The energy deposited at pulse durations shorter than T_r is 'thermally confined' to a thin layer at the enamel surface. On the other hand, pulse durations much longer than T_r result in ablation, which is not a desirable laser effect when caries prevention is intended.

With respect to the optimum number of pulses, Kantorowitz et al.[17] found that the best inhibitory effect of a TEA CO_2 laser on caries-like lesion development was obtained when 25 pulses of 0.1–0.2 μs were used.

In a separate study, considerable surface damage following laser irradiation over 200 mJ/pulse at a 9.6 μm wavelength was observed by scanning electron microscopy (SEM). The surface damage apparently was detrimental and provided less resistance to the acid challenge.[35] This pattern is consistent with the view that there is a point at which a further increase in the energy density of pulses significantly reduces the inhibition of caries progression. In the same way, an increase in the number of pulses can also induce an undesirable cumulative energy deposition, a consequent temperature elevation and possible pulp damage.[16,17]

In conclusion, in order to prevent dental caries it is more appropriate to use 9.6 μm CO_2 lasers, with pulsed operation, low energies (hundreds of mJ) and pulses of 100 μs or less. The total energy deposition would be of the order of a few Joules. These conditions are normally obtained with TEA CO_2 lasers working at a low Hz repetition rate, pulses of 0.1–0.2 μs, and GigaWatts peak powers. Another alternative, not yet explored, is the use of waveguide CO_2 lasers, operating at high repetition rates (kHz), and pulses in the 100 μs duration and low peak powers (100 W).[36] The advantage would be simplification of the technology and low cost of this system compared to the TEA CO_2 laser system, which has a more complex configuration. TEA lasers present a different source of supply and optic cavity and due to these differences it is more expensive and difficult to operate.

CO_2 laser in dental caries prevention

Carbon dioxide, Nd:YAG, Ho:YAG and Argon lasers have become more popular among dentists after being approved by the Food and Drug Administration for use on soft-tissue.[30] The Er:YAG laser was the first laser which was approved by FDA for limited hard-tissue procedures in 1997.[17] One potential application of dental lasers is preventive laser treatment of dental hard substrates to increase their resistance to caries. The role of CO_2 lasers in dental caries prevention has been explored since the 1960s. These studies used different types of CO_2 lasers: cw and pulsed lasers. Research on the effects of CO_2 lasers have focused on increasing the resistance to caries by reducing the rate of subsurface enamel and dentin demineralisation.[11–13,16–19,21,37–43] Furthermore, some studies have combined the effects of lasers with

Table 2 Wavelength, energy density and percentage inhibition of enamel caries by CO_2 laser in combination or not in combination with fluoride.

Author	Year	Wavelength	Beam	Energy density (J/cm^2)	With fluoride	Percentage inhibition
Nelson et al.[60]	1986	9.3	Pulsed	50	No	50
Nelson et al.[11]	1987	9.3	Pulsed	50	No	50
Kantorowitz et al.[17]	1998	10.6	Pulsed	12 per pulse	No	87
Featherstone et al.[16]	1998	9.6	Pulsed	2.5 per pulse	No	70
Phan et al.[48]	1999	9.6	Pulsed	1 per pulse	Yes	87
Young et al.[20]	2000	9.6	Pulsed	6.1	No	50
Hsu et al.[21]	2000	10.6	Pulsed	0.3 per pulse	No	98
Hsu et al.[49]	2001	10.6	Pulsed	0.3 per pulse	Yes	98
Nobre dos Santos et al.[50]	2001	9.6	Pulsed	1.5	Yes	76

fluoride.[44-51] A compilation of the main studies, which were performed to measure the CO_2 laser preventive effect on enamel with or without fluoride, is presented in Table 2. This table also gives the wavelengths and energy density used, which showed the greatest percentage of caries inhibition. For any procedure using lasers, the optical interactions between the laser light and enamel or dentin must be thoroughly understood to ensure a safe and effective treatment. However, the mechanisms of caries inhibition remain unclear. In the next two sections, we summarize the main kinds of experiments and explanations for the mechanism of caries inhibition by CO_2 lasers.

Effect of CO_2 laser on enamel

A variety of explanations have been given for the alteration of the dental enamel acid reactivity rate by treatment with CO_2 laser irradiation.

One explanation focused on the decrease in enamel permeability to chemical agents caused by physical fusion of the enamel surface microstructure.[37] However, this hypothesis seems to be unlikely, since the only study that has performed permeability experiments was carried out by Borggreven et al.[52] and they found that laser irradiation increased the permeability of enamel rather than decreasing it. These authors suggested that the reported resistance of lased enamel to subsurface demineralization might be due to chemical changes, such as the loss of organic matter and carbonate.

Another explanation has focused on the combination of reduced enamel permeability with a reduced solubility with melting, fusion and re-crystallization of enamel crystallites, sealing the enamel surface.[11,12] In addition, a less soluble compost (tetracalcium diphosphate monoxide) was identified as being a component of the melting surface and this layer presented reduced carbonate content.[12] On the other hand, a cross-sectional transmission electron microscopy examination revealed that the melting of the enamel surface was not homogeneous and usually occurred in limited areas.[53,54] Therefore, it seems that surface melting and fusion are not necessary to increase enamel resistance to demineralization,[17,35] which weakens this theory.

Stern et al.,[37] Ferreira et al.[53] and Kantola[39] carried out studies utilizing cw CO_2 lasers and demonstrated ultra-structural crystallographic effects, such as apatite crystals with a different shape and larger size, and loss of prismatic structure. The authors suggested that these effects could be responsible for the increased enamel acid resistance.

Fowler and Kuroda[41] found that the laser treatment at temperatures ranging from 100 to 650 °C may convert acid phosphate to pyrophosphate to inhibit demineralization, since Christoffersen[55] reported that pyrophosphate reduced the hydroxyapatite dissolution rate. Besides, the water content decreased and an overall reduction in total carbonate (CO_3^{2-}) content occurred. Another temperature range tested by these researchers (650–1100 °C) caused modifications in tooth enamel, which could increase or decrease the solubility depending on the Ca/P ratio and the resultant amounts of alpha-tricalcium phosphate and beta-tricalcium phosphate formed. However, treatments using temperatures over 1100 °C formed products that are expected to increase solubility in those regions that contain considerable amounts of these products.

In the same way, after producing artificial caries-like lesions in human enamel specimens heated at temperatures ranging from 100 to 600 °C, Sato[56] showed that those samples heated at temperatures below 300 °C had shallower caries lesions and a lower amount of dissolved calcium than enamel heated at temperatures ranging from

350 to 600 °C. Additionally, a temperature increase above 400 °C led to formation of pores in the enamel.

Yamamoto et al.[57] proposed a different model to explain the increase of the dental enamel resistance. The authors described a decrease in the apparent solubility of enamel after heat treatment to temperatures higher than 1200 °C. The decrease in solubility was attributed to the change in the rate of the dissolution kinetics due to the change from the more accessible dissolution site of hydroxyapatite (HAP) to a less reactive site of heat-treated apatite. The dissolution of synthetic apatites is explained by a model in which the dissolution behavior is governed by two types of dissolution sites (site Nos 1 and 2). Site No. 1 has an ion activity product (IAP) of around 10^{-121} and site No. 2 an IAP of 10^{-130}.[57] These IAPs represent the threshold IAP above which dissolution from a determined site will not occur. The reader should note that the word 'site' refers to dissolution rates which correspond to the IAP (based on hydroxyapatite stoichimetry). Heat-treated HAP has shown to possess only dissolution site No. 2, which is less soluble, while dissolution of the non-heated HAP is dominated by the more labile site No. 1.[44] Therefore, it can be speculated that heating HAP would provide a greater acid resistance. However, the dissolution studies estimate the acid resistance of the enamel surface by determining the dissolved calcium content, a phenomenon that takes place in the enamel surface and the caries process occurs also in the enamel subsurface. This fact may partly explain why the reduced dissolution rate obtained by CO_2 laser irradiation of dental enamel is higher than the caries inhibition effect observed when irradiated enamel is submitted to a cariogenic challenge. In addition, a recent study carried out by Tsai et al.,[58] showed that laser-treated enamel by a CO_2 laser tended to be more resistant to an acid challenge to a depth about 54 μm. Thus, the effect of laser irradiation is limited to the tooth surface area.

Another explanation to the caries preventive effect of the CO_2 laser is carbonate loss, which is a soluble mineral that is lost from the carbonated apatite tooth mineral during specific laser irradiation.[16,31,34] The reduced carbonate content could decrease demineralization of the substrate, because of a poorer fit of carbonate in the lattice, generating a less stable and more acid-soluble apatite phase.

Finally, the organic matrix in enamel has been shown to reduce enamel demineralization during an acid attack.[21] The laser irradiation effect, using very low energy density (0.3 J/cm^2) may heat enamel to a temperature lower than 400 °C. This effect can cause a partial decomposition of the organic matrix and could lead to a blockage of the inter- and intraprismatic spaces. Consequently, ion diffusion in enamel is compromised resulting in the reduction of enamel demineralization. This theory disagrees with the inorganic block theory, which advocates the melting of hydroxyapatite to block the enamel diffusion pathway.[21]

Therefore, it is possible to conclude that further studies are necessary to clarify the action mechanisms of lasers, which have a caries preventive effect, on dental enamel.

Effect of CO_2 laser on dentin

Since Kantola[59] showed that laser irradiation could be used to increase the dentin mineral content by preferential removal of the inherent water and protein, researchers have evaluated the susceptibility of dentin modified by various lasers systems to artificial caries-like lesion formation. However, a limited number of articles have been published concerning the changes in dentin irradiated by a CO_2 laser.

Dentin has a much higher content of water and protein than enamel, decreasing the contribution of the mineral phase and emphasizing the role of water and protein in the light absorption.[31] Like enamel, dentin absorption is low in the visible region, but the tissue scatters more than enamel,[30,31] which may have negative consequences such as subsurface vaporization, cracking and pulpal necrosis.

In dentin, the exact reasons for the caries lesion inhibition by laser treatments are also unknown. However, some hypotheses have been proposed. Kantola[59] suggested the first theory that investigated crystallographic changes in lased dentin by a cw CO_2 laser and showed that re-crystallization occurred due to laser irradiation. Simultaneously, growth in the crystal size of the crystallites was observed, and dentin of a low order of crystallinity, structurally changed in such a way as to closely resemble the crystalline structure of the hydroxyapatite of normal enamel. On the other hand, no investigation was performed in this study to verify the effects of the crystal growth on dentin resistance to demineralization.

Another action mechanism was proposed by Nelson et al.[60] who showed that fusion and melting occurred in the root dentin surface lased with a CO_2 laser at $\lambda = 9.3$ μm; these alterations were related to a caries-like lesion inhibition up to 50% with 50 J/cm^2 density energy. However, recent studies carried out by this research group have shown that the suitable energy densities for caries prevention in enamel should be lower than those used in this study.[16,31] Since dentin is more sensitive to laser irradiation than enamel, the incident energy would be even lower.

Nammour et al.[13] found good caries inhibition results in irradiated dentin. The authors used a cw CO_2 laser at $\lambda = 10.6\ \mu m$ operating at very high intensities and showed a sealed layer at the dentin surface, which delayed the diffusion of acid to the underlying sound dentin and reduced the extent of the caries lesion to a significant degree. However, in this study, laser irradiation did not completely seal the dentinal tubule lumen, thus allowing acid to diffuse through the large surface of the cracks created during the irradiation process. In addition, the energy density used was the same as that applied for caries removal, which is not appropriate for using in caries prevention, since ablation typically occurred with the first few laser pulses.

In another experiment, which used atomic analyses, the calcium (Ca) and phosphorus (P) contents of the dentin surfaces increased significantly after low-level laser irradiation, but the ratio of Ca to P was not altered and remained almost at the same level as the control (non-irradiated). The authors suggested that not only re-crystallization, but also an increase of inorganic content occurred in the laser-irradiated dentin surface, and this might be related to the increased resistance to demineralization.[18]

González et al.[42] and Kimura et al.[61] found conflicting results when they studied the CO_2 laser effect on human dentin. The first study showed[42] by SEM that the CO_2 laser effect with a wavelength of $10.6\ \mu m$ (2 W, 10 J, 0.2 s, 25 pulses) varied from charring, cratering, poring, fissuring, fracturing and cracking up to melting. It was also found that the dentinal tubules were not sealed. On the other hand, Kimura et al.[61] observed no craters or cracks, but documented many small molten and re-hardened particles on the sample surface. Some small cracks were seen in the subsurface layer, and the authors suggested that laser irradiation, using a $\lambda = 9.3\ \mu m$ and low energy density, affect the dentin surface minimally (less than $20\ \mu m$) and would be less likely to cause thermal dental pulp damage. However, these studies are not suitable for determining the optimum laser parameters for caries inhibition in dentin, because they did not investigate the effect of laser irradiation on the dentin acid resistance.

In conclusion, dentin is a less mineralized substrate than enamel and presents different characteristics. In order to modify dentin positively, the energy density necessary seems to be lower than that used for enamel. However, even the beneficial effect of dentin irradiation is not well established and its irradiation parameters still have to be determined.

CO_2 laser and fluoride on caries prevention

The decline in dental caries over the last few decades has been attributed to the widespread use of fluoride.[62] Furthermore, there is consensus that the main effect of fluoride is to interfere physically and chemically with caries development by reducing demineralization and enhancing remineralization of dental enamel.[63] Nevertheless, the fluoride effect is partial, since it cannot completely block dental caries development. Thus, the combined effects of laser and fluoride have been investigated in order to develop more effective procedures for caries prevention and control.[44–51,64–68] Some of these investigations were carried out using CO_2 lasers.[44–51]

In 1991, Featherstone et al.[43] observed that low energy laser treatment coupled with fluoride treatment entirely inhibited subsequent lesion progression in a pH-cycling model. The combination of laser irradiation and topical fluoride application decreased the enamel demineralization more than either fluoride treatment or laser treatment alone. In this study, fluoride was applied after the irradiation.

Tagomori and Morioka[65] suggested that laser-modified enamel has an enhanced uptake of acidulated phosphate fluoride and that this fluoride uptake was greater when laser treatment was performed before the fluoride treatment. This could decrease enamel demineralization by retention of fluoride. Other authors in agreement with this hypothesis are Hossain et al.[51] These studies suggested that the combination of CO_2 laser irradiation with NaF solution was more effective in preventing dental caries than CO_2 laser irradiation alone. In addition, they suggested that the retention of fluoride solution may also influence the caries inhibition effect and that laser irradiation might prolong the retention of fluoride in the enamel or dentin microstructures by increasing their adhesion to the underlying surface, keeping the effect for a longer time.

Fox et al.[44] proposed a different theory for the efficiency of this combination of CO_2 laser and fluoride. These authors treated sound enamel with cw $\lambda = 10.6\ \mu m$ radiation followed by treatment of fluoride or dodecylamine HCl or ethane-1-hydroxyl-1, 1-diphosphonic acid and observed a significant synergism between laser treatment and these chemical dissolution rate inhibitors. It was hypothesized that thermal treatment with lasers converts the carbonated hydroxyapatite of tooth enamel to a less soluble mineral, and chemical inhibitors work by a common ion effect of the fluorapatitic surface, which is more effective on the less soluble laser-modified enamel. In the same way, Meurman et al.[46] showed that it is possible to transform HAP crystals to fluorapatite (FAP) crystals instantaneously in the presence of fluoride using a CO_2 laser, and the threshold energy density needed was 38 J/cm^2. In this work, there was no investigation about the acid dissolution of the end product, because the researchers assumed that FAP is more resistant to acid attack than HAP. Additionally, the energy density used to transform HAP to FAP was very high.

Hsu et al.[47] tested the combined effects of CO_2 laser ($\lambda = 10.6\ \mu m$) irradiation and solution fluoride ion on enamel demineralization. The authors found that lased enamel had an increased acid-resistance with increasing laser energy density and, at the highest energy density of 170 J/cm^2, there was little or no lesion development in the fluoride-free dissolution medium. In the presence of fluoride, there was only modest caries development in the unlased enamel and, at an energy density 50% lower (85 J/cm^2) than the highest energy density used without fluoride, the enamel surface was found to be completely protected. Although, the combination of the methods resulted in a lower energy density being used, the effective energy density used could still be considered as being high.

Phan et al.[48] proposed that the mechanism for FAP transformation is as follows. During the fluoride gel treatment, fluoride ions diffuse through the pores between the enamel rods to deposit and form an F-veneer layer covering all the enamel rods. Following the CO_2 laser ($\lambda = 9.6\ \mu m$) irradiation, this thin F-veneer layer, along with a few additional outer micrometer of enamel surface were thermally melted and recrystallized to rearrange themselves into a new structure, the FAP mineral. This study found that the dissolution rates showed some synergistic benefits from combining fluoride and laser treatment, and that the concentrations of fluoride content incorporated into enamel structure is much higher when demineralized enamel is irradiated than when sound enamel is irradiated.

Hsu et al.[49] investigated the interaction among CO_2 laser irradiation, fluoride and the organic matrix on the human enamel demineralization. A microradiograph analysis performed after a pH-cycling procedure indicated that the combined fluoride-laser treatment led to 98.3 and 95.1% reductions in mineral loss for enamel with and without an organic matrix, respectively, when compared to sound enamel. It was demonstrated that the reduced effect (74%) of laser irradiation on enamel without an organic matrix could be compensated by the presence of fluoride during laser irradiation.

Another recent work revealed that the combination of a new $\lambda = 9.6\ \mu m$ TEA CO_2 laser and acidulated phosphate fluoride produced a significant protective effect against caries progression and caries development in smooth surfaces.[50] This research tested the ideal fluoride application time, before or after laser treatment. The best result was obtained when the fluoride was used before irradiation at an energy density of 1.5 J/cm^2 per pulse.[50]

It can be seen that there is no consensus with regard to whether the fluoride treatment should be performed before or after laser irradiation. However, all experiments associating CO_2 laser irradiation and fluoride treatment showed better results in caries prevention when compared to one single treatment. Therefore, such 'combination therapy' may be clinically effective while, at the same time, involving only moderate daily doses of both fluoride and low energy levels of laser irradiation.

Conclusions

- Irradiation of dental enamel by specific wavelengths and energy densities of CO_2 laser alters the hydroxyapatite crystals reducing the acid reactivity of the mineral;
- CO_2 laser irradiation in combination with fluoride treatment is more effective in inhibiting caries-like lesions than CO_2 laser irradiation or fluoride alone;
- When a CO_2 laser and fluoride are combined, it is possible to reduce laser energy density and fluoride levels;
- If this CO_2 laser technology becomes available at a reasonable cost and the results can be applied in clinical practice, there is a promising future for this laser in caries prevention.

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CAPÍTULO 2

Caries inhibition around composite restorations by pulsed carbon dioxide laser application

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Caries inhibition around composite restorations by pulsed carbon dioxide laser application

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This *in vitro* study aimed to evaluate whether laser irradiation of cavity margins reduces enamel demineralization around composite restoration. Enamel cavities were prepared in 33 human enamel slabs, which were randomly divided into three groups. One group was kept as a control, and the cavosurface margin of the cavities of the other groups were irradiated, using a CO_2 laser ($\lambda = 10.6\ \mu$m), at 8 J·cm^{-2} or 16 J·cm^{-2}. The cavities were restored with a resin-based composite, according to the manufacturer's specifications. Before restoration, scanning electron microscopy was performed on one specimen of each group. The remaining slabs were submitted to thermal and pH-cycling models. Enamel mineral loss, at 50 and 100 μm from the restoration margin, was assessed by cross-sectional microhardness analyses. Fusion and melting were observed in the irradiated groups. Mineral loss at 50 μm from the restoration margin was significantly inhibited in the irradiated groups compared to the control group, but at 100 μm from the restoration margin, mineral loss at only the highest laser energy density differed statistically from the control group. The difference between the irradiated groups was not statistically significant at either 50 or 100 μm from the restoration margin. In conclusion, irradiatiation of the cavosurface margin of cavities, using a pulsed CO_2 laser, is able to inhibit enamel demineralization around composite restorations, and an energy density of 16 J·cm^{-2} is efficient, even at 100 μm from the cavity margin.

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Clinical studies have demonstrated that secondary caries lesion is one of the main reasons for replacing restorations (1–4). The limited durability of dental restorations puts some patients in repetitive restorative cycles that make the restorations larger and the therapy required more complex (5). Thus, preventing or slowing down lesion progression could reduce the rate of restoration replacement, thereby reducing the need for additional restorative treatment and costs.

The tooth structure immediately adjacent to restorations is susceptible to secondary caries (6, 7) owing to the imperfect adaptation of restorative materials and subsequent micro leakage. Other factors, such as the chemical composition of enamel and dentin in the cavity wall, and the characteristics of the restorative material used, affect the progression of this type of caries lesion (8). Therefore, in order to identify methods of preventing secondary caries and increasing the durability of clinical dental restoration, different technologies have been introduced and applied in the dental clinic.

Since the development of the ruby laser by Maiman (9) in 1960, several studies have demonstrated that laser pretreatment of enamel can inhibit subsequent artificial caries-like lesions. However, the wavelengths (λ) of argon lasers ($\lambda = 488$–514 nm) and Nd:YAG lasers ($\lambda = 1064$ nm) seemed to fail to be effectively absorbed by enamel (10). Furthermore, cavity preparation performed by Er:YAG ($\lambda = 2940$ nm) and Er,Cr:YSGG ($\lambda = 2780$ nm) was found not to be capable of reducing the susceptibility of the prepared enamel to *in vitro* demineralization (11). On the other hand, studies carried out to evaluate the effect of a CO_2 laser on enamel and dentin structures showed its absorption by dental tissues to be high. This is a result of the fact that the CO_2 laser produces radiation in the infrared region, which coincides closely with some of the apatite absorption bands, mainly phosphate and carbonate group absorption bands (12). Therefore, it seems that the CO_2 laser is the most appropriate for preventing dental caries (12–14).

Hence, CO_2 laser radiation at safe energy levels can be used to thermally modify carbonated hydroxyapatite (the mineral component of dental hard tissues) to form a purer hydroxyapatite phase that is more resistant to acid dissolution. Konishi *et al.* (8) demonstrated that caries removal by a pulsed CO_2 laser produced cavity walls that were more resistant to secondary caries than those produced by mechanical removal. Thus, the irradiation of surfaces adjacent to restorations might act as a prophylactic measure against the formation of secondary caries. However, the energy density necessary for removing caries lesions is higher than that used for caries prevention. As there are threshold conditions above which a pulsed CO_2 laser may cause detrimental changes to dental tissues (15), the irradiation of cavity walls seems to be an appropriate alternative for using to prevent secondary caries without causing risk of pulp damage.

Therefore, the present research aimed to investigate, *in vitro*, the effect of a CO_2 laser ($\lambda = 10.6$ μm), at two energy densities, on enamel demineralization inhibition around composite restoration.

Material and methods

Ethical aspects

The teeth used in this investigation were collected from adults living in Piracicaba, Brazil, in conformity with the norms of the Research and Ethics Committee of FOP-UNICAMP (Process no. 90/2000).

Experimental design

Thirty-three extracted impacted human third molars were used to perform this *in vitro* study. The teeth were stored in 0.1% thymol solution and were sterilized by using 4.08 kGy of gamma radiation (16). The teeth had more than two-thirds of formed root, and were free of apparent caries, macroscopic cracks, and abrasions and staining, as assessed by visual examination.

The factor in study was the laser energy density used to irradiate the cavosurface margins of the cavities. These cavities were mechanically prepared in 33 enamel slabs, which were randomly allocated to three groups, containing 11 slabs each, by using the lottery method (17). One group was retained as the control and the other two groups were laser treated with 8 J·cm^{-2} (group Laser8) or 16 J·cm^{-2} (group Laser16) irradiation. Before being restored, one slab from each group was kept for analysis by scanning electron microscopy (SEM). All groups were submitted to thermal and pH-cycling models. At the end of the study, specimens were sectioned and mineral loss from enamel was determined around the restoration by the analysis of cross-sectional microhardness.

Slab and cavity preparations

One enamel slab ($4 \times 4 \times 2$ mm) was obtained from the buccal surface of each tooth by using a water-cooled diamond saw and a cutting machine (Isomet; Buehler, Lake, Bluff, IL, USA). A #3100 cylindrical diamond bur (KG Sorensen, Barueri, Brazil) in a high-speed turbine with air-water spray was used. A standard cavity was prepared with all margins in enamel on buccal surface (diameter 1.7 ± 1 mm, and depth 1.5 mm). All tooth surfaces of the slabs, except the prepared surface, were protected with acid-resistant nail varnish (Fig. 1).

Laser irradiation

The cavity margins of the slabs of groups Laser8 and Laser16 were irradiated by pulsed CO_2 laser (Opus 20 series number SA 3101004; Opus Dent, Tel Aviv, Israel) emitting photons at 10.6 μm wavelength. Group Laser8 was irradiated with 1 W output power and Group Laser16 was irradiated with 2 W output power. The pulse duration was 50 ms, and the pulse repetition rate was 2 Hz. The beam spot size was 0.8 mm^2 with the use of a 0.6-mm diameter fibre. Irradiation was performed in a non-contact mode with a focused beam at 0.5 cm of working distance. A power meter Coherent Radiation-Palo Alto Model-201 (Coherent Radiation, Palo Alto, CA, USA) was used to measure the peak powers, and 0.8 and 1.6 W peak powers were determined. These output powers are considerably lower than those displayed by the setting on the laser (1 and 2 W, respectively). The tip of the laser waveguide was clamped to guarantee that the diameter of the focal spot at the distance used was 0.8. Thus, the energy densities used were ≈ 8 and 16 J·cm^{-2}.

The cavosurface margin of each preparation was irradiated with the tip of the laser in a fixed position and the enamel slab being moved manually. As the cavity diameter was 1.7 mm, irradiation was carried out for ≈ 30 s. This procedure was repeated once more, so that each 'spot' in a cavity was irradiated twice to provide uniform coverage of the cavity surface. The precision of the method seemed to be commensurate with the clinical procedure.

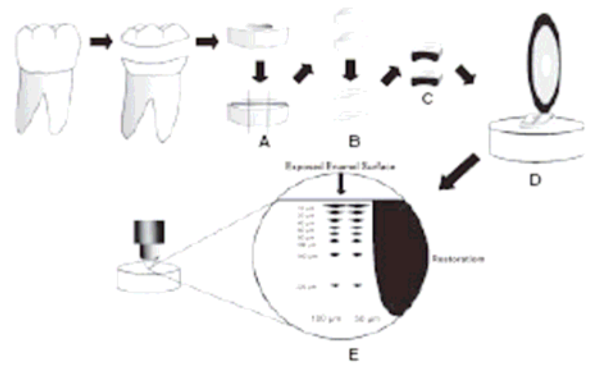

Fig. 1. (A) Human enamel slab preparation. (B) Resin restoration on slab. (C) Enamel slab protected by nail varnish. (D) The enamel slab was sectioned in the restoration central area. (E) Pictorial representation of cross-sectional microhardness measurements.

Sem

The cavity of one slab from each group was analysed by SEM. These specimens were longitudinally fractured through the enamel cavity preparation and the cut side was coated with a thin layer of gold ($\approx$ 10–12 nm thickness). Observations were then made with a JEOL JSM-5600 LV Scanning Electron Microscope (Jeol, Peabody, MA, USA) at 15 kV and with magnifications up to $\times 2{,}000$.

Restoration of enamel cavities

All cavities were restored using a resin-based composite Filtek Z-250 (3M Dental Products Division, St Paul, MN, USA; A3 shade) according to the manufacturer's specifications. A dental adhesive Single Bond (3M Dental Products Division) was used according to the manufacturer's instructions. Cavities were restored in one increment and light-polymerized for 40 s (Optilux 400; Demetron Research, Danbury, CT, USA). The light output was tested (480 ± 30 mW cm^{-2}) before each use by using a Demetron Model 100 radiometer (Demetron Research). All slabs were stored in 100% humidity for 24 h and then polished using the Sof-lex disk system for 15 s with each disk (3M Dental Products Division).

Thermal cycling and pH cycling

The restored slabs were subjected to 1,000 thermal cycles, each cycle alternating between 5°C ± 1°C and 55°C ± 1°C, for 60 s at each temperature and with 7 s transfer intervals between the baths. All groups were thermal cycled at the same time.

Next, the specimens were submitted to five demineralization–remineralization cycles at 37°C, using the model originally described by FEATHERSTONE *et al.* (18) and modified by ARGENTA *et al.* (19), which induces a typical subsurface caries-like lesion of ≈ 50–60 μm in depth. Each cycle comprised 3 h of immersion in demineralizing solution followed by ≈21 h of immersion in remineralizing solution. The demineralizing solution was composed of 75 mM acetate buffer, pH 4.3, containing 2.0 mM calcium and 2.0 mM phosphate. The composition of the remineralizing solution was 20 mM cacodylate buffer, pH 7.4, containing 1.5 mM calcium, 0.9 mM phosphate and 0.15 M KCl. The proportion of demineralizing and remineralizing solutions per area of exposed enamel was 0.73 and 1.45 ml·mm^{-2}, respectively. The pH-cycling process started with the demineralizing phase. Between the demineralizing and remineralizing stages, and at the end of the pH-cycling, the specimens were washed with deionized and distilled water for 10 s and wiped with tissue paper. There was no change of demineralizing or remineralizing solutions during this 5-day period. Both solutions contained thymol crystals to avoid microbial growth. After completing the cycling procedure, the specimens were stored in constant relative humidity of 100%.

Cross-sectional microhardness analysis

Each enamel slab was longitudinally sectioned by a cut through the centre of the restoration. The segments were embedded in acrylic resin and polished using an Arotec APL-4 polishing machine with 400, 600 and 1,200-grade sandpaper, followed by 6 μm, 3 μm and 1 μm diamond abrasive paste (Buehler) on polishing cloths. Cross-sectional microhardness measurements were performed by using a microhardness tester (Future Tech FM-ARS; Tokyo, Japan) with a Knoop diamond under a 25 g load for 5 s. Two lanes of eight indentations each were made, one lane being 50 μm distant from the preparation margin and the other, 100 μm distant. The indentations were made at the following depths: 10, 20, 40, 60, 80, 100, 140 and 220 μm from the outer enamel (Fig. 1).

The mean Knoop hardness number values at each distance from the surface, and at 50 and 100 μm from the enamel–restoration interface, were obtained and converted into volume percentage mineral, according to FEATHERSTONE *et al.* (20). Volume percentage mineral was plotted against depth for each specimen and the integrated mineral content of the lesion was calculated. A mean of volume percentage mineral for depths greater than 100 μm was used as a measure of the integrated mineral content of inner sound enamel. To compute ΔZ parameters, the integrated mineral content of the lesion was subtracted from the value obtained for sound enamel (21–23). Based on the mean ΔZ parameter, the inhibition percentage of caries-lesion progression, at 50 and 100 μm from the restoration margin, was calculated for the irradiated groups as follows:

$$\text{Percentage inhibition} = [(\Delta Z\text{Control} - \Delta Z\text{Treatment}) \div \Delta Z\text{Control}] \times 100.$$

Statistical analysis

In order to assess the effect of the treatments, the dependent variables ΔZ and volume percentage of mineral at different depths from the surface, were independently analysed by analysis of variance (ANOVA). The volume percentage of mineral was evaluated at eight depths and at two distances. Each depth analysis was performed independently in order to compare the treatment effects per layer. ANOVA was followed by the Tukey test to evaluate the significance of all pairwise comparisons. The software SAS system (version 8.02, SAS Institute Inc., Cary: NC, 1999) was used and the significance limit was set at 5%.

Results

The SEM observations showed evidence of melting and fusion in specimens treated with the CO_2 laser (Fig. 2).

The ANOVA results showed that CO_2 laser irradiation had a significant effect on the caries process at 50 μm and at 100 μm from the cavity margin. At 50 μm, the ΔZ values were statically significantly lower in irradiated groups than in non-irradiated groups. However, there were no statistically significant differences between the irradiated groups (Table 1). At 100 μm from the cavity margin, only the Laser16 group presented a statistically significant difference when compared with the control

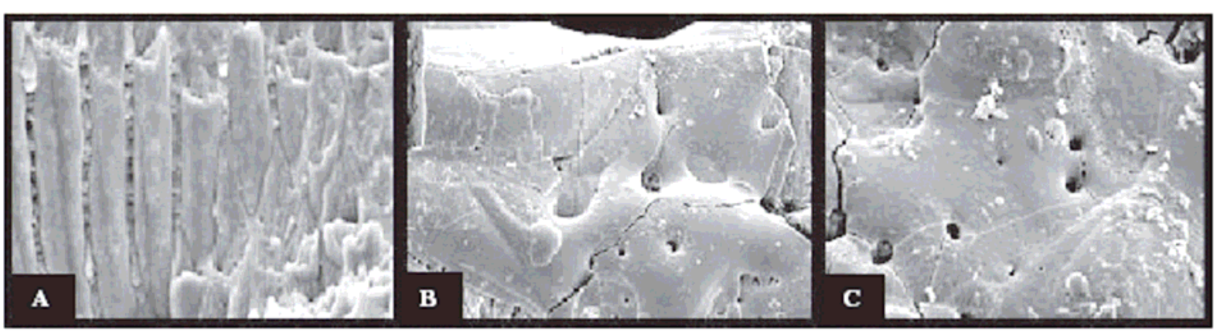

Fig. 2. Representative scanning electron micrographs of irradiated and non-irradiated specimens. Surface morphology of enamel cavity preparation with a diamond drill (A). Enamel cavities irradiated with a CO_2 laser using (B) 8 J·cm^{-2} or (C) 16 J·cm^{-2}. Magnification ×2000.

Table 1

Mineral loss (ΔZ) and percentage of caries inhibition, according to the treatments, for each studied distance

Treatment/groups	Distance from the restoration margin (μm) 50		100	
	ΔZ	Demineralization inhibition (%)	ΔZ	Demineralization inhibition (%)
Control	1222.71 ± 409.8[a]	–	1330.52 ± 624.1[a]	–
Laser8	576.02 ± 475.8[b]	52.8	761.33 ± 429.2[a,b]	42.7
Laser16	440.31 ± 374.4[b]	64.0	692.17 ± 639.0[b]	48.0

Data are expressed as mean value ± standard deviation ($n = 10$). Superscript letters indicate statistically significant differences among treatment groups at each depth ($P < 0.05$).

group. Moreover, there were no statistically significant differences between the irradiated groups (Table 1). In this investigation, the percentage of caries inhibition ranged from 42.7 to 52.8% for the Laser8 group and from 48.0 to 64.0% for the Laser16 group (Table 1).

At 50 μm from the enamel–restoration interface (Fig. 3), the irradiated groups showed, at 20 and 40 μm depths, a statistically lower volume percentage mineral loss than the control group ($P < 0.05$). However, at 100 μm from the restoration margin (Fig. 4), the statistically significant difference ($P < 0.05$) between the irradiated groups and the control group was only observed at 20 μm depth for the Laser16 group. The irradiated groups did not differ statistically ($P > 0.05$) between each other either at any distance from the restoration margin or at any depth from the enamel surface (Figs 3 and 4).

Discussion

The results of this study show that the irradiation of cavity walls by a pulsed CO_2 laser reduces the enamel mineral loss around composite restorations submitted to thermal and cariogenic challenges. This may be attributed to the effect of this type of laser treatment, which induces thermal decomposition of the carbonated hydroxyapatite mineral in the irradiated enamel, forming a less soluble hydroxyapatite phase that is more resistant to acid dissolution (10, 13). The findings also show that the enamel adjacent to composite restorations of the irradiated groups has a lower mineral loss than that found in the control group, mainly near the restoration margin. Previous studies have also shown significant inhibition of enamel demineralization following treatment with a CO_2 laser (12, 24–29). In these studies, the percentage of caries inhibition ranged from 17 to 98% and varied according to the type of laser beam, wavelength, operational mode and energy output (27, 28). In the present study we achieved a maximum inhibition of 64%, which is lower than the 87% reported by Kantorowitz *et al.* (25) who used similar parameters and laser wavelength. This discrepancy might have been caused by the higher susceptibility of restoration margins to caries development than enamel smooth surface, making caries inhibition more difficult (8).

However, no studies report an evaluation of the inhibition of secondary caries by laser irradiation of the cavosurface margin of the cavity preparation. The present data are in agreement with those of Konishi *et al.* (8) who demonstrated that laser treatment used to remove artificial caries was also able to inhibit cavity wall demineralization. The benefit of cavity irradiation would be the

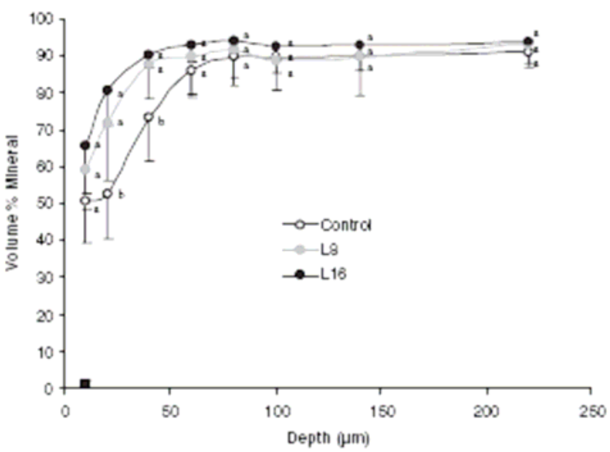

Fig. 3. Enamel volume % mineral at 50 μm from the margin restoration according to the treatments and the distance from the surface. Different letters show statistically significant differences among treatment groups at each depth (Tukey test; $P < 0.05$). Bars denote standard deviation.

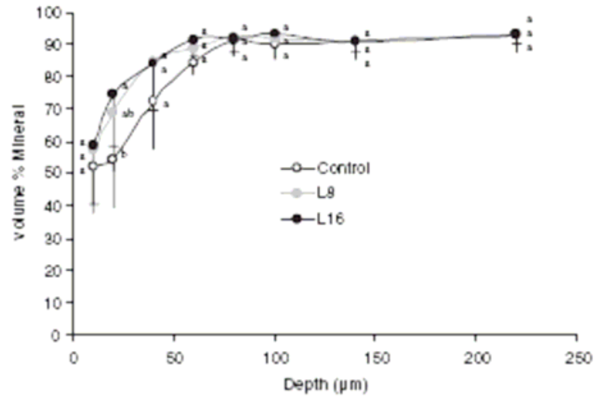

Fig. 4. Enamel volume % mineral at 100 μm from the margin restoration according to the treatments and the distance from the surface. Different letters show statistically significant difference among treatment groups at each depth (Tukey test; $P < 0.05$). Bars denote standard deviation.

possibility of using lower laser energy than that used for tissue ablation, resulting in minimal pulp damage.

Another important point of the findings was the use of a 10.6 CO_2 laser, which is a commercially available medical CO_2 laser. This may represent an advantage in view of the fact that the best results described in the scientific literature were obtained by using CO_2 laser prototypes (12, 24, 27, 30). This would represent technology simplification and a lower cost of treatment compared to the complex TEA CO_2 laser system (10).

There was a trend for better demineralization inhibition when the cavities were irradiated with 16 $J{\cdot}cm^{-2}$ energy density (i.e. the Laser16 group), as lower values of ΔZ were found compared to the Laser8 group (Table 1). Furthermore, only the Laser16 group presented a statistically significant lower mineral loss, when compared to the control group, at 100 μm from the restoration (Table 1, Fig. 4). Some factors may have contributed to the non-statistically significant difference between the irradiated groups, such as laser beam diameter and variability of the substrate.

The phenomena of fusion and melting, observed in the present study (Fig. 2), are in agreement with data reported by Nelson *et al.* (24), Kantorowitz *et al.* (25) and McCormack *et al.* (31), who used energy densities similar to or higher than those used in the present study. The most frequently mentioned hypothesis for laser effect states that caries inhibition is caused by the melting and fusion of hydroxyapatite (32, 33). Therefore, it seems that surface melting and fusion, as evidenced by SEM analyses in this study, may have increased the resistance of enamel to demineralization by sealing the enamel surface, as previously reported by Nelson *et al.* (24, 30). In addition to presenting reduced solubility, this melt layer presents reduced carbonate content when compared to normal surface enamel, as well as different components, such as tetracalcium diphosphate monoxide and denatured organic material (30). Completely different processes can occur at the enamel surface, depending on the pulse duration and its deposited pulse energy. Thus, it is difficult to compare the data from the several published reports of the CO_2 laser effects on enamel.

Besides obtaining a tissue more resistant to acid dissolution surrounding the restoration, other possible advantages of using lasers for the prevention of secondary caries are sterilization (34) and microretention of prepared enamel, thus avoiding the adverse effects of acid etching and contribution to resin adhesion (35). Fluoride has also been shown to inhibit secondary caries, either when it is released from the restorative material (36) or as a cavity wall pretreatment (37). However, the fluoride effect is time-limited, while the duration of the laser effect remains unknown. In addition, many possibilities also exist for combining laser treatment with fluoride treatments or fluoride-releasing restorative materials, which may provide synergistic effects on secondary caries resistance. Determination of the optimal combination of various caries-preventive regimens would be especially useful in view of the fact that secondary caries lesion is one of the main reasons for replacing restorations (1–4).

Conclusion

In conclusion, the findings of the present investigation suggest that the irradiation of dental cavities by using a CO_2 laser reduces enamel demineralization around composite restoration, but further *in situ* and *in vivo* investigations should be conducted to confirm these results and, subsequently, its clinical application.

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CAPÍTULO 3

In Situ Evaluation of the Effects of CO_2 Laser and Fluoride Dentifrice on Caries Development in Human Enamel*

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ABSTRACT

This study assessed *in situ* the effect of a TEA (Transversely Excited Atmospheric-pressure) CO_2 laser (λ = 9.6 µm) and use of fluoridated dentifrice on enamel demineralization. During two phases of 14 days each, 17 volunteers wore palatal appliances containing slabs of human enamel, which were submitted to four groups of treatments, as follows: non-fluoride dentifrice; CO_2 laser irradiation plus non-fluoride dentifrice; fluoride dentifrice and CO_2 laser irradiation plus fluoride dentifrice. A 20% sucrose solution was dripped onto the slabs 8 times per day. The specimens treated with laser and/or fluoridated dentifrice presented a significantly lower mineral loss when compared to those from the non-fluoride dentifrice group. The combined fluoride-laser treatment showed additional effect against enamel demineralization and caries inhibition ranged from 35% to 84%. The results suggested that CO_2 laser treatment of enamel inhibits demineralization in the human mouth, being more effective when associated with the use of fluoride.

INTRODUCTION

In spite of the decline observed in dental caries, it still represents the most prevalent chronic childhood oral disease (U.S. Department of Health and Human Services). In addition, the disease became polarized, with carious surfaces being centralized in certain groups of children, who present high caries activity (Tayanin *et al.*, 2005). Consequently, the use of combined therapies for this population might be a promising method to prevent and control dental caries.

The efficacy of CO_2 laser irradiation combined with fluoride in inhibiting enamel demineralization has been demonstrated by several investigations (Featherstone *et al.*, 1991; Fox *et al.*, 1992; Hsu *et al.*, 1998; Hsu *et al.*, 2001; Nobre dos Santos *et al.*, 2001).

However, there is no report about the *in situ* or *in vivo* caries preventive effect of CO_2 laser combined with fluoride dentifrice on dental enamel. Furthermore, no study has tested a TEA CO_2 laser operating at 9.6 µm wavelength combined with fluoride dentifrice used under intra-oral conditions. It must be emphasized that the most efficient wavelengths for preventing dental caries, are 9.3 and 9.6 µm due to the high absorption coefficient in dental enamel at these wavelengths (Featherstone *et al*., 1998).

Moreover, the mechanism of CO_2 laser for inhibiting enamel demineralization is not completely clear. In this context, a more sensitive analysis should be performed in order to clarify the enamel modifications induced by laser irradiation, mainly concerning the chemical aspect. Raman spectroscopy is known to be a useful non-destructive tool, both for studying the molecular composition and obtaining information on the structure of substrates (Suzuki *et al*., 1991).

Thus, the objective of this study was to assess *in situ* the combined effects of a 9.6 µm TEA CO_2 laser and fluoride dentifrice on the inhibition of human dental enamel demineralization.

MATERIALS AND METHODS

Experimental Design

This study was approved by the Research and Ethics Committee of Faculty of Dentistry of Piracicaba-UNICAMP (Protocol No. 114/2003). It was performed in two phases of 14 days each (Paes Leme *et al*., 2004), during which 17 volunteers, 20-33 years old, wore acrylic palatal appliances containing two human enamel slabs. In each phase, each slab was submitted to one of the treatments: NFD- non-fluoride dentifrice; LNFD - CO_2 laser irradiation plus non-fluoride dentifrice; FD- fluoride dentifrice and LFD- CO_2

laser irradiation plus fluoride dentifrice. The volunteers were randomly allocated to treatments and those who applied treatments NFD and LNFD in the first phase applied F and LFD in the second one, and vice versa (Fig. 1). The use of two treatments (split-mouth) in the same intra-oral palatal appliance was supported by the absence of cross-effect between irradiated and non-irradiated enamel slabs. On the 14th day of each phase, the slabs were collected, sectioned and enamel mineral loss was determined by cross-sectional microhardness analyses. For statistical analysis, the volunteer was considered as a unit and the treatment as an individualized block. This study was triple-blind since the examiner, the volunteers as well as the technician who performed the analyses could identify the dentifrice type and irradiated slabs.

Enamel slabs and palatal device preparation

Forty extracted impacted human third molars, which were sterilized by gamma radiation (Amaechi *et al*., 1999), were used to perform this *in situ* study. Eighty enamel slabs (5 x 5 x 2 mm) were obtained and coated using an acid-resistant varnish, leaving a window (12.25 mm^2) of exposed enamel, and randomly divided into four groups of 20 specimens according to the treatments. Acrylic custom-made palatal devices were made in which 2 cavities (6 x 6 x 3 mm) were confectioned on the left and right sides, and into each of them, one slab was placed. In order to allow plaque accumulation, and protect it from mechanical disturbance a plastic mesh was positioned on the acrylic resin, leaving a 1-mm space from the slab surface (Benelli *et al*., 1993). To avoid carry-across effect, both fluoride treatments were performed in the same phase. Within each palatal device, the side of the slab was randomly determined.

Laser Irradiation

The exposed enamel area of 40 slabs was irradiated using a TEA CO_2 (Argus Photonics Group, Jupiter, FL) laser at 9.6 µm wavelength, 5 µs pulse duration, 10 Hz repetition rate, 1.5-mm beam diameter and 1.5 J/cm^2 per pulse (Nobre dos Santos *et al*., 2001). The laser energy was measured and calibrated using a calorimeter and the laser spot size was measured by scanning the beam with a razor blade. In order to provide a uniform coverage of each window, a computer-operated micrometer-driven x-y stage was used and each spot size was irradiated with 25 overlapping pulses.

Raman Spectroscopy and Scanning Electron Microscopy

Six irradiated and six non-irradiated slabs were evaluated by Fourier Transform Raman Spectroscopy followed by SEM.

Spectra of the specimens were obtained using an F-T Raman Spectrometer (RFS 100/S – Bruker Inc., Karlsruhe, Germany) with one Ge diode detector cooled by liquid N_2. To excite the spectra, the focused λ = 1,064.1 nm line of an air cooled Nd:YAG laser source was used. The maximum laser power incident on the sample surface was about 77 mW and the spectrum resolution was 4 cm^{-1}. An IR354 lens collected radiation scattered over 180°. The FT-Raman spectra were obtained using 150 scans. The explored frequency ranged from 50 to 4,000 cm^{-1}.

After Raman spectroscopy analyses, the specimens were coated with a thin layer of gold (approximately 10-12 nm in thickness). Observations were then made with a JEOL JSM-5600 LV Scanning Electron Microscope (JEOL, Tokyo, Japan) at 15 kV and magnifications up to 3,000 ×.

Intra-oral Phase

During the lead-in and washout periods of 7 days each, the volunteers brushed their teeth with a non-fluoride silica-based dentifrice prepared for this study (FGM Dentistry Product, Joinville/SC, Brazil). Next, all volunteers started to wear palatal devices and appropriate dentifrice according to the treatments.

In order to provide a cariogenic challenge, the volunteers were instructed to remove the appliance and drip one drop of 20% sucrose solution onto each enamel slab, eight times per day at predetermined times (8.00, 9.30, 11.00, 14.00, 15.30, 17.00, 19.00 and 21.00) (Pecharki *et al*., 2005). As stated by Paes Leme *et al*., 2004, this model is able to simulate a high caries risk situation.

Fluoridated dentifrice (silica-based, containing 1,100 μg F/g, w:w, as NaF, FGM Dentistry Product, Joinville/SC, Brazil) was used by the volunteers in the phase in which they were submitted to FD and LFD treatments. In the other phase, NFD and LNFD treatments were carried out and the previously described non-fluoridated dentifrice was used. The dentifrice treatment was performed 3 times a day, after main meal-times and when volunteers' habitually performed oral hygiene. The appliances were extra-orally brushed, except the enamel slabs, and volunteers were asked to brush carefully over the covering meshes, to avoid disturbing the plaque. All volunteers consumed fluoridated water (0.70 mg F/l) and received oral and written instructions to wear the appliances all time, including at night. They were allowed to remove the appliances only during meals and when performing oral hygiene (Cury *et al*., 2000).

Microhardness analysis

Each enamel slab was longitudinally sectioned by a cut through the center of the exposed enamel area. The segments were embedded in acrylic resin and serially polished. Cross-sectional microhardness measurements were taken with a microhardness tester (Future Tech FM-ARS) with a Knoop diamond under a 25 g load for 5 s. Three lanes of thirteen indentations each were made in the central region of the slab and distance between them was set at 100 µm. The indentations were made at the following depths: 10, 20, 30, 40, 50, 60, 80, 100, 120, 140, 160, 180 and 200 µm from the outer enamel (Hara *et al.*, 2003).

The mean Knoop hardness number values at each distance from the surface were obtained and converted into volume percent mineral (Featherstone *et al.* 1983). Volume percent mineral was plotted against depth for each specimen and the integrated mineral content of the lesion was calculated. A mean of volume percent mineral for depths greater than 100 µm was used as a measure of the integrated mineral content of sound inner enamel. To compute ΔZ parameters, the integrated mineral content of the lesion was subtracted from the value obtained for sound enamel (Featherstone et al., 1988). Based on the mean ΔZ parameter, the percentage of caries inhibition was calculated for experimental groups.

Statistical Analysis

In order to assess the effect of the treatments, the dependent variable ΔZ was analyzed; the assumptions of equality of variances and normal distribution of errors were checked. The assumptions were satisfied and analysis of variance followed by Tukey test were applied. The software SAS system (version 8.02, SAS Institute Inc., Cary: NC, 1999) was used and the significance limit was set at 5%. The intensity of all bands found in the spectra of selected slabs was compared using Origin Pro software system. In addition, the ratio between the enamel components and phosphate was analyzed.

RESULTS

Raman spectra of irradiated and non-irradiated enamel are shown in Figure 2. A statistically significant decrease in the intensity of all bands was found in the irradiated slabs. However, when ratio between the enamel components and phosphate was analyzed, only the bands that showed organic grouping vibration modes, amide and CH in the 1,200-3,000 cm^{-1} region, showed a statistically significant decrease in irradiated specimens.

The SEM observations showed evidence of melting and fusion in specimens treated with CO_2 laser (Figure 3).

The ΔZ values in experimental groups were lower, to a statistically significant extent, than the values found for the control group. However, there was no statistically significant difference between the NFDL and FD groups (Table 1). In addition, when laser irradiation was associated with fluoridated dentifrice use, a statistically significant lower mineral loss value was found.

DISCUSSION

Enamel specimens exposed to CO_2 laser irradiation, whether or not associated with fluoridated dentifrice, presented significantly less demineralization than specimens treated with non-fluoridated dentifrice (Table 1). In addition, the combination of CO_2 laser irradiation with fluoride dentifrice provided significantly more protection against caries development than laser irradiation or fluoride dentifrice use alone. These *in situ* data confirm several *in vitro* studies, which found an additional effect when this laser was combined with fluoride (Featherstone *et al.*, 1991; Fox *et al.*, 1992; Hsu *et al.*, 1998; Hsu *et al.*, 2001; Nobre dos Santos *et al.*, 2001, Nobre dos Santos *et al*., 2002). This effected was attributed not only to enamel changes promoted by the CO_2 laser, but also to a possible increase in fluoride uptake in irradiated enamel, as previously reported by Tepper *et al*., 2004 and Chin-Ying *et al.,* 2004. There are two possible mechanisms involved in the laser-induced increase of fluoride uptake. One suggestion is that laser-fluoride treatment produces numerous spherical precipitates that morphologically resemble calcium fluoride-like deposits on dental surfaces, which serve as a reservoir to replenish fluoride (Chin-Ying et al., 2004). Another mechanism emphasizes the role of lasers in enhancing fluoride uptake into the tooth crystalline structure in the form of firmly bound fluoride (Meurman *et al*., 1997).

Since fluoride was not present at the time of irradiation, it was supposed that heat was not supposed to be responsible for fluoride uptake. As evidenced by SEM analysis, laser-induced surface changes, such as an increase in cracks and roughness, might have played an important role in increasing fluoride uptake (Putt *et al*., 1978).

The laser treatment alone produced inhibition of enamel demineralization comparable to that found by three-time-a-day use of fluoridated dentifrice, which was also shown by an *in situ* study (Featherstone *et al*., 2001). However, these authors found caries inhibition by laser irradiation only in those individuals who were considered demineralizers, which means, those who were exposed to a high cariogenic diet daily and consequently showed higher enamel mineral loss expressed as the ΔZ parameter. Thus, it seems that laser treatment did not enhance remineralization in the absence of fluoride, but only inhibited demineralization. This may explain why fluoride dentifrice use presented a higher percentage of caries inhibition than laser treatment, since fluoride can interfere physicochemically with caries development by reducing demineralization and enhancing remineralization (Dawes *et al*., 1990).

As expected, the fluoridated dentifrice inhibited enamel demineralization in this intra-oral model. This confirms previous *in situ* studies evaluating the role of fluoridated dentifrice in caries inhibition (Paes Leme *et al*., 2004). However, no statistically significant difference was found between FD and LNFD groups, showing that laser treatment may be a good alternative in those cases where fluoride is apparently not as effective as it is on smooth surfaces, such as in pit and fissures regions. Previous *in vitro* study showed caries inhibition in occlusal surfaces treated by CO_2 laser similar to that one achieved for smooth surfaces (Nobre dos Santos *et al*., 2002).

Although fusion and melting do not seem to be necessary to increase enamel resistance to demineralization, the SEM data in the present study suggest that these phenomena might be responsible for the inhibition of demineralization found in irradiated groups. Fusion and melting observed in the present study (Figure 3) are in agreement with Nelson *et al*., 1986; Kantorowitz *et al*., 1998 and McCormack *et al*., 1995 who used higher energy densities than those used in the present study.

With regard to the FT-Raman spectroscopy results, the strongest bands of phosphate v_1 (962 cm^{-1}) and carbonate (1070 cm^{-1}) modes, which have been previously reported (Tsuda *et al*., 1996, Nelson *et al*., 1985) were immediately identified. However, the organic phase (Fig. 2 area from 1,200 to 3,000 cm^{-1}) could systematically be observed in the dentin spectrum but never in the enamel (Tramini *et al*., 2000). These different spectral results could be explained due to the use of FT-Raman spectroscopy, since FT-Raman spectroscopy presents a reduction in the fluorescence background making it possible to identify bands in that region, even in the enamel spectrum, although enamel is a substrate that has a small organic component content. The spectra showed that no change in the position of bands was found. Furthermore, the relative organic component/phosphate intensity ratio decreased in irradiated specimens. Therefore, since organic matrix phase decreased, the caries inhibition effect of laser may not be explained by the organic matrix hypothesis, which states that, this substrate may lead to a blockage of the inter- and intraprismatic spaces (Hsu *et al*., 2000).

In conclusion, CO_2 laser treatment, using the 9.6 μm wavelength, whether or not associated with fluoride use, inhibits subsequent caries lesion in the human mouth.

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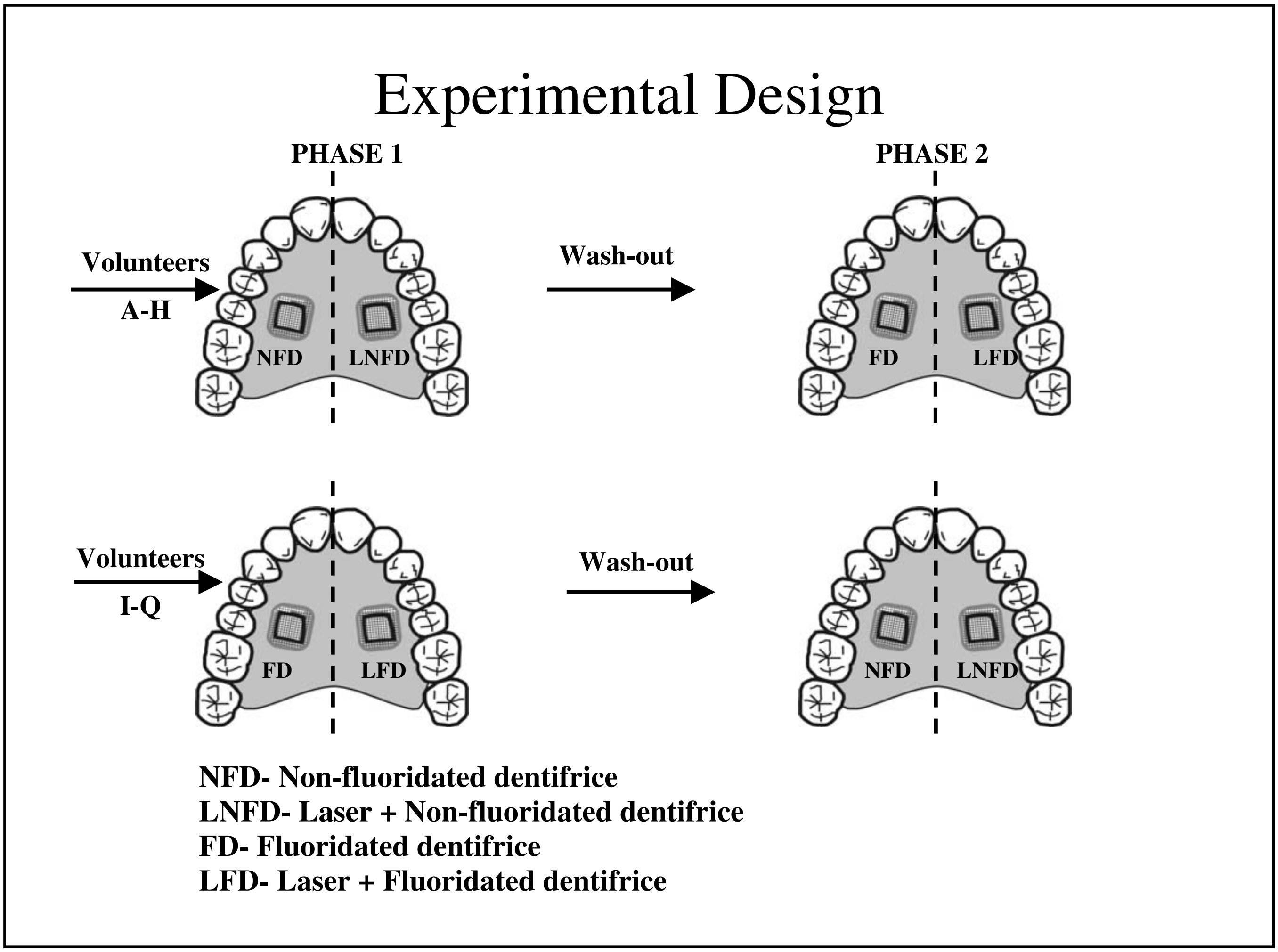

Figure 1. Experimental design used in the study.
Addapted from Pecharki *et al*., 2005.

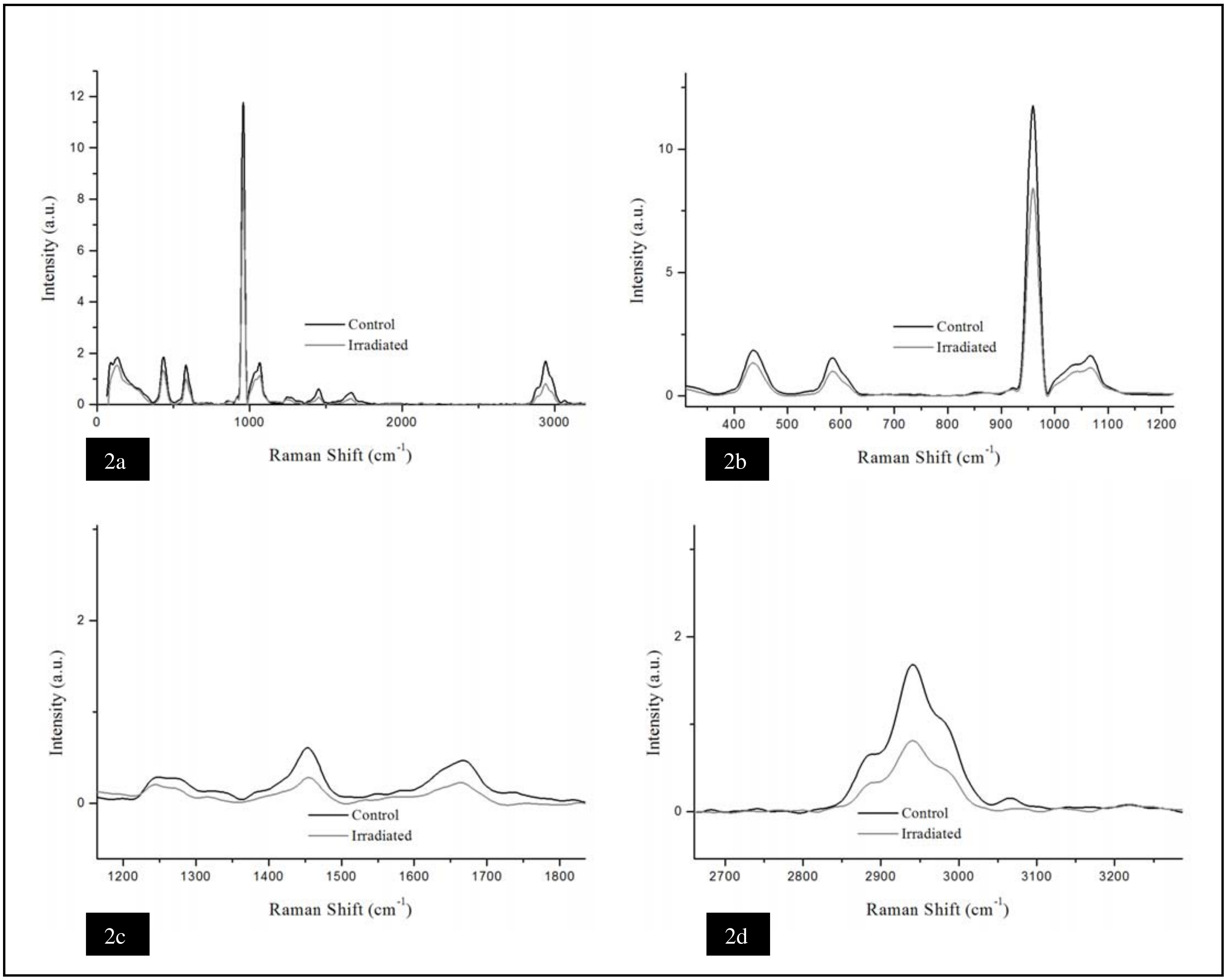

Figure 2. **2a** Whole averaged Raman spectra of the non-irradiated and irradiated specimens; **2b** Averaged Raman spectra of the mineral components of non-irradiated and irradiated specimens; **2c** Averaged Raman spectra of the organic components of non-irradiated and irradiated specimens; **2d** Averaged Raman spectra of the organic components and water of non-irradiated and irradiated specimens.

Figure 3. Representative Scanning Electron Micrographs of irradiated specimen. Surface morphology of transition area between normal and irradiated enamel 100x magnification (3.a) irradiated enamel 200x magnification (3.b), irradiated enamel 500x magnification (3.c) and irradiated enamel 3,000x magnification (3.d).

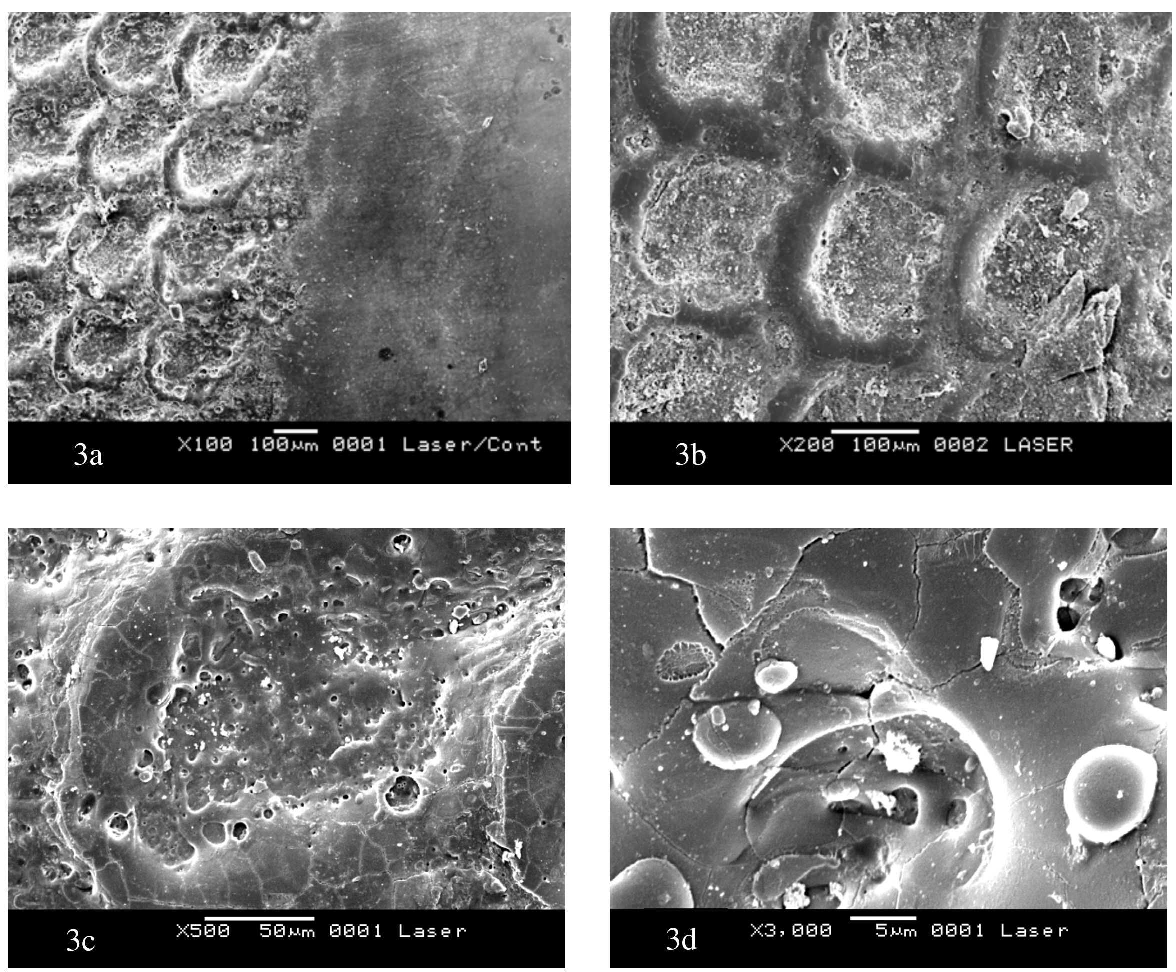

Table 1. Mineral loss (vol% x µm) in human dental enamel, according to the groups (mean ± SD, n = 17).

Group/Treatment	Mineral Loss – ΔZ (vol. % min. x µm)
Non-fluoride Dentifrice	1523.1±939.8a
Non-fluoride Dentifrice+Laser	982.7±445.5b
Fluoride Dentifrice	801.7±533.4b
Laser+Fluoride Dentifrice	235.8±164.3c

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

IV – DISCUSSAO GERAL

Vários estudos mostraram que os lasers de CO_2 podem ser usados para modificar a composição química e/ou a morfologia da superfície do esmalte dental e inibir *in vitro* e *in situ* o desenvolvimento e a progressão de lesões de cárie (Nelson *et al*., 1986, 1987, Featherstone *et al*., 1998, Kantorowitz *et al*., 1998, Hsu *et al*., 2000, 2001, Featherstone et al., 2001, Nobre dos Santos *et al*., 2001, 2002, Tepper *et al*., 2004). Durante a irradiação do esmalte dental com laser de CO_2 há eficiente conversão da luz laser em calor o que causa um aumento da temperatura nas camadas mais externas deste substrato (Featherstone, 2000). Isto pode promover a redução do conteúdo de carbonato superficial (Nelson *et al*., 1987), modificação da matriz orgânica (Hsu *et al*., 2000) e melhorar a cristalinidade do mineral, resultando em uma redução na dissolução do esmalte durante desafios ácidos subseqüentes (Hsu *et al*., 1998). Segundo Featherstone (2000), os comprimentos de onda mais indicados para uso na prevenção de cárie são 9,3 µm e 9,6 µm com duração de pulso menor ou igual a 100 µs e densidades de energia por pulso, iguais ou inferiores a 4 J/cm^2. No entanto, até o momento, não existem aparelhos de laser que estejam comercialmente disponíveis e que possam reproduzir tais condições, de modo que as pesquisas realizadas com estes parâmetros utilizaram protótipos (Featherstone *et al*., 1998, Phan *et al*., 1999, Featherstone *et al*., 2001, Nobre dos Santos *et al*., 2001, 2002).

Consequentemente, em busca de simplificação de tecnologia, aproveitamento de tecnologia já existente e diminuição de custos, muitas pesquisas têm empregado o comprimento de onda 10,6 µm (Kantorowitz *et al*., 1998, Hsu *et al*., 2000, 2001, Tepper *et al*., 2004). Desta forma, estudos realizados com este comprimento de onda, emitido em modo contínuo e utilizando densidades de energia variadas, demonstraram a ocorrência de microfendas superficiais (Stewart *et al*., 1985), redução em torno de 97,2% na perda mineral do esmalte dental (Hsu *et al*., 1998) e, por outro lado, dissolução mais rápida de hidroxiapatita sintética (Meurman *et al*., 1992). Adicionalmente, técnicas como a espectroscopia infravermelha e/ou difração por raios-X demonstraram haver redução do conteúdo de água, proteína e carbonato nos espécimes irradiados (Kuroda & Fowler, 1984), bem como formação de compostos fosfatados no esmalte dental, como ortofosfato α-cálcico, fosfato α-tricálcico e fosfato tetracálcico (Kantola *et al*., 1973; Kuroda & Fowler, 1984; Meurman *et al*., 1992). A redução do conteúdo de carbonato decorrente da irradiação laser é um fator positivo visto que, o carbonato torna a apatita mais instável e solúvel em ácido (Robinson *et al*., 2000). Por outro lado, Fowler & Kuroda (1986) salientaram que fosfato tetracálcico e fosfato α-tricálcico são substancialmente mais solúveis em ácido que o esmalte dental e a hidroxiapatita. Deste modo, a ação do laser na superfície irradiada pode ser benéfica ou prejudicial dependendo dos parâmetros de irradiação empregados. Neste contexto, são particularmente importantes a quantidade de energia e a forma como esta energia é depositada no substrato.

Na emissão pulsada do comprimento de onda 10,6 µm, os períodos de relaxamento térmico, inexistentes no modo contínuo, resultam em uma elevação mínima de temperatura na profundidade de 10 a 20 µm da superfície e, conseqüentemente, menor probabilidade de ocorrência de injúrias teciduais (Nelson *et al*., 1986). Assim, densidades de energia menores do que aquelas necessárias no modo contínuo promovem alterações no mineral de hidroxiapatita sem promover efeitos indesejáveis como aquecimento ou ablação da subsuperfície (McComarck *et al*., 1995). Adicionalmente, McCormack *et al*. (1995) salientaram que a deposição de uma grande quantidade de energia no dente pode resultar em dano térmico à periferia do local irradiado, dano pulpar, carbonização de tecido e rachaduras ou fendas no esmalte ou dentina. Além disso, enquanto a irradiação com baixa densidade de energia tende a aumentar a resistência ácida do esmalte, altas densidades de energia, de modo geral, promovem efeito inverso (Fowler & Kuroda, 1986).

De fato, estudos nos quais foram utilizadas densidades de energia de 0,3 a 50 J/cm^2 mostraram uma inibição de cárie que variou de 40% a 98% (Nelson *et al.*, 1986, 1987; Featherstone *et al.*, 1998; Kantorowitz *et al.*, 1998; Hsu *et al.*, 2000). Esses resultados, juntamente com a possibilidade de utilizar a tecnologia laser na prevenção de cárie, têm estimulado o desenvolvimento de novas pesquisas com o comprimento de onda 10,6 μm.

Dessa forma, empregou-se no estudo do Capítulo 2, o laser de CO_2 com comprimento de onda 10,6 μm em modo pulsado, do aparelho Opus 20, um sistema que incorpora dois lasers cirúrgicos em um único aparelho: Er-YAG e CO_2. Segundo as especificações deste laser, o modo de emissão pode ser contínuo com potência média de 1 a 10 W, ou pulsado com potência entre 1 e 6 W e a duração de pulso que pode variar de 50 a 500 ms. A seleção dos parâmetros de irradiação baseou-se em estudos preliminares bem como na tentativa de que as menores densidades de energia produzidas pelo aparelho fossem usadas, acarretando assim em menores possibilidades de ocorrência de danos teciduais. Neste mesmo capítulo, observou-se nos espécimes irradiados com 8 ou 16 J/cm^2, uma inibição de cárie adjacente à restauração de resina composta que variou de 42,7% a 64,0% e foi observada a ocorrência de derretimento e fusão. Na condição experimental do estudo, tais fenômenos são até desejáveis uma vez que podem aumentar a resistência adesiva da interface dente restauração (Walsh *et al.*, 1994) uma vez que aumenta o embricamento mecânico do material na estrutura mecânica. Já no estudo do capítulo 3, quando foi utilizado um protótipo de laser de CO_2 com comprimento de onda 9,6 μm, uma menor porcentagem de inibição de cárie foi obtida quando apenas o tratamento com laser foi utilizado (35%). Tal discrepância pode ser atribuída à diferença nos modelos empregados para produção de lesões de cárie, já que o último estudo foi realizado com um modelo intra-oral onde há a interferência do fator microrganismo, o qual não estava presente no estudo 2, onde foi empregado um sistema de ciclagem de pH. No estudo *in situ,* a ablação ocorrida em algumas regiões do esmalte, evidenciada pela microscopia eletrônica de varredura, pode ter aumentado a área de superfície facilitando a colonização bacteriana nos estágios iniciais de formação do biofilme. Uma alternativa para esta limitação do laser seria a busca de parâmetros de irradiação que não causassem mudanças estruturais evidentes na superfície do esmalte e sim, apenas modificações na sua composição química. Os parâmetros de irradiação do estudo 3 foram determinados em estudos anteriores nos quais altas taxas de porcentagem de inibição forma encontradas (Nobre dos Santos *et al*., 2001, 2002).

Um grande número de pesquisas científicas evidenciou o efeito protetor do laser de CO_2 no esmalte dental quando submetido a desafios cariogênicos *in vitro*. É importante salientar, contudo, que a comparação direta dos resultados destas pesquisas com aqueles relatados na literatura pode ser difícil, visto que as condições de irradiação empregadas são bastante distintas, com diferentes comprimentos de onda, densidades de energia, duração de pulso, taxa de repetição, entre outros. Apesar disso, verifica-se que os achados destes estudos ratificam relatos anteriores nos quais, a inibição de desmineralização no esmalte variou de 40% a 98% com emprego do comprimento de onda 10,6 μm e densidades de energia de 0,3 a 83,33 J/cm^2 e de 28% a 73% utilizando os comprimentos de onda 9,3 μm, 9,6 μm e 10,3 μm com 1 a 50 J/cm^2 (Nelson *et al*., 1986, 1987; Featherstone *et al*., 1998;

Kantorowitz *et al*., 1998; Hsu *et al*., 2000; Tange *et al*., 2000; Tsai *et al*., 2002, Tepper *et al*., 2004).

Com relação às alterações que podem ocorrer na superfície do esmalte dental, o derretimento e a fusão podem não ser necessários para aumentar a resistência do esmalte ao desafio ácido (McCormack *et al*., 1995; Kantorowitz *et al*., 1998; Hsu *et al*., 2000). De fato, o emprego de densidades de energia de 0,3 a 12 J/cm^2 com o comprimento de onda 10,6 μm, em modo pulsado, têm causado pouca ou nenhuma mudança morfológica (Kantorowitz *et al*., 1998), ausência de derretimento (McCormack *et al*., 1995; Hsu *et al*., 2000, 2001) ou formação de crateras nas superfícies de esmalte avaliadas ao microscópio eletrônico de varredura (Hsu *et al*., 2000) e a inibição de cárie tem sido da ordem de 48% a 98%. Em contrapartida, densidades de energia iguais ou maiores que 20 J/cm^2 têm reduzido de 23,9% a 46,1% a profundidade de lesões *in vitro* (Tsai *et al*., 2002), promovido derretimento e alguma fusão dos cristais e levado a formação de fendas no esmalte (Nelson *et al*., 1987; McCormack *et al*., 1995; Tsai *et al*., 2002). Tais fendas podem ocorrer como resultado da incompleta vitrificação do esmalte ou devido aos rápidos e repetidos ciclos térmicos sofridos pela superfície do mineral durante a irradiação pulsada. Assim, a resistência do esmalte ao desafio ácido também tem sido relatada como resultante de alterações químicas como a redução do conteúdo de carbonato na superfície do esmalte (Nelson *et al*., 1987) ou da decomposição parcial da matriz orgânica (Hsu *et al*., 2000). Os resultados da microscopia eletrônica de varredura (Capítulos 2 e 3) evidenciaram derretimento e fusão da superfície do esmalte sugerindo a ocorrência de selamento da superfície do esmalte. Baseando-se nestes resultados, pode-se sugerir que os mecanismos de inibição de cárie promovidos pelo laser de CO_2 foram semelhantes nos dois estudos.

Com relação ao efeito sinérgico da associação flúor e laser de CO_2, na literatura, várias pesquisas têm analisado os efeitos desta combinação, embora nenhuma delas tenha empregado condições intra-orais. Os resultados do presente estudo (Capítulo 3) corroboram os encontrados por Hsu *et al*. (2001) que também encontraram expressiva redução da perda mineral no esmalte (98,3%) quando associaram flúor na forma de NaF, embora a irradiação tenha sido realizada com comprimento de onda 10,6 µm e densidade de energia de 0,3 J/cm^2. A inibição de cárie observada no presente estudo confirma os resultados inibição de desmineralização *in vitro* obtida por Nobre dos Santos *et al*. (2001) que variou de 49% a 76% quando associaram aplicação de flúor fosfato acidulado por 5 min e irradiação do esmalte com comprimento de onda 9,6 µm e densidade de energia de 1 e 1,5 J/cm^2. Da mesma forma, Fox *et al*. (1992), Meurman *et al*. (1997) e Hsu *et al*. (1998) encontraram resultados de inibição de cárie promissores quando associaram o comprimento de onda 10,6 µm, em modo contínuo, ao flúor ou outros agentes químicos, mesmo empregando densidades de energias maiores, de até 500 J/cm^2.

Deste modo, pode-se concluir que o laser de CO_2 foi efetivo na inibição da desmineralização do esmalte dental, apresentando efeito sinérgico quando utilizado em associação a dentifrício fluoretado.

V – CONCLUSÃO GERAL

1. A irradiação do esmalte dental, por laser de CO_2 altera os cristais de hidroxiapatita reduzindo a dissolução ácida do mineral; a irradiação com laser de CO_2 combinada com o tratamento com flúor é mais efetiva na inibição de cárie que a irradiação com laser de CO_2 ou tratamento com flúor aplicados isoladamente; se a tecnologia de utilização do laser de CO_2 tornar-se disponível a um custo razoável e os resultados encontrados *in vitro* puderem ser aplicados clinicamente, há um futuro promissor para este laser na prevenção de cárie dentária.

2. A irradiação das margens do preparo cavitário com laser de CO_2 (λ = 10,6 µm) reduz a desmineralização *in vitro* do esmalte ao redor de restaurações de resina composta.

3. O tratamento do esmalte com laser de CO_2 associado ou não ao uso de dentifrício fluoretado, com comprimento de onda 9,6 µm, inibiu o desenvolvimento de lesão de cárie em esmalte dental humano, apresentando efeito sinérgico quando utilizado em associação ao flúor.

VI – REFERÊNCIAS*

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

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ANEXO 1

DELIBERAÇÃO CCPG – 001/98

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

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

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

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

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

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

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

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

ANEXO 2

COMITÊ DE ÉTICA EM PESQUISA

UNICAMP

UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE ODONTOLOGIA DE PIRACICABA

CERTIFICADO

Certificamos que o Projeto de pesquisa intitulado "Efeito do laser de cO_2 no desenvolvimento de cárie em esmalte adjacente à restaurações de resina composta. - estudo *in vitro*", sob o protocolo nº **90/2000**, do Pesquisador ***André Luiz Lux Klein***, sob a responsabilidade da Profa. Dra. **Marinês Nobre dos Santos Uchôa**, está de acordo com a Resolução 196/96 do Conselho Nacional de Saúde/MS, de 10/10/96, tendo sido aprovado pelo Comitê de Ética em Pesquisa – FOP.

Piracicaba, 22 de novembro de 2000

We certify that the research project with title "Effect of co_2 laser in the development of caries-like lesion adjacent to composite restaurations – an in vitro study", protocol nº **90/2000**, by Researcher ***André Luiz Lux Klein***, responsibility by Prof. Dr. **Marinês Nobre dos Santos Uchôa**, is in agreement with the Resolution 196/96 from National Committee of Health/Health Department (BR) and was approved by the Ethical Committee in Resarch at the Piracicaba Dentistry School/UNICAMP (State University of Campinas).

Piracicaba, SP, Brazil, November 22 2000

Prof. Dr. Pedro Luiz Rosalen
Secretário - CEP/FOP/UNICAMP

Prof. Dr. Antonio Bento Alves de Moraes
Coordenador - CEP/FOP/UNICAMP

ANEXO 3

COMITÊ DE ÉTICA EM PESQUISA

UNIVERSIDADE ESTADUAL DE CAMPINAS

FACULDADE DE ODONTOLOGIA DE PIRACICABA

UNICAMP

CERTIFICADO

Certificamos que o Projeto de pesquisa intitulado "Avaliação in situ dos efeitos do laser de CO_2 e do flúor no desenvolvimento de cárie em esmalte", sob o protocolo nº **114/2003**, da Pesquisadora ***Lidiany Karla Azevedo Rodrigues***, sob a responsabilidade da Profa. Dra. **Marinês Nobre dos Santos Uchôa**, está de acordo com a Resolução 196/96 do Conselho Nacional de Saúde/MS, de 10/10/96, tendo sido aprovado pelo Comitê de Ética em Pesquisa – FOP.

Piracicaba, 15 de outubro de 2003

We certify that the research project with title "In situ evaluation of effects of CO_2 laser and fluoride on caries development in enamel", protocol nº **114/2003**, by Researcher ***Lidiany Karla Azevedo Rodrigues***, responsibility by Prof. Dr. **Marinês Nobre dos Santos Uchôa**, is in agreement with the Resolution 196/96 from National Committee of Health/Health Department (BR) and was approved by the Ethical Committee in Research at the Piracicaba Dentistry School/UNICAMP (State University of Campinas).

Piracicaba, SP, Brazil, October 15 2003

Prof. Dr. Antonio Fernando Martorelli de Lima
Secretário
CEP/FOP/UNICAMP

Prof. Dr. Antonio Bento Alves de Moraes
Coordenador
CEP/FOP/UNICAMP