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"Efeitos do Laser de Dióxido de Carbono (CO_2) no esmalte dentário"

Tese apresentada à Faculdade de Odontologia de Piracicaba, da Universidade Estadual de Campinas, para obtenção do Título de Doutor em Odontologia.
Área de Odontopediatria.

Orientadora:
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PIRACICABA
2010

FICHA CATALOGRÁFICA ELABORADA PELA

BIBLIOTECA DA FACULDADE DE ODONTOLOGIA DE PIRACICABA

Bibliotecária: Marilene Girello – CRB-8ª. / 6159

V673e

Vieira, Karlla Almeida.

Efeitos do Laser de Dióxido de Carbono (CO_2) no esmalte dentário / Karlla Almeida Vieira. -- Piracicaba, SP: [s.n.], 2010.

Orientador: Marinês Nobre dos Santos Uchôa.

Tese (Doutorado) – Universidade Estadual de Campinas, Faculdade de Odontologia de Piracicaba.

1. Desmineralização. 2. Análise espectral Raman. 3. Testes de dureza. 4. Microscopia Eletrônica de Varredura. 5. Lasers. I. Nobre dos Santos, Marinês. II. Universidade Estadual de Campinas. Faculdade de Odontologia de Piracicaba. III. Título.

(mg/fop)

Título em Inglês: Effects of carbon dioxide (CO_2) laser on dental enamel

Palavras-chave em Inglês (Keywords): 1. Desmineralization. 2. Spectrum analysis, Raman. 3. Hardness tests. 4. Microscopy, Electron, Scanning. 5. Lasers.

Área de Concentração: Odontopediatria

Titulação: Doutor em Odontologia

Banca Examinadora: Josimeri Hebling Costa, Marinês Nobre dos Santos Uchôa, Natanael Barbosa dos Santos, Patrícia Moreira de Freitas, Regina Maria Puppin Rontani

Data da Defesa: 29-04-2010

Programa de Pós-Graduação em Odontologia

UNICAMP

UNIVERSIDADE ESTADUAL DE CAMPINAS
Faculdade de Odontologia de Piracicaba

A Comissão Julgadora dos trabalhos de Defesa de Tese de Doutorado, em sessão pública realizada em 29 de Abril de 2010, considerou a candidata KARLLA ALMEIDA VIEIRA aprovada.

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DEDICATÓRIA

Aos meus pais, Antônio Vieira e Maria Almeida,
por fazerem dos sonhos dos filhos seus sonhos.
Ao meu irmão, Rodrigo Almeida, pela nossa
amizade e companheirismo.
Ao meu marido, Thiago Moura, por suportar minha
ausência e entender o quanto esta etapa era
importante para mim.

AGRADECIMENTOS

A Deus e a todos os meus Guias Espirituais, por me protegerem e me fortalecerem em todos os momentos de minha vida, e em especial nesta jornada.

À Universidade Estadual de Campinas, na pessoa do seu Magnífico Reitor Prof. Dr. Fernando Ferreira Costa.

À Faculdade de Odontologia de Piracicaba, na pessoa do seu Diretor, Prof. Dr. Francisco Haiter Neto.

Ao Prof. Dr. Jacks Jorge Júnior, Coordenador Geral do Curso de Pós-Graduação;
À Profa. Dra. Maria Beatriz Duarte Gavião, Coordenadora do Curso de Pós-Graduação em Odontologia.

À Universidade Federal de Alagoas, na pessoa da sua magnífica Reitora, Profa. Dra. Ana Dayse Rezende Dorea.

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

À Profa. Dra. Marinês Nobre dos Santos Uchôa, minha orientadora, por acreditar na minha capacidade desde o curso de mestrado, por me acolher como orientada, pela troca de idéias e pelas conversas. Obrigada por entender meu lado pessoal.

Às Profas. Dras. Maria Beatriz Duarte Gavião e Regina Maria Puppin Rontani por todos esses anos de convívio, pelos estímulos e desafios.

À Profa. Dra. Cecília Gatti Guirado (*in memoriam*). Tudo foi possível graças a sua acolhida. Obrigada pelos ensinamentos clínicos, pelo convívio, apoio em todas as

etapas, pelo carinho, por fazer de mim uma pessoa melhor, e me mostrar que somos fortes em qualquer circunstância da vida. Sinto uma saudade imensa!

Ao Prof. Dr. Milton Fernando de Andrade Silva, por estar ao meu lado sempre, acolhendo minhas inquietações, com carinho, paciência e amizade. Sei que o senhor está “presente” neste momento, e se estou aqui hoje, é devido ao seu incentivo e suporte desde a graduação. Espero poder retribuir um pouco tudo o que o senhor fez por mim.

À Profa. Dra. Lidiany Karla de Azevêdo Rodrigues, pela disponibilidade em ajudar, pelo exemplo de pessoa e profissional.

À Profa. Dra. Regina Célia Rocha Peres, pesquisadora, professora e amiga. Obrigada pelas conversas, pela ajuda e pelo companheirismo.

Ao Prof. Dr. Érico Barbosa Lima, pela troca de idéias e exemplo de dedicação à Odontopediatria.

Às Professoras: Dra. Eliana Rodrigues, Dra. Lívia Maria Andaló Tenuta e a Dra. Maria Beatriz Duarte Gavião pelas considerações sugeridas na qualificação que contribuíram sobremaneira para a melhoria deste trabalho.

Ao meu amigo e Prof. Dr. Natanael Barbosa dos Santos pela amizade, conversas e estímulos.

Ao Prof. Dr. Frederico Barbosa de Sousa (UFPB) por me orientar e me ensinar novas técnicas. Espero que possamos dar continuidade ao nosso trabalho.

Ao Adriano Luis Martins, do Centro de Microscopia Eletrônica de Varredura da FOP/UNICAMP, por me ensinar tudo que sei, hoje, sobre MEV e por tornar tudo mais fácil. Obrigada pelas conversas e ajuda.

Às secretárias da pós-graduação da FOP-UNICAMP, Érica A. Pinho Sinhoreti e Raquel Q. Marcondes Cesar Sacchi pela ajuda e atenção em todas as fases administrativas.

À Marilene Girello pela orientação bibliográfica e auxílio na elaboração desta tese, e à Sueli Ferreira Julio de Oliveira pelas conversas e auxílio na buscas dos artigos.

À secretária Maria Elisa dos Santos e a Técnica do Laboratório de Farmacologia Eliane Melo de Franco por viabilizarem os procedimentos administrativos, torcerem por mim e pelo apoio de sempre.

À Cristina, Cleone e Flávia, funcionárias do Laboratório de Odontologia Preventiva da FOUFAL, pela assistência, apoio e conversas durante a execução da análise de microdureza.

Ao Técnico do Laboratório de Odontopediatria da FOP/UNICAMP, Marcelo Corrêa Maistro pela ajuda e parceria ao longo desses anos.

A todos os funcionários da Faculdade de Odontologia de Piracicaba pela colaboração.

Às minhas colegas de Curso de Doutorado do Programa de Pós-Graduação em Odontologia, Área de Odontopediatria: Carolina Steiner Oliveira, Fernanda Miori Pascon, Flávia Riqueto Gambareli, Kamila Rosamilia Kantovitz, Márcia Diaz Serra Vicentin, Moara de Rossi e Renata Andréa Salvitti de Sá Rocha, que a nossa

convivência saudável perdure não só no âmbito profissional, mas na vida. O nosso companheirismo foi essencial.

À Fernanda Miori Pascon (Fê) por me auxiliar em várias etapas desta tese. Sempre prestativa e companheira. Obrigada por participar desta e de outras etapas da minha vida. Espero que possamos ser cada vez mais amigas. Agradeço também aos seus pais, Sebastião e Neusa Pascon, por me tratarem como filha e me ajudarem em diversos momentos.

À Kamila Rosamilia Kantovitz (Kami) por me mostrar sempre o lado prático da vida e que somos “mulheres maravilhas” – damos conta de tudo! Obrigada por ser você e ser minha amiga.

À Carolina Steiner Oliveira (Carol) por ser minha amiga e parceira nas pesquisas, estar sempre disponível para ajudar e pelas conversas. Alguns momentos são só nossos como: comer o cachorro-quente na USP e ir na Igreja dos Monges na Sé comprar pão de mel!

Às colegas do Programa de Pós-Graduação em Odontologia, Área de Odontopediatria, Éfani Caroline de Freitas Banzi, Fernanda Frasseto, Larissa Ferreira Pacheco, Marcela Pinto Monteiro de Oliveira, Marina Severi Leme, pela amizade, convivência e parcerias.

Às amigas do Programa de Pós-Graduação em Odontologia, Área de Odontopediatria, Annicele da Silva Andrade, Thaís Manzano Parisotto e Eliana Rodrigues, obrigada por me acolherem em sua casa, por dividirem comigo as angústias e alegrias desta jornada. Sentirei falta dos nossos momentos. Vocês se tornaram grandes companheiras!

À Milena Schaaf Teixeira Bruno e a todos os seus familiares. Obrigada por estar comigo no início desta etapa e, hoje, apesar da distância, a amizade prevalece. Boa sorte! Tenho muitas saudades!

Ao seu Zezinho, meu mecânico. Obrigada por ser solícito e me ajudar com o meu Fiestinha!

À Rose, minha faxineira. Obrigada por me ajudar a manter minha casa sempre acolhedora.

Aos meus companheiros da Radiologia e da Prótese da FOP/UNICAMP, que estão no mundo, por tudo que vivemos e aprendemos.

À Dona Janete, que me guia e me ilumina.

Ao Grupo de Recife por me dá força e amparo para seguir adiante.

Aos meus Pais, Vieira e Maria. Obrigada pela dedicação aos filhos de maneira incondicional. O apoio e zelo de vocês me permitiram chegar aqui. Obrigada pela educação, disciplina, pela vivência familiar e pelo suporte em todos os âmbitos.

Ao Meu irmão Rodrigo, por me incentivar, apoiar, dividir comigo muitas etapas da vida, e sobretudo por ser meu melhor amigo.

À Daniela Toscano, minha cunhada, por participar e torcer por mim sempre.

À minha avó, Maria José, pelas orações, apoio e dedicação.

Ao Meu Marido, Thiago. Pelos sacrifícios ultrapassados ao longo destes quase 13 anos de convivência. Ter você ao meu lado me faz mais feliz e me dá forças para ir adiante. Amo você. Que nossa família cresça em breve!

A todas as pessoas que direta ou indiretamente auxiliaram na concretização desta etapa de minha vida ...muito obrigada!

EPÍGRAFE

“O valor das coisas não está no tempo em que elas duram,
mas na intensidade com que acontecem.
Por isso existem momentos inesquecíveis,
coisas inexplicáveis e pessoas incomparáveis.”

(Autor desconhecido)

RESUMO

A irradiação do esmalte dentário com o laser de Dióxido de Carbono (CO_2) modifica a superfície deste substrato e traz benefícios no que diz respeito a um aumento na resistência aos ácidos quando ocorre um desafio cariogênico. Sendo assim, esta tese constituída por 2 artigos, teve os seguintes objetivos: (1) revisar sistematicamente o efeito da irradiação do esmalte (mudanças morfológicas e de composição) pelo laser de CO_2 com comprimento de onda 10,6 μm e sua resistência a desmineralização; (2) investigar, *in vitro*, o efeito de quatro aplicações sobrepostas do laser de CO_2 (10,6 μm) na redução da desmineralização do esmalte dentário humano. Para o estudo 1, foi realizada uma busca na literatura científica, em base de dados universais no que concerne ao tema. No estudo 2, uma a quatro aplicações sobrepostas do laser de CO_2 (10,6 μm) com 10 J/cm^2 foram realizadas no esmalte dentário e os efeitos dessas aplicações foram avaliados pelas análises de Espectroscopia FT-Raman, Espectrometria de Fluorescência de Raio-X de Energia Dispersiva, Microdureza e Microscopia Eletrônica de Varredura. No estudo 2, a perda mineral do esmalte foi determinada pelo teste de microdureza. Os resultados foram analisados estatisticamente pelos testes ANOVA e Tukey ($p<0,05$). A análise da literatura apresentada no artigo 1 mostrou que existe uma moderada evidência entre as modificações estruturais e químicas no esmalte, produzidas pela irradiação com o laser de CO_2 (10,6 μm), e sua resistência à dissolução ácida quando exposto a um alto desafio cariogênico. O estudo 2 mostrou que uma aplicação do laser de CO_2 (10,6 μm) foi suficiente para produzir modificações na superfície do esmalte e que 3 aplicações promoveram um aumento à resistência do esmalte à desmineralização. Sendo assim, a irradiação do esmalte com o laser de CO_2 (10,6 μm) modifica a superfície do esmalte tornando este substrato mais resistente à desmineralização frente a um desafio cariogênico.

Palavras-chave: Desmineralização; Análise espectral, Raman; Testes de dureza; Microscopia Eletrônica de Varredura; Lasers.

ABSTRACT

The irradiation of dental enamel with Carbon Dioxide (CO_2) laser modifies the surface of this substrate and increases its acid-resistance under a cariogenic challenge. Thus, this thesis comprises 2 manuscripts with the following purposes: (1) to review the existing literature about the effect of a CO_2 laser (10.6 μm) irradiation on enamel (structural and compositional changes) and its resistance to demineralization; (2) to investigate, *in vitro*, the effect of four CO_2 laser (10.6 μm) applications on the reduction of human dental enamel demineralization. In study 1, the scientific literature related to the theme was searched using universal databases. In study 2, one to four CO_2 laser (10.6 μm) applications (10 J/cm^2) were performed on dental enamel and the results of the effects of these applications were evaluated by FT-Raman spectroscopy, Energy-dispersive X-ray fluorescence spectroscopy, Microhardness analysis and Scanning electron microscopy. In study 2, the enamel mineral loss was determined by microhardness test. Results were analyzed by ANOVA and Tukey's test ($p<0.05$). The literature review showed moderate evidence between the structural and chemical modifications, occurring on the enamel surface induced by with CO_2 laser (10.6 μm) irradiation, and its reduction to demineralization when exposed to a high cariogenic challenge. The study 2, showed that one CO_2 laser (10.6 μm) application was able to modify the enamel surface and that three irradiations did enhance the enamel resistance to demineralization. Therefore, irradiation of enamel with CO_2 laser (10.6 μm) was able to promote modifications on its surface, which leads this a substrate more resistant to the demineralization process when a cariogenic challenge occurs.

Key words: Desmineralization; Spectrum analysis, Raman; Hardness tests; Microscopy, Electron, Scanning; Lasers.

SUMÁRIO

INTRODUÇÃO

O uso da irradiação a laser em Odontologia tem sido estudado por mais de 40 anos. A primeira referência na literatura de irradiação dos tecidos dentários foi feita por Stern e Sognnaes em 1964. Dentre os diferentes tipos de lasers utilizados em Odontologia, os lasers de CO_2 tem sido um dos mais estudados por causa de sua absorção pelos tecidos dentários duros (Fried *et al*., 1996; 1997). O emprego eficiente dos lasers com o objetivo de prevenir ou reduzir a desmineralização do esmalte dentário, requer o conhecimento dos processos físicos que levam a absorção da energia depositada pela irradiação com laser. O nível de interação que ocorre entre o tecido dentário e o laser é dependente das propriedades estruturais do tecido irradiado, bem como do comprimento de onda, densidade de energia, duração de pulso e taxa de repetição do laser (Gimbel, 2000).

Em relação as propriedades do tecido dentário, o esmalte possui matriz mineral de 97% sendo os outros 3% formados por água e matriz orgânica. Esta matriz mineral (hidroxiapatita) é formada por uma rede cristalina hexagonal e diferentes íons(Na^+, K^+, MG^{2+}) podem substituir o cálcio e outros radicais, íons ou moléculas (CO_2^{-3}, F^-, HPO_2^{-4}, Cl^-, H_2O) que substituem o fosfato e a hidroxila (Fried *et al*., 2001). A hidroxiapatita, é geralmente chamada de hidroxiapatita carbonatada porque o carbonato é o seu maior substituinte, em torno de 3,5%. A quantidade e natureza desses constituintes dependem do processo de mineralização dos tecidos. A ação térmica, irradiação a laser, altera as características cristalográficas da matriz mineral do esmalte, tais como os parâmetros da rede cristalina e o tamanho dos cristais. Há ainda a formação de novos compostos cristalinos (fosfato tricálcio na fase beta, fosfato tricálcio na fase alfa, fosfato tetracálcio) decorrente da ação térmica sobre o tecido dentário (Bachmann e Zezell, 2005).

Com o laser de CO_2, a maior parte da luz é absorvida nos poucos micrometros externos da superfície do esmalte e convertida em calor, causando

perda de carbonato do mineral, fusão/derretimento dos cristais de hidroxiapatita, tendo como consequência diminuição na dissolução desta estrutura aos ácidos (Featherstone e Nelson, 1987). O coeficiente de absorção do laser pelo tecido dentário deve ser alto o suficiente para modificar a superfície sem danificar o tecido circunjacente e o tecido pulpar.

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 dentário, pois produzem radiação na região do infravermelho que coincide com algumas bandas de absorção da hidroxiapatita, principalmente os grupamentos fosfato e carbonato (Featherstone *et al*., 1998, Kantorowitz *et al*., 1998).

Segundo Featherstone (2000), os comprimentos de onda mais indicados para uso na prevenção de cárie dentária são 9,3 μm e 9,6 μm com duração de pulso de 100 μs ou menos. No entanto, até o momento, não existem aparelhos de laser comercialmente disponíveis que possam produzir tais condições, de modo que as pesquisas realizadas com estes parâmetros utilizaram protótipos. Consequentemente, em busca de simplificação, e aproveitamento da tecnologia já existente, muitas pesquisas tem empregado o comprimento de onda 10,6 μm (Kantorowitz *et al*., 1998; Hsu *et al*., 2000; Klein *et al*., 2005; Steiner-Oliveira *et al*., 2006; Tagliaferro *et al*., 2007).

Para aplicações em Odontologia, os aparelhos de laser de CO_2 operam em um modo de não contato (Rodrigues *et al*., 2004) podendo ser contínuo ou pulsado (Coluzzi, 2004).

O laser de CO_2 pode ser utilizado visando-se dois aspectos no controle da cárie dentária: o preventivo e o curativo. No aspecto preventivo, tem-se a utilização da irradiação deste laser no diagnóstico de cáries, no selamento de cicatrículas e fissuras e na maior penetração do flúor nos tecidos dentários, tornando-os mais resistentes e menos permeáveis ao ataque ácido bacteriano (Longbottom e Pitts, 1993; Tagliaferro *et al*., 2007; Steiner-Oliveira *et al*., 2008). As modificações morfológicas e estruturais que ocorrem na superfície do esmalte dentário (fusão e derretimento) são fenômenos de importância em relação a

obtenção do efeito preventivo (Ferreira *et al*., 1989). No aspecto curativo, remoção de tecido cariado, tem-se vaporização e consequente redução da microbiota do local infectado (Jeffrey *et al*.,1990; Fortes e Villela, 2000; Ana *et al*., 2006).

Muitas são as hipóteses que tentam explicar esse aumento na resistência aos ácidos para o esmalte irradiado. Dentre elas podemos citar: (1) a diminuição da permeabilidade do esmalte a agentes químicos causada pelo derretimento da superfície (Stern *et al*., 1966) – hipótese sem suporte, uma vez que o estudo de Borggreven *et al*. (1980) executou experimentos em relação a permeabilidade do esmalte e observou que a mesma aumentava e não diminuía; (2) diminuição da solubilidade do esmalte resultante da alteração na fase mineral (Kuroda e Fowler, 1984), redução do conteúdo de carbonato, formação de compostos fosfatados como fosfato tricálcio na fase beta, fosfato tricálcio na fase alfa, fosfato tetracálcio (Nelson *et al*., 1986, 1987), ou seja, alterações químicas (Stern *et al*., 1972; Fowler e Kuroda, 1986). A redução do conteúdo de carbonato decorrente da irradiação com laser é um fator positivo visto que, o carbonato torna a apatita mais instável e solúvel em ácido (Rodrigues *et al*., 2004). No entanto, a formação de compostos fosfatados, como os citados acima, são mais solúveis em ácido; (3) derretimento e recristalização dos cristais de hidroxiapatita; (4) mudança da solubilidade da apatita aquecida pela formação de compostos fosfatados menos solúveis em ácido (pirofosfato). O pirofosfato inibe a dissolução dos cristais de hidroxiapatita, na medida em que o ânion P_2O_7 tendo alta afinidade pela hidroxiapatita bloqueia os principais sítios de dissolução (Hirota e Furumoto, 2003); (5) alteração/redução da matriz orgânica podendo causar uma decomposição (pirólise). Esta decomposição poderia bloquear os espaços intra e interprismáticos com consequente comprometimento da difusão de íons (Hsu *et al*., 2000). O exato mecanismo ou associação de mecanismos de como se dá essa diminuição da desmineralização no esmalte não está ainda elucidado.

Apesar dos lasers de CO_2 serem objeto de estudo desde 1960, os trabalhos científicos ainda apresentam discrepâncias no modo de atuação destes lasers em relação aos efeitos benéficos obtidos no esmalte dentário irradiado na

prevenção da cárie dentária, principalmente quando se leva em conta o comprimento de onda utilizado. Consequentemente, para que esta tecnologia possa ser empregada clinicamente com segurança, torna-se necessária a realização de estudos e revisões sistemáticas que comprovem sua eficácia em situações de alto desafio cariogênico. Além disso, não está estabelecido se o efeito cumulativo de várias aplicações do laser de CO_2 aumentaria o seu efeito preventivo.

Sendo assim, os objetivos desta Tese foram: (1) revisar a literatura disponível que aborda os efeitos do laser de CO_2, no que concernem as modificações morfológicas e estruturais da superfície do esmalte e consequentemente, a redução da desmineralização, com ênfase no comprimento de onda 10,6 μm; (2) investigar se o aumento no número de aplicações do laser de CO_2 (10,6 μm) promoveria um maior efeito na diminuição da desmineralização da superfície do esmalte dentário humano quando submetido a um desafio cariogênico *in vitro*.

CAPÍTULOS

Esta tese está baseada na Resolução CCPG/002/06/UNICAMP que regulamenta o formato alternativo para dissertações de Mestrado e teses de Doutorado e permite a inserção de artigos científicos de autoria ou co-autoria do candidato (Anexo 1 e 2). Por tratar-se de pesquisa envolvendo seres humanos ou material biológico extraídos deles (dentes), o projeto de pesquisa do capítulo 2, foi submetido à apreciação do Comitê de Ética em Pesquisa da Faculdade de Odontologia de Piracicaba, tendo sido aprovado (Anexo 3). Assim sendo, esta tese é composta por dois capítulos contendo artigos científicos completos e/ou em redação, conforme descrito abaixo:

CAPÍTULO 1

Effects of CO_2 laser (10.6 μm) irradiation on enamel demineralization. A systematic review.

Vieira KA, Rodrigues LKA, Nobre-dos-Santos M.

CAPÍTULO 2

In vitro evaluation of several overlapping CO_2 laser applications on enamel demineralization.

Vieira KA, Steiner-Oliveira C, Soares LES, Rodrigues LKA, Nobre-dos-Santos M.

CAPÍTULO 1

Effects of CO_2 laser (10.6 µm) irradiation on enamel demineralization. A systematic review.

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This article will be submitted to Compendium of Continuing Education in Dentistry.

Abstract

Lasers were introduced into the field of clinical dentistry with the hope of improving dental procedures and to modify dental enamel surface to increase its resistance to caries lesions. Among the many types of lasers commercially available, Carbon dioxide (CO_2) lasers are presently thought to have a great potential for use in dentistry. The aim of this paper was to undertake a systematic review of the literature with regard to the effects of a CO_2 laser (10.6 μm) on enamel surface (structural/compositional changes) and its resistance to demineralization. The literature from 1960 to 2010 regarding the preventive effect of laser irradiation on enamel was searched on Pubmed, Cochrane Library and Scopus databases. The main terms used in the search were "CO_2 laser(s) and enamel", "CO_2 laser(s) 10.6 μm wavelength, and enamel", "CO_2 laser(s) and cariogenic challenge", "CO_2 laser(s) and enamel demineralization", "CO_2 laser(s) and fusion", "CO_2 laser(s) and melting", "CO_2 laser(s) and recrystallization", "CO_2 laser(s) and morphological changes". Also, Carbon dioxide laser was searched. The inclusion criteria were studies that: related the structural/chemical changes on enamel, used human or bovine teeth as research specimens, had clear laser parameters, used the CO_2 laser irradiation at 10.6 μm wavelength as independent variable, and also performed a cariogenic challenge simulation. Case reports, reviews, protocols, brief/short communications, and also papers that had dentin, cementum, root or occlusal surfaces as specimens were excluded. The selected papers were assigned with a score (A to C), according to predetermined criteria. Of the 112 papers, 62 were critically assessed and 20 papers were included in the project critical appraisal. One paper was classified as grade A, 16 as grade B and 03 as grade C. Available data about irradiation of enamel with a CO_2 laser at 10.6 μm wavelength, suggest that there is a moderate evidence showing that structural/compositional changes occurring on enamel surface may be related to it's resistance to demineralization. Thus, further studies are required to standardize the CO_2 lasers parameters before its recommendation for use as a method of dental caries prevention. **Keywords:** CO_2 laser, enamel, demineralization, review

Introduction

Laser technology for caries removal, cavity preparation, increase acid resistance of dental enamel and soft tissue surgery is at a high state of refinement, having had several decades of development up to the present time. Since the development of a ruby laser by Maiman[1] in 1960s, several studies have demonstrated the potential of laser pre-treatment of enamel to inhibit subsequent acid induced. The use of laser irradiation as a mean of reducing enamel demineralization was first suggested by Stern and Sognnaes[2] (1964) and Stern et al[3] (1972).

Patel et al[4] were the first to develop a Carbon dioxide (CO_2) laser in 1964. The CO_2 laser is a gas-active medium laser that incorporates a sealed tube containing a gaseous mixture with CO_2 molecules pumped via electrical discharged current.

Carbon dioxide lasers present over a hundred different emission laser lines with wavelengths ranging from 9 to 11 μm. The wavelength of 10.6 μm is placed at the end of the mid-infrared invisible nonionizing portion of the spectrum, and it is delivered through a hollow tube-like waveguide in continuous or gated pulsed mode.[5]

Dental hard tissues strongly absorb light in certain regions of the infrared spectrum because of its crystal structure. Enamel is known to be 97% mineralized by weight and composed of a singular crystalline structure, hydroxyapatite (HA). The physical properties of enamel are unique due to its structural components.

Carbon dioxide laser interacts with the phosphate groups in the dental mineral, is preferentially absorbed and efficiently transformed to heat. As the carbonated HA is more soluble (low thermal stability) in acid solution than pure hydroxyapatite, this process can raise the temperature to levels that drive off the carbonate.[6]

Mechanistically, what occurs is that the carbonated HA in the surface and immediate subsurface of the enamel is heated to temperatures greater than

400°C, decomposing the carbonate and leaving behind a hydroxyapatite-like mineral that is much less soluble than the original mineral. It has been shown that a variety of conditions can be used to produce this effect.[7]

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.[5] 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.[5,8,9,11]

As a result of laser interaction with dental enamel, melting, fusion and recrystallization can occur and change the morphology/structure of enamel[3,8,9,10]. Also, compositional changes such as decomposition of the organic material of enamel, loss of carbonate and water, alteration on the Ca/P ratio and as consequence resultant amount of more soluble phosphates [8,9] ($\propto$ and β tricalcium phosphate) are presented as a result of this relation. Both morphological and compositional changes are related in literature as phenomena that could improve enamel resistance to demineralization.

What condition or the association of conditions are related with the exact action mechanism of CO_2 laser in the inhibition of enamel demineralization are still unclear. Some theories focus on enamel mineral phase changes[3,8,9]. However, others studies[10,11] showed no conclusive evidence that such physical changes are necessary to increase enamel resistance to demineralization. Therefore, the aim of this paper was to review the literature with regard to the effects a CO_2 laser (10.6 μm) on enamel (structural and compositional changes) and its resistance to demineralization.

Materials and Methods

Question Addressed by this Review

Are the structural or compositional changes occurring on enamel surface irradiated with a CO_2 laser at 10.6 μm related to its resistance to demineralization?

Literature Searching

Papers, from January 1960 to January 2010, were sought and obtained from online databases, including Pubmed, Cochrane Library and Scopus. The main terms used in the search were "CO_2 laser(s) and enamel", "CO_2 laser(s) 10.6 μm wavelength and enamel", "CO_2 laser(s) and cariogenic challenge", "CO_2 laser(s) and enamel demineralization", "CO_2 laser(s) and fusion", "CO_2 laser(s) and melting", "CO_2 laser(s) and recrystallization", "CO_2 laser(s) and morphological changes". Also, Carbon dioxide laser was searched. Eligibility of the selected studies was determined by reading the title and then the abstract of the articles identified by the search. If the abstracts were missing, the full-text articles were printed. All papers that appeared to meet the inclusion criteria were selected. Papers were included if the language was English, Spanish or Portuguese.

Inclusion and exclusion criteria

The search was limited to randomized, clinical trials, *in situ* and *in vitro* studies, which were considered as relevant papers. Case reports, reviews, protocols and brief/short communications were dismissed. Also, papers reporting on the effect of CO_2 in animals (rats, dogs) and in dentin, cementum, root and occlusal surfaces were discharged.

In a second step, two authors (KAV-MNS) performed a detailed screening of the papers. Papers were included only if a CO_2 laser at 10.6 μm wavelength was used, the laser parameters were described, and the structural or compositional changes on enamel and/or reduction of enamel demineralization were reported. A cariogenic challenge simulation was also required. The enamel slabs/specimens should have been buccal and/or lingual surfaces.

Evaluation of Scientific Papers

Papers that met the inclusion criteria were submitted to critical appraisal, independently carried out. Data were extracted using a pilot-tested form and each paper was assed with a score from A to C, according to predetermined criteria for methodology and performance[13], as defined in Table 1. If, for any reason, a selected paper was found to be irrelevant for the research question, the paper was excluded. Based on the evaluated literature, the final level of evidence was judged according to the protocol of the Swedish Council on Technology Assessment in Health Care[12,13], as described in Table 2.

Table 1. Criteria for grading the assessed papers

Grade A All criteria stated on the right side should be met	Irradiation of enamel with a CO_2 laser at 10.6 μm wavelength; Laser parameters completely described; Cariogenic challenge simulation; Study group representative; Clinical trial and *in situ* studies; Randomization of teeth; Reliability of evaluation methods described; Control group; Statistical Analysis.
Grade B All criteria stated on the right side should be met	Irradiation of enamel with a CO_2 laser at 10.6 μm wavelength; Laser parameters completely described; Cariogenic challenge simulation; Study group representative; *In vitro* studies; Randomization of teeth; Reliability of evaluation methods described; Control group; Statistical Analysis.
Grade C One or more of the conditions stated on the right side	Irradiation of enamel with a CO_2 laser at 10.6 μm wavelength; Laser parameters not completely described; Cariogenic challenge simulation; Study group not representative; Randomization of teeth; Reliability of evaluation methods not described; No control group; No statistical Analysis.

Table 2. Definitions of evidence level[12,13]

1. Strong evidence	At least two studies with high level of evidence (grade A)
2. Moderate evidence	One study with high level of evidence (grade A) and at least two studies with a moderate level of evidence (grade B)
3. Limited evidence	At least two studies with a moderate level of evidence (grade B)
4.Inconclusive evidence	Fewer than two studies with a moderate level of evidence (grade B)

Results

The systematic search for papers on the databases led to the identification of 112 articles. From those, 62 were selected. Of these articles that remained, only 20 fulfilled all the selection criteria and for this reason, they were used for the systematic review. Almost all the 20 articles were in *vitro studies* with just one *in situ* study. Since the heterogeneity of the studies did not allow a meta-analysis, they were qualitatively analyzed to obtain evidences that would clarify the question addressed.

One paper that reported an *in situ* study, fulfilled all criteria and was graded A. The papers (16) that were given grade B fulfilled all the grading criteria but were *in vitro* studies. The following papers by Hsu et al[14] (1998), Fox et al[15] (1992) and Stern et al[3] (1972) were graded C as they failed on some grading criteria as: study group not representative or missing (n=?) and no statistical analysis was performed (Table 3).

Table 3. Results of references appraised

Reference number/First author	Year	Study design	Number of teeth	Type of teeth	Pulsed or Continuous-wave	Irradiation condition	Treatment with fluoride	Cariogenic challenge simulation	Analysis	Structural Changes on enamel	Conclusion	EL
20.Esteves-Oliveira *et al.*	2009	*In vitro*	276 slabs	B	P	0.3 J/cm^2 5 µs	1.23%APF (positive control)	pH Cycling	Lesion Depth SEM	No ablation, no melting, no surface damages (craks/fissures)	Irradiation with 0.3J/cm^2, 5 µs increased caries resistance by up to 81% compared to control.	B
43.Steiner-Oliveira *et al.*	2008	*In vitro*	45	H	P	10 J/cm^2 10ms	0.05% NaF	Acetate Buffer pH Cycling	PLM, Microhardness	–	The inhibition of demineralization ranged from 48-60%.	B
44.Steiner-Oliveira *et al.*	2008	*In situ*	80	H	P	10 J/cm^2 10ms	–	20% sucrose	Microhardness	–	Reduction of enamel demineralization on cavity margins around composite restorations.	A
36.Schmidlin *et al.*	2007	*In vitro*	60	H	CW	2W 15s	1 %AMF solution	pH Cycling Lactic acid	SEM, Fluoride uptake	Melting, Cracks	The combination of laser irradiation with a topical application of AMF resulted in a higher fluoride uptake and better acid resistance.	B
33.Steiner-Oliveira *et al.*	2006	*In vitro*	90	H	P	10.0,11.5 J/cm^2 10ms	–	pH Cycling	Thermal Analysis, Raman, Microhardness, SEM	Cracks, Melting, Fusion Tetracalcium diphosphate monoxide	Laser energy densities in the range of 10.0 and 11.5 J/cm^2 could be applied in enamel to produce chemical/morphological changes and reduce the acid reactivity (68%).	B
32.Klein *et al.*	2005	*In vitro*	33	H	P	16 J/cm^2 50ms	–	Thermal and pH Cycling	SEM, Microhardness	Melting, Fusion	Irradiation of the cavosurface margin of cavities is able to inhibit enamel demineralization (64%) around composite restorations.	B
37.Tepper *et al.*	2004	*In vitro*	20	H	CW	2W 15s	1% AMF solution	Lactic acid	Acid resistance, SEM, Fluoride uptake	Melting, Cracks, Fusion	Beneficial effect of combining enamel laser irradiation with topically applied AMF, resulting in high fluoride uptake and acid resistance.	B

34.Tsai *et al.*	2002	*In vitro*	10	H	SP	1,33m J/pulse	–	Lactic acid	Calcium analysis, PLM, SEM	Etching pattern, Cracks Fissures	CO_2 laser-reated enamel was more resistance (12.6%) to acid challenge than was Nd-YAG laser-treated enamel.	B
41. Hossain *et al.*	2002	*In vitro*	20	H	CW	1W 30s	2% NaF	Lactic acid	Atomic Measurement, Morphological study	Carbonization, Melting	CO_2 laser irradiation with NaF solution has more caries-preventive effect than CO_2 laser irradiation alone.	B
42. Hsu *et al.*	2001	*In vitro*	24	H	P	0.3 J/cm^2 5ms	2% NaF	pH Cycling	Microradiography, SEM	No melting	The combined fluoride-laser treatment led to 98.3% reduction in mineral loss.	B
10. Lakshmi *et al.*	2001	*In vitro*	36	H	P	8 J/cm^2 25 pulses	–	Lactic Acid	PLM	Melting, Recrystallization	CO_2 laser irradiation can inhibit caries like lesion up to 82.7% and it was optimal at 25 pulses.	B
11. Hsu *et al.*	2000	*In vitro*	24	H	P	0.3 J/cm^2 5ms	–	pH Cycling,	PLM, Lesion Characterization, Microradiography, SEM	No melting, No craters	The laser irradiation resulted in a greater than 98% reduction in mineral loss.	B
38. Hossain *et al.*	1999	*In vitro*	40	H	CW	3 W 5s	–	Lactic acid	Atomic measurement, SEM	Melting, Solidification	CO_2 laser irradiation could sufficiently melt and solidify the enamel surface and thus enhance resistance to artificial caries-like formation.	B
14. Hsu *et al.*	1998	*In vitro*	–	H	CW	170 J/cm^2 2-8s	0.2ppm Fluoride	Saturated demineralized solution	Light Microscopy, Microradiography	–	Laser irradiation of dental enamel results in significant reduction (92,7%) of solubility of enamel mineral, also there is a synergism between laser and fluoride.	C
31.Featherstone *et al.*	1998	*In vitro*	160 slabs	H	P	10 J/cm^2 100µs	–	pH Cycling	Microhardness	No fusion, No crystallization	Inhibition of caries progression of 57% was achieved for 10.6µm.	B
39. Kantorowitz *et al.*	1998	*In vitro*	120	H	P	12 J/cm^2 50-500µs	–	pH Cycling	Microhardness, SEM	No morphological changes	Pulsed CO_2 laser – preventive treatment inhibited caries like lesion progression by up to 87%.	B

15. Fox *et al.*	1992	*In vitro*	–	H	CW	130 J/cm^2 4 s	–	Acetate buffer	X-ray of Mineral density	–	The combination of laser irradiation and chemical agents has been shown to be very effective in increasing resistance to a strong acid challenge.	C
9. Nelson *et al.*	1987	*In vitro*	51	H	P	50 J/cm^2 100-200ns	–	Lactic Acid	PLM, Microradiography Refelected Light Microscopy, SEM, Raman	Melting, Roughening, Fusion, Fractures, Tetracalcium diphosphate monoxide	Tetracalcium diphosphate monoxide was identified as being a component of the surface melt together with an apatite phase that had a reduced carbonate content when compared to normal surface enamel.	B
8. Nelson *et al.*	1986	*In vitro*	106	H	P	10-50 J/cm^2 100-200ns	–	Lactic Acid	Microhardness, Calcium and Phosphorus	Melting, Tetracalcium diphosphate monoxide	Laser-treated enamel produced lesions that were 25 to 50% less demineralized than the controls.	B
3. Stern *et al.*	1972	*In vitro*	03	H	P	13, 25, 50 J/cm^2 80µs	–	Lactic Acid	Microradiographs, SEM	Crazed, Motled, Cracks, Fissures, Melting	Surface changes associated with reduced demineralization could be achieved at energy densities of 13 to 50 J/cm^2	C

EL = Evidence Level; **H= Human enamel; B= Bovine enamel;** P= Pulsed Mode; CW= Continuous wave; SP = Super Pulsed mode; **NaF= Sodium Fluoride; APF= Acidulated Phosphate Fluoride Gel; AMF= Amine Fluoride;** SEM= Scanning Electron Microscopy; PLM= Polarized Light Microscopy; TEM= Transmission Electron Microscopy; **TCP= Tri-calcium Phosphate.**

Discussion

Systematic Review

The validity of a review article depends on its methodological quality. While a traditional review articles or narrative reviews can be useful when properly conducted, there is evidence that they are usually of poor quality[16]. In contrast to a narrative review, the systematic review provides a summary of reports on a specific question, using explicit methods to search, critically appraise, and systematically synthesize the literature[17].

The present systematic review searched and provided a comprehensive and contemporary appraisal of substantial literature in order to achieve conclusions about the effects of the CO_2 laser irradiation at (10.6 μm wavelength) on enamel surface and its consequence on dental demineralization. Therefore, with regard to Dentistry based on scientific evidences, systematic reviews play a very important role. Moreover, subsequent quality assessments of included papers are now well-established measures for evidence-based dentistry[13]. In this way, this review may be important for aiding clinical decision-making and may contribute to the development of new methodologies in preventive dentistry.

Hierarchy of evidence – study design

It is well recognized that some research designs are more powerful than others in their ability to answer research questions on the effectiveness of interventions. The hierarchy provides a framework for ranking evidence that evaluates health care interventions (opinion, *in vitro* studies, case reports, case series, case-control studies, cohort studies, systematic review of randomized clinical trials with or without meta-analysis)[18].

In this review, two types of models-studies were found as tools to assess scientific questions: *in situ* study (01 paper) and *in vitro* studies (19 papers). There are some important differences that should be considered when these different models are used.

While *in vitro* studies have limitations, such as sample size and possibly decreased clinical relevance, they have a high level of scientific control and low variation[13,19]. The *in situ* model uses the mouth as a laboratory and is therefore much more realistic than *in vitro* models, but on the other hand, there are many more variables in the mouth.

Although *in vitro* studies are adequate for the evaluation of caries-preventive methods, only *in situ* and *in vivo* studies are capable of offering the clinical evidence necessary to prove the efficacy of a therapy.

Type of Teeth

Only the study of Esteves-Oliveira et al[20] (2009) used bovine teeth as the research specimen. The advantages of using bovine teeth are the better availability and the possibility of obtaining bigger areas of plane enamel. Although, these teeth present composition and optical properties similar to human teeth, as well as, the same mechanism of caries formation[21,22], the rate of demineralization progression is higher[23] and should be taken into account.

The CO_2 laser 10.6 µm wavelength choice

Carbon dioxide laser irradiation should occur with absorbed energies (fluence = energy/surface area) in the order 9.6<9.3<10.3<10.6 µm. The 9.6 µm wavelength has a 10 times higher absorption in enamel (8,000 cm^{-1}) than the 10.6 µm (825 cm^{-1}) and has therefore been considered the most promising for use in caries prevention. However, the lower absorption of the 10.6 µm wavelength results in a higher penetration depth and can therefore affect a thicker enamel layer.[20] Consequently, it has been suggested that most of the caries-preventive effect obtained with 10.6 µm wavelength could be longer-lasting.[24] Moreover, the 10.6 µm laser line is the commercially available medical CO_2 laser[25].

Continuous X Pulsed wavelength

This review showed that 14 papers used a pulsed mode laser and only 06 papers used a continuous wave (cw) laser.

Many researchers have studied the interaction of the CO_2 laser 10.6 µm continuous-wave with dental hard tissues. Usually, they used relatively high

fluences (>50 J/cm^2) and long interactions times of 5 ms to 15s[26,27,28]. These interactions times are far in excess of the thermal relaxation time for enamel (approximately 60-90 μs)[29]. Nevertheless, pulse duration much longer than the thermal relaxation time of enamel, results in ablation, which is an undesirable laser effect when caries prevention is intended[5].

Pulsed lasers provide a way of increasing the peak power density while keeping the pulse energy density at low levels (hundred of mJ/cm^2)[11,20]. The energy deposited at pulse durations shorter than the thermal relaxation time is thermally confined to a thin layer at the enamel surface (cooling between exposures pulses). The studies [20,31,39] which used pulses in the range of 5-500 μs were able to obtain an inhibition varying from 57-87% and used a pulsed wavelength.

The pulsed lasers are better than cw lasers because they can provide a way for increasing the peak power density while keeping the pulse energy density constant. Using high power densities and short interaction times the fusion, melting and recrystallization of the enamel crystallites are confined to the surface region without affecting the underlying enamel or more importantly the underlying dentin or pulp[30]. For a given pulse energy, shorter pulse time means that, the peak power density deposited increases.[8]

Irradiation Conditions

The irradiation condition showed that the preventive effect of enamel irradiation with a CO_2 laser (10.6 μm) varied for energy densities (0.3 – 170 J/cm^2) and for pulse durations (5 μs – 15s)[8,9,20,31,32,33,34]. It is important to note that low energy densities and short pulse durations are said to cause less thermal damage to the surface and less irreversible pulp inflammation, which can improve *in vivo* application[20].

Treatment with fluoride

The use of CO_2 laser (10.6 μm) irradiation and its interaction with fluoride were related by 06 papers. The consequences of this interaction were not the aim of this present review. However, some observations are related below.

There is still no consensus with regard to the mechanisms of laser and fluoride interaction aimed at caries prevention was achieved, or even whether fluoride treatment should be performed before or after laser irradiation[14,36,37,41,42,43]. This finding emphasizes the need for more researches to be performed under well-controlled conditions in order to provide evidences about the effects of the combination of laser irradiation and fluoride. All papers showed better results in the inhibition of enamel demineralization when the combined therapy was used compared to one single treatment. The use of 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[14,25].

Cariogenic Challenge Simulation – acid resistance

Two models were predominantly found among the papers: pH Cycling and the use of lactate buffer solution. The use of the acetate buffer was only employed in two papers. The pH cycling delivers results more comparable to clinical outcomes, because this process can be delineated in order to mimic a situation more closely related to the oral conditions. This attribute differentiates it from all others methods of artificial caries induction (such as lactate or acetate buffer), whose static behavior simulates a condition different from what occurs in the oral environment. Thus, when the substrate is kept in the demineralizing solution, it simulates the several moments of pH decrease that happens between the plaque and enamel. The remineralization stage is simulated by using solutions that contains calcium and phosphate in a degree of saturation similar to that of saliva[31,35].

Relating the use of acetate and lactic buffers, it was found that only 02 studies[43,15] used it as cariogenic challenge and 09 studies[36,37,34,41,10,38,9,8,3] employed the lactic buffer. It is important to remember that the buffer effect depends on two parameters, the pK of the acid and the ratio of salt to acid, according to the equations: pH= pK + log [salt] / [acid]. The buffer effect is maximum when [salt] / [acid] equals 1, in which case log [salt] / [acid] = 0 and pH = pK. Hence, the buffer effects of acetate and lactate, with pKs of 4.75 and 3.86, are

optimal at pH 4.75 and 3.86, respectively. Since the pH of dental plaque rarely falls below 4.0, acetate would be expected to be a more effective intra-oral buffer than lactate[45].

Analysis methods

Two quantitative methods were used for evaluation of enamel demineralization: microhardness and microradiography. Both are known to be very sensitive methods.

Microhardness test was the most (7 papers) [8,31,32,33,39,43,44] used method when the mineral loss or percentage of inhibition of enamel demineralization was studied. The advantages of this test are: determine the mineral content quantitatively, estimate the mineral loss and mineral gain values, obtain the mineral profile (volume % of mineral as a function of the distance from the outer surface. Even of being a sensitive and an easy-learn test, and be accessible in most laboratories, it has disadvantages in that the method does not allow direct measurement of the mineral content and also requires a flat polished surface to perform accurate results [46,47].

Microradiography analysis was also reported[42,14,11,9,3] as the method of choice for quantifying enamel mineral loss. It gives a direct quantitative analysis of the amount of mineral seen by the x-ray beam. However, this methodology is only available in laboratories of developed countries, and excludes the organic content. It is important to commit to memory that the hardness values are presumably created by the combination of inorganic material and organic matrix[48].

Comparative data from microhardness and microradiography measurements are scarce but have shown some correlation[48]. In this review, none of the studies performed the two tests so the association was not possible.

Extensive studies[3,9,10,11,20,32,33,34,36,37,38,39] used Scanning Electron Microscopy (SEM) to investigate the effects of CO_2 laser irradiation on enamel surface[33]. In this review, SEM analysis was performed in 12 studies but only 08 reported surface changes[3,9,32,33,34,36,37,38] such as melting, cracks, fusion, recrystallization and carbonization. As the temperature induced modifications on

enamel, the absence of surface changes showed in the others studies[11,20,31,42] means that the temperature achieved was not sufficient to modify the enamel surface (<800°C).

After laser irradiation the chemical characteristics of enamel structure are also important. Ft-Raman analysis gives information about chemical state without damage. In this review, the FT-Raman Spectroscopy was performed in only two studies[9,33]. In both, Tetracalcium diphosphate monoxide was identified as being a component of the melting surface indicating that enamel achieved high temperatures.

Changes that can occur in dental enamel surface after the laser irradiation

As a result of laser interaction with dental enamel, melting, fusion, and recrystallization are structural changes that can modify enamel morphology. Melting occurs when the enamel surface is changed to a liquid condition by heat. It is defined as the initial stage of individual crystals beginning to coalesce. Fusion is defined as the stage when melting is complete and individual crystals have fused together to form large, new crystals. Recrystallization occurs when a liquid phase resulting from the heat process is exposed to the environment[40]. For hydroxyapatite, the melting temperature is in the range of ~800–1620ºC. Although using different irradiation parameters, the studies of Schmidlin et al[36] (2007), Steiner-Oliveira et al[33] (2006), Klein et al[32] (2005), Tepper et al[37] (2004), Hossain et al [38,41] (2002; 1999), Lakshmi et al[10] (2001), Nelson et al[9,8] (1987; 1986) and Stern et al[3] (1972) reported this surface change (melting). Therefore, this phenomenon depends on the melting point of carbonated hydroxyapatite that varies with carbonate content[40,41], in a way that the lower the carbonate content, the lower the dissolution rate[26].

The relation between the melting and carbonate content must be remembered for the reason that during the heating and melting process hydroxyapatite looses carbonate[49], up to a complete loss, becoming more resistant demineralization. Of the carbonate 30% is lost between 400 and 600 °C and it is completely removed only after repeated irradiation beyond the melting temperature

of enamel[50]. Complete carbonate removal derives both from the absorption depth of the surface and from the pulse intensity and duration[51].

The studies of Featherstone et al [31] (1998); Kantorowitz et al[39] (1998); Hsu et al[11,42] (2000; 2001) and Esteves-Oliveira et al[20] (2009) did not relate surface changes. The absence of these changes, especially melting, implies that the maximum temperature reached by enamel in these studies was lower than 800°C. This implies that melting of enamel surface may occur as a consequence of enamel irradiation but it is not a necessary change for laser irradiation to inhibit enamel demineralization.

Inhibition of enamel demineralization – mineral loss

The percentage of caries inhibition had a wide range varying from 25 to 98%. A search in the scientific databases showed that the studies that used an energy density between 8 and 16 J/cm^2 achieved a maximum inhibition of 87%[8,31,32,34,39,41,43]. In the other side, Esteves-Oliveira et al[20] (2009) and Hsu et al[11] (2000) related an inhibition of caries progression of 81% and 98%, respectively, using an energy density of 0.3 J/cm^2. However, the first authors assessed enamel demineralization by lesion depth measurement while in most of the others studies a quantitative analysis of mineral loss was performed (microhardness or microradiography). Moreover, Hsu et al [11] (2000) did not analyze their specimens to determine how much of the organic matrix was removed, and neither did they verify whether the mineral phase had been altered by sodium hypochlorite (NaClO). As some magnesium and carbonate ions could be removed from the dental substrate upon NaClO treatment[52], the inhibition showed by Hsu et al [11] (2000), might be the effect of the NaClO rather than the laser effect.

When concerning pulse duration, short pulses allow the thermal relaxation (~60-90 μs) time of enamel. In this way, the second pulse interacts differently with the surface than the first one and the optical and thermal properties of the surface changes until a certain balance is achieved. The interaction between enamel surface and each additional pulse are not clear and additional studies are needed to further understand the sequence of events[41,44]. The overlapping of a

higher number of pulses on the same area has the potential to increase the resistance of enamel to demineralization, until a threshold of stabilization is reached[39].

In summary, the effectiveness of CO_2 laser in turning enamel more resistant to acid dissolution is directly related to the way the laser parameters are defined (energy density/effect relationship), and the main limitation when selecting these laser parameters is the operating mode of the laser equipment.

Conclusions

Beyond the limits of this systematic review, and the criteria proposed for the evidence level, there is moderate evidence that the structural or compositional changes occurring on enamel surface irradiated with a CO_2 laser at 10.6 μm are related to its resistance to demineralization.

In spite of this moderate evidence, further well-designed and randomized *in vitro* and *in situ/in vivo* studies are needed to establish and validate a standard protocol for CO_2 laser (10.6 μm) application of before it can be recommended for caries prevention in dental practice.

References

1. Maiman TH. Stimulated optical radiation in ruby. *Nature* 1960; 187 (4736): 493-494.
2. Stern RH, Sognnaes RF. Laser beam effects on dental hard tissues (abstract). *J Dent Res* 1964; 43: 873.
3. Stern RH, Vahl J, Sognnaes RF. Lased enamel: ultrastructural observations of pulsed carbon dioxide laser effects. *J Dent Res* 1972; 51 (2): 455-460.
4. Patel CKN, McFarlane RA, Faust WL. Selective excitation through vibrational energy transfer and optical laser action in N_2-CO_2. *Physiol Rev* 1964; 13: 617-619.
5. Coluzzi DJ. Fundamentals of dental lasers: a science and instruments. *Dent Clin N Am* 2004; 48 (4): 751-770.

6. Featherstone JDB, Fried D, Bitten ER. Mechanism of laser induced solubility reduction of dental enamel. In: *SPIE*, Bellingham, WA, 1997: 112-116.
7. Fowler BO, Kuroda S. Changes in heated and laser-irradiated human tooth enamel and their probable effects on solubility. *Calc Tiss Int* 1986; 38 (4): 197-208.
8. Nelson DGA, Shariati M, Glena R, shields CP, Featherstone JDB. Effect of a pulsed low energy infrared laser irradiation on artificial caries-like lesion formation. *Caries Res* 1986; 20 (4): 289-299.
9. Nelson DGA, Wefel JS, Jongebloed WL, Featherstone JDB. Morphology, histology and crystallography of human dental enamel treated with pulsed low-energy infrared laser radiation. *Caries Res* 1987; 21 (5): 411-426.
10. Lakshmi A, Shobha D, Lakshminarayanan L. Prevention of caries by pulsed CO_2 laser pre-treatment of enamel: an *in vitro* study. *J Indian Soc Pedo Prev Dent* 2001; 19(4): 152-156.
11. Hsu CY, Jordan TH, Dederich DN, Wefel JS. Effects of low-energy CO_2 laser irradiation and the organic matrix on inhibition of enamel demineralization. *J Dent Res* 2000; 79 (9): 1725-1730.
12. Britton M. Evidence-based medicine. Grading the scientific values and strength of conclusions in clinical trials. *Läkartidningen* 2000; 97(40): 4414-4415. [In Swedish].
13. Pascon FM, Kantovitz KR, Sacramento PA, Nobre-dos-Santos M, Puppin-Rontani RM. Effect of sodium hypochlorite on dentine mechanical properties. A review. *J Dent* 2009; 37 (12): 903-908.
14. Hsu J, Fox, JL, Wang Z, Powell GL, Otsuka M, Higuchi WI. Combined effects of laser irradiation/solution fluoride ion on enamel demineralization. *J Clin Laser Med Surg* 1998; 16 (2): 93-105.
15. Fox JL, Yu D, Otsuka M, Higuchi WI, Wong J, Powell G. Combined effects of laser irradiation and chemical inhibitors on the dissolution of dental enamel. *Caries Res* 1992; 26 (5): 333-339.

16. Akobeng AK. Principles of evidence based medicine. *Arch Dis Child* 2005; 90 (8): 837-840.
17. Sackett DL, Strauss SE, Richardson WS. Evidence-based medicine: how to practice and teach. EBM. London: Churchill-Linvingstone, 2000.
18. Rychetnik L, Hawe P, Waters E. A glossary for evidence based public health. *J Epidemiol Comm Health* 2004; 58 (7): 538-545.
19. Bowen WH. The influence of dietary patterns and de and remineralization of tooth enamel. In: *Leach SA, Edgar WM (eds) Demineralization and remineralization of the teeth,* Oxford 17-24, 1983.
20. Esteves-Oliveira M, Zezell DM, Meister J, Franzen R, Stanzel S, Lampert F, Eduardo CP, Apel C. CO_2 laser (10.6 μm) parameters for caries prevention in dental enamel. *Caries Res* 2009; 43 (1): 261-268.
21. Arends J, Schuthof J, Jongebloed WG. Lesion depth and microhardness indentations on artificial white spot lesions. *Caries Res* 1980; 14 (4): 190-195.
22. Bachman L, Diebolder R, Hibst R, Zezell DM. Infrared absorption bands of enamel and dentin tissues from human and bovine teeth. *Appl Spectrosc Rev* 2003; 38 (1): 1-14.
23. Featherstone JD, Mellberg JR. Relative rates of progress of artificial carious lesions in bovine, ovine and human enamel. *Caries Res* 1981; 15 (1): 109-114.
24. Fox JL, Yu D, Otsuka M, Higuchi WI, Wong J, Powell GL. Initial dissolution rate studies on dental enamel after CO_2 laser irradiation. *J Dent Res* 1992; 71(7): 1389-1398.
25. Rodrigues LKA, Nobre-dos-Santos M, Pereira D, Assaf AV, Pardi V. Carbon dioxide laser in dental caries prevention. *J Dent* 2004; 32 (7): 531-540.
26. Kuroda S, Fowler BO. Compositional, structural and phase changes *in vitro* laser-irradiated human tooth enamel. *Calcif Tissue Int* 1984; 36 (4): 361-369.

27. Ferreira JM, Palamara J, Phakey PP, Rachinger WA, Orams HJ. Effects of continuous wave CO_2 laser on the ultrastructure of human dental enamel. *Arch Oral Biol* 1989; 34 (7): 551-562.
28. Walsh LJ, Perham SJ. Enamel fusion using a carbon dioxide laser: a technique for sealing pits and fissures. *Clin Prev Dent* 1991; 13 (3): 16-20.
29. Fried D, Borzillary SF, McComarck SM, Glena R, Featherstone JDB. The thermal effects on CO_2 laser irradiated dental enamel at 9.3,9.6, 10.3,10.6 μm. In: *Laser surgery, advanced characterization, therapeutics and system IV.* Vol. 2128. Bellingham, WA: SPIE; 1994: 319-328.
30. Palamara J, Phakey PP, Orams HJ, Rachinger WA. The effect on the ultrastructure of dental enamel of excimer-dye, argon-ion and CO_2 lasers. *Scannig Microsc* 1992; 6 (4): 1061-1071.
31. Featherstone JDB, Barret-Vespone NA, Fried D, Kantorowitz Z, Seka W. CO_2 laser inhibition of artificial caries-like lesion progression in dental enamel. *J Dent Res* 1998; 77 (6): 1397-1403.
32. Klein ALL, Rodrigues LKA, Eduardo CP, Nobre dos Santos M, Cury JA. Caries inhibition around composite restorations by pulsed carbon dioxide laser application. *J Oral Sci* 2005; 113 (3): 239-244.
33. Steiner-Oliveira C, Rodrigues LKA, Soares LES, Martin AA, Zezell DM, Nobre-dos-Santos M. Chemical, morphological and thermal effects of 10.6μm CO_2 laser on the inhibition of enamel demineralization. *Dent Mat J* 2006; 25 (3): 455-462.
34. Tsai C-L, Lin Y-T, Huang S-T, Chang H-W. *In vitro* acid resistance of CO_2 and Nd-YAG laser treated human tooth enamel. *Caries Res* 2002; 36 (6): 423-429.
35. Serra MC, Cury JA. The *in vitro* effect of glass-ionomer cement restoration on enamel subjected to a demineralization and remineralization model. *Quintessen Int* 1992; 23 (2): 143-147.

36. Schmidlin PR, Dorig I, Lussi A, Roos M, Imfeld T. CO_2 laser-irradiation through topically applied fluoride increases acid resistance of demineralized human enamel *in vitro*. *Oral Health Prev Dent* 2007; 5 (3): 201-208.
37. Tepper SA, Zehnder M, Pajarola GF, Schmidlin PR. Increased fluoride uptake and acid resistance by CO_2 laser-irradiation through topically applied fluoride on human enamel *in vitro*. *J Dent* 2004; 32 (8): 635-641.
38. Hossain M, Nakamura Y, Kimura Y, Mitsuhiro I, Yamada Y, Matsumoto K. Acquired acid resistance of dental hard tissues by CO_2 laser irradiation. *J Clin Laser Med Surg* 1999; 17 (5): 223-226.
39. Kantorowitz Z, Featherstone JDB, Fried D. Caries prevention by CO_2 laser treatment: dependency on the number of pulses used. *JADA* 1998; 129 (5): 585-591.
40. McCormack SM, Fried D, Featherstone JDB, Glena RE, Seka W. Scanning electron microscope observations of CO_2 laser effects on dental enamel. *J Dent Res* 1995; 74 (10): 1702-1708.
41. Hossain MMI, Hossain M, Kimura Y, Kinoshita J-I, Yamada Y, Matsumoto K. Acquired acid resistance of enamel and dentin by CO_2 laser irradiation with sodium fluoride solution. *J Clin Laser Med Surg* 2002; 20 (2): 77-82.
42. Hsu CY, Jordan TH, Dederich DN, Wefel JS. Laser matrix fluoride effects on enamel demineralization. *J Dent Res* 2001; 80 (9): 1797-1801.
43. Steiner-Oliveira C, Rodrigues LKA, Lima EB, Nobre-dos-Santos M. Effect of the CO_2 laser combined with fluoridated products on the inhibition of enamel demineralization. *J Contemp Dent Pract* 2008; 9 (2): 113-121.
44. Steiner-Oliveira C, Vieira KA, Sousa RP, Rodrigues LKA, Nobre-dos-Santos M. *In situ* effect of the CO_2 laser on enamel caries adjacent to composite restorations. *Rev Assoc Paul Cir Dent* 2008; 62 (3): 189-194. [In Portuguese]
45. Brudevold F, Tehrani A, Attarzadeh F, Goulet D, van Houte J. Effect of some salts of calcium, sodium, potassium, ans strontium on intra-oral enamel demineralization. *J Dent Res* 1985; 64 (1): 24-27.

46. Arends J, ten Bosch JJ. Demineralization and Remineralization evaluation techniques. *J Dent Res* 1992; 71 (Spec Iss): 924-928.
47. Clasen ABS, ∅gaard B. Experimental intra-oral caries models in fluoride research. *Acta Odontol Scand* 1999; 57 (6): 334-341.
48. Featherstone JDB, ten Cate JM, Shariati M. Comparison of artificial caries-like lesions by quantitative microradiography and microhardness profiles. *Caries Res* 1983; 17 (5): 385-391.
49. Featherstone JD. Caries detection and prevention with laser energy. *Dent Clin North Am* 2000; 44 (4): 955-969.
50. Zuerlein MJ, Fried D, Featherstone JDB. Modeling the modification depth of carbon dioxide laser-treated dental enamel. *Lasers Surg Med* 1999; 25 (4): 335-347.
51. Palamara J, Phakey PP, Rachinger WA. The ultrastructure of human dental enamel heat-treated in the temperature range 200°C to 600°C. *J Dent Res* 1987; 66 (12): 1742-1747.
52. Sakae T, Mishima H, Kozawa Y. Changes in bovine dentin mineral with sodium hypochlorite treatment. *J Dent Res* 1988; 67 (9): 1229-34.

CAPÍTULO 2

In vitro evaluation of several overlapping CO_2 laser applications on enamel demineralization.

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Keywords: CO_2 laser, dental enamel, demineralization, SEM, FT-Raman

* This article was submitted to Lasers in Surgery and Medicine. Manuscript ID: LSM-10-111.

Abstract

Background and Objective: Studies have shown that CO_2 (10.6 μm) laser can be used to modify the morphological and chemical composition of tooth enamel to render it less soluble. Among the different laser sources designed to be utilized in the dental clinical practice, CO_2 lasers have attracted considerable attention due to their high absorption coefficient by dental hard tissues. The aim of this study was to evaluate the effects of repeated CO_2 laser applications on the inhibition of enamel demineralization. The hypothesis was if the increased in the number of CO_2 laser irradiations would increase the effect of this laser in reducing enamel demineralization.

Study Design/Material and Methods: Sixty-five human dental enamel slabs were randomly assigned to the following 5 groups (n=13): Control (C), 1 application of CO_2 laser (L1), 2 applications of CO_2 laser (L2), 3 applications of CO_2 laser (L3) and 4 applications of CO_2 laser (L4). Enamel slabs were irradiated by a 10.6 μm CO_2 laser at 10 J/cm^2, for 10 ms, with a repetition rate of 50 Hz. They were submitted to a pH-cycling regimen and then analyzed by FT-Raman Spectroscopy, Energy-Dispersive X-Ray fluorescence spectrometry, Cross Sectional Microhardness and Scanning Electron Microscopy. Statistical analysis was performed by ANOVA and Tukey's test ($p<0.05$).

Results: The main change showed by FT-Raman spectroscopy was on the phosphate (585cm^{-1}) band for control and 1 irradiation, 2 irradiations and 3 irradiations groups ($p<0.05$). Cross sectional microhardness data showed statistically significant difference between the control and all irradiated groups ($p<0.05$), but no difference was found among the irradiated groups ($p>0.05$). The 3 irradiations group showed a reduction in demineralization until even the 50 μm. The SEM results showed that with repeated applications of CO_2 laser, a progressive melting and recrystallization of enamel surface occurred.

Conclusions: Three repeated irradiations of dental enamel with a CO_2 laser at 10.6 μm, at an energy density of 10 J/cm^2, enhanced the inhibition of enamel demineralization.

Introduction

In spite of reduction in the prevalence of dental caries, it still remains a major public health problem in some strata of the population[1]. Thus, the development of new methods to prevent dental caries is important to control the disease.

Since the development of the ruby laser by Maiman[2] in 1960, several studies have demonstrated that laser (pre)treatment of enamel can inhibit subsequent artificial caries-like lesions[3,4]. For more than 40 years, several studies have shown that irradiation of dental enamel with Carbon dioxide (CO_2) laser, created by Patel et al.[5] in 1964, turns this substrate more resistant to demineralization. Nelson et al.[6] (1986) described in their study that a pulsed CO_2 laser irradiation (10-50 J/cm^2) of enamel caused marked surface fusion and inhibited the progression of subsurface caries-like lesions by as much as 50%. This could be explained by the presence of hydroxyapatite, which has absorption bands in the infrared region (9.0 - 11.0 μm) due the presence of phosphate, carbonate and hydroxyl groups in its crystal structure[6]. This absorption coincides closely with the radiation produced by the CO_2 laser[4], which implies that enamel should efficiently absorb this radiation[7,8,9].

Irradiation of enamel by a CO_2 laser usually involves the melting, fusion and recrystallization process if the proper laser energy is applied, and this process produces apparent morphological and crystallographic changes in enamel.[10,11,12,13,14] These changes of enamel crystals can be coffined to a thin surface region without affecting the underlying dentin or pulp.[8,9]

In all previous studies, the inhibition of enamel demineralization effect was obtained when CO_2 laser irradiation was performed over the enamel only one time. However, at least to our knowledge, it is not known if more than one laser application could bring some additional effect on the inhibition of enamel demineralization. Thus, the aim of this *in vitro* study was to evaluate the effects of cumulative irradiation of CO_2 laser on the inhibition of enamel demineralization.

Materials and Methods

This study was approved by the Research and Ethics Committee of the Piracicaba Dental School at University of Campinas in Piracicaba, SP, Brazil (Protocol Nº 017/2009).

Sample preparation

To perform this *in vitro* study, fifty extracted impacted human third molars, stored in 0.1% thymol solution, were selected, cleaned and sterilized using gamma radiation[15]. These teeth were sectioned using a water-cooled diamond saw cutting machine (Isomet 1000, Buehler, Lake Bluff, IL, USA) to obtain 65 enamel slabs (4 x 4 mm). All slabs were obtained from the medium third of buccal or lingual coronal surfaces of each tooth. Then, the enamel surface was screened for cracks and white spots under a stereoscopic microscope. The enamel slabs were kept moist before experimentation was begun. The slabs were randomly assigned according to a computer generated list to one of the following 5 groups (n=13): Control (C), 1 application of CO_2 laser (L1), 2 applications of CO_2 laser (L2), 3 applications of CO_2 laser (L3) and 4 applications of CO_2 laser (L4).

Laser treatment

The enamel surface irradiation was carried out using a X-Y position platform. They were scanned at a distance of 10 mm from the tip of the handpiece for approximately 30 seconds. The scanning speed was approximately 1 mm/s. A commercially available Model UM-L30 pulsed CO_2 laser (Union Medical Engineering Co., Yangju-si, Gyeonggi-Do, Korea) at a wavelength of 10.6 μm was used for irradiating the groups L1, L2, L3 and L4 with the following parameters: 0.7 W of power, 10 ms pulse duration, 10 ms of time off, 50 Hz repetition rate, 0.3 mm beam diameter. Using a power meter (Scientech Inc., Boulder, CO, USA), the average power output was measured and found to be 0.7 W. Thus, the laser fluence applied on enamel was approximately 10.0 J/cm^2 per pulse. These parameters have been previously tested[10] and showed no possibility of pulp

damage. No cooling process was performed in the slabs during the laser irradiation, and also there was no waiting time between the laser applications. The irradiated slabs (16 mm^2) were coated with an acid-resistant varnish leaving a window (4 mm^2) of exposed enamel surface for the artificial caries-like lesion production.

Fourier Transform Raman Spectroscopy (FTRS)

After laser treatment, the samples of each group were evaluated by Fourier Transform Raman Spectroscopy (FTRS). Spectra of the samples were obtained using a FT-Raman spectrometer (RFS 100/S, Bruker Inc., Karlsruhe, Germany) both before and after irradiation with one Ge diode detector cooled by liquid N_2. To excite the spectra, a focused λ= 1,064.1 nm beam of an air-cooled Nd:YAG laser source was used. Maximum incident laser power on the sample surface was about 150 mW and spectrum resolution was 4 cm^{-1}. The samples were positioned in a sample holder, and an IR354 lens collected radiation scattered over 180º on the exposed surface. The FT-Raman spectra were obtained using 100 scans. The selected point was located in the central region of the dental slab. Frequency ranged from 300 to 4,000 cm^{-1}, thereby allowing a characterization of both mineral (hydroxyapatite) and organic (essentially collagen) constituents.

Energy-dispersive X-ray fluorescence spectrometry (EDXRF) Measurements

Semiquantitative elemental analyses of calcium (Ca) and phosphorus (P) were carried out by an energy-dispersive micro X-ray fluorescence spectrometer, model μ-EDX 1300, Shimadzu (Kyoto, Japan), equipped with a rhodium X-ray tube and a Si (Li) detector cooled by liquid nitrogen (N_2). The spectrometer was coupled to a computer system for data acquisition and processing. The voltage in the tube was set at 15 kV, with automatic adjustment of the current and incident beam diameter of 50 μm.

One point located in the center of the irradiated enamel slab was chosen for the measurement. The measurements were performed with a count rate of 100

s per point (live time) and a dead time of 25%. The energy range of scans was 0.0–40.0eV. The equipment was calibrated and adjusted using certified commercial stoichiometric hydroxyapatite (Aldrich, synthetic $Ca_{10}(PO_4)_6(OH)_2$, grade 99.999%, lot10818HA) as a reference. The measurements were collected using classic parameters for Ca and P X-ray emission. The elements oxygen (O) and hydrogen (H) were used as a chemical balance. Energy calibration was performed using equipment-specific internal standards.

Caries-like lesion formation (pH cycling process)

The pH-cycling model used in this study was based on the one described by Featherstone et al.[16] (1988) with modifications by Steiner-Oliveira et al.[17] (2008). The enamel slabs were kept individually in a demineralizing solution (2.0 mM calcium, 2.0 mM phosphate, in 75 mM acetate buffer, pH 4.6) for 3 hours (20 mL per slab - 5 mL/mm^2), and in a remineralizing solution (1.5 mM calcium, 0.9 mM phosphate, 150 mM of KCl, in 20 mM cacodylic buffer, pH 7.0) for approximately 21 hours (10 mL per slab -2.5 mL/mm^2) each day. After each cycle, the slabs were returned to the solutions. This cycle was repeated daily for 5 days and the enamel slabs remained in the remineralizing solution for 2 days. Both solutions were changed daily. This pH-cycling regimen was carried out at 37ºC. Between immersions in demineralizing and remineralizing solutions and at the end of the pH-cycling regimen, the enamel slabs were rinsed with deionized distilled water for 10 seconds and wiped with tissue paper. The demineralizing and remineralizing solutions contained thymol to avoid bacterial and fungal growth. The enamel slabs were kept moist until the analyses.

Cross-sectional enamel microhardness (CSEM) analysis

For cross-sectional enamel microhardness analysis, each enamel slab was longitudinally sectioned with a cut through the center of the exposed area (one half for CSEM and the other for SEM). The segments to be used for CSEM analysis were embedded in a thermo-acrylic resin Vipicril Plus (VIPI Ind e Com,

Pirassununga, SP, Brazil) and heated in the PRE 30MI (Arotec SA Ind. E Com, Cotia, SP, Brazil). Then they were serially flattened and polished using an AROTEC APL-4 (Arotec SA Ind. E Com, Cotia, SP, Brazil) polishing machine and sandpapers, followed by diamond abrasive paste on polishing cloths. The slabs were assessed for microhardness test with a Knoop diamond under a 25g load for 5 seconds mounted on a HMV 2000 microhardness testing device (Shimadzu, Japan) and read at 200X magnification. Thirty-six indentations (three rows of 12 indentations each) were made with the long axis of the Knoop diamond parallel to the outer surface, maintaining a 10-μm interval between 10 μm and 60 μm and then a 20 μm interval from 80 μm to 180 μm across the lesion and into the underlying enamel. The distance between the rows was 100 μm. The mean Knoop hardness number (KHN) values at each distance was obtained.

Scanning Electron Microscopy (SEM) analysis

Morphological investigation was performed in order to verify the effects of repeated applications of CO_2 laser on the enamel surface. Thus, the other half of the cut enamel slab was examined under scanning electron microscopy. All the specimens were previously submitted to an ultra-sound system for 10 seconds twice and then air-dried. They were mounted on aluminum stubs and sputter-coated with gold (~10-12nm thickness) by the BAL-TEC SCD 050 (Wetzlar, Liechtenstein/Vienna, Austria). Observations were made with a JEOL JSM-5600 LV Scanning Electron Microscope (Jeol, Peaboy, MA, USA) at 15 kV with magnifications up to x2000.

Statistical Analysis

The Kolmorogov-Smirnov test verified the normal distribution of the sample data, and the Levene's test verified the variance homogeneity.

FTRS - In order to normalize measurements and allow their comparison, the band surface parameter was used, which corresponds to the area under the curve for "n" analyzed band. Fluorescence spectrum was removed with a

polynomial fitting with varying degrees from the spectra, by means of Origin 5.0® software (Microcal Software, Northampton. USA). Areas under the curve were calculated for each band using Microcal Origin 5.0®. Changes in mineral and organic components were evaluated by comparing the relative peak areas in enamel in the different groups. Statistical analysis was performed by ANOVA and Tukey's test ($p<0.05$) using BioEstat 5.0® software.

EDXRF - The Calcium and Phosphorus percentages obtained by X-ray analysis were statistically compared among the control and lasers groups. Those comparisons were performed using the Tukey's test ($p<0.05$).

CSEM - A one-way analysis of variance (ANOVA) model was constructed to assess the enamel mineral loss effects of the laser irradiations. Next, the Tukey's test was chosen to evaluate the significance of all pair-wise comparisons. Values of $p<0.05$ were accepted as statistically significant using the SAS (Statistical Analysis System).

Results

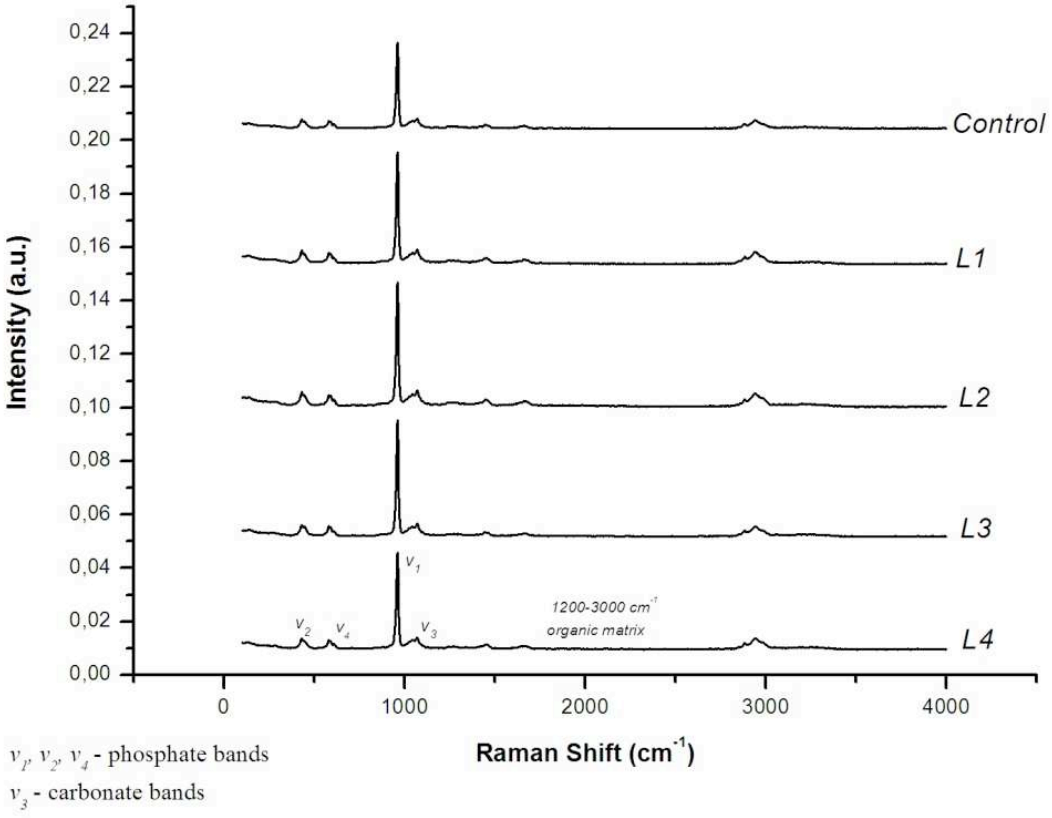
Fig. 1 shows the average Raman spectra of all groups. The region spanning from 300 to 1,100 cm^{-1} was characteristic of phosphate groups and representative of the mineral phase of enamel. The FT-Raman bands at ν_2 (430-450 cm^{-1}), ν_4(585-612 cm^{-1}), ν_1(960 cm^{-1}), represent the phosphate vibrations in hydroxyapatite (Fig. 1). The band in the range of 1026-1072 cm^{-1} can also represent the ν_3 carbonate vibration (type B carbonate). For the bands ν_2 (430-450 cm^{-1}), 612 cm^{-1} e 1072 cm^{-1}, no statistically significant differences in enamel spectrum could be observed among all groups (Table 1). For the 585 cm^{-1} band, the control group was statistically different from the L1 ($p<0.05$); and L1 differ statistically from L2 and L3 ($p<0.05$). For the 960 cm^{-1} band, only the L2 was statistically different from L4 ($p<0.05$). For the 1026 cm^{-1} band, just L1 was statistically different from L4. For the 1045 cm^{-1} band, the control group was different from L1, L3 and L4 groups ($p<0.05$).

The EDXRF measurements showed no statistical difference between the control and irradiated groups for Calcium and Phosphorus components ($p>0.05$) (Table 2).

Mean HKN represented the severity of average caries-like lesion that was developed in each group. Our results showed a statistically significant inhibition of enamel demineralization in all irradiated groups when compared to control group (Table 3). The effect of laser was observed from 10-30 μm depths, and for the group L3, the irradiation effect was deeper (until 50 μm depths). However, no statistically significant difference in the mean of the HKN number was evidenced among the irradiated groups.

The SEM observations showed evidence of melting and fusion in the specimens treated with the CO_2 laser in all the laser applications (Fig. 2). Enamel surfaces showed fusion across the prisms boundaries and melted structures several times larger than prismatic structures (Fig.2.d; Fig.2.e). Recrystallization was observed specially in group L3 (Fig.2.d.). Fusion and melting of the enamel surface were more frequent in the groups submitted to more than one laser application.

Fig1. Raman spectra for all groups



v_1, v_2, v_4 - phosphate bands

v_3 - carbonate bands

Table 1. Areas under the peaks bands (means± SD) for all groups

Raman Peaks	430 cm^{-1}	450 cm^{-1}	585 cm^{-1}	612 cm^{-1}	960 cm^{-1}	1026 cm^{-1}	1045 cm^{-1}	1072 cm^{-1}
Groups								
Control	0.08 ± 0.02^{a}	0.08 ± 0.01^{a}	0.13 ± 0.03^{ab}	0.05 ± 0.03^{a}	0.69 ± 0.10^{ab}	0.03 ± 0.02^{ab}	0.07 ± 0.02^{a}	0.12 ± 0.01^{a}
L1	0.07 ± 0.01^{a}	0.06 ± 0.02^{a}	0.07 ± 0.04^{c}	0.03 ± 0.01^{a}	0.60 ± 0.10^{ab}	0.04 ± 0.02^{a}	0.02 ± 0.02^{b}	0.08 ± 0.03^{a}
L2	0.07 ± 0.01^{a}	0.08 ± 0.01^{a}	0.13 ± 0.01^{ab}	0.02 ± 0.00^{a}	0.81 ± 0.14^{a}	0.02 ± 0.01^{ab}	0.05 ± 0.01^{ab}	0.12 ± 0.05^{a}
L3	0.07 ± 0.02^{a}	0.06 ± 0.02^{a}	0.13 ± 0.01^{ab}	0.02 ± 0.01^{a}	0.67 ± 0.19^{ab}	0.03 ± 0.01^{ab}	0.02 ± 0.01^{b}	0.10 ± 0.04^{a}
L4	0.05 ± 0.01^{a}	0.06 ± 0.01^{a}	0.09 ± 0.02^{ac}	0.02 ± 0.01^{a}	0.56 ± 0.10^{b}	0.01 ± 0.01^{b}	0.02 ± 0.01^{b}	0.08 ± 0.02^{a}

Different letters indicate statistically significant differences by *Tukey*'s test ($p< 0.05$).

Table 2. Mean and standard deviations of calcium, phosphorus and oxygen percentages obtained by EDXRF

Components	Groups	Mean (DV)	p_value_{anova}
Calcium	Control	32.93 (1.91)	0.865
	L1	32.85 (9.33)	
	L2	30.27 (8.98)	
	L3	33.98 (6.34)	
	L4	33.12 (7.80)	
Phosphorus	Control	17.53 (0.78)	0.840
	L1	16.87 (5.49)	
	L2	16.28 (3.55)	
	L3	18.04 (2.26)	
	L4	17.49 (2.85)	
Oxygen	Control	49.54 (2.67)	0.861
	L1	50.28 (14.71)	
	L2	53.45 (12.50)	
	L3	47.98 (8.60)	
	L4	49.39 (10.64)	

Table 3. Mean (and standard deviation) of Knoop hardness number (KHN x μm) of each group

Groups	Depths (μm)											
	10μm	20μm	30μm	40μm	50μm	60μm	80μm	100μm	120μm	140μm	160μm	180μm
Control	151.29 (47.72) B	246.76 (88.64) B	280.81 (84.13) B	317.53 (80.70) B	324.20 (70.10) B	339.23 (71.06) A	372.74 (58.07) B	390.08 (51.80) B	389.64 (42.20) B	391.46 (48.02) B	406.59 (41.55) A	411.87 (47.53) B
L1	323.00 (46.91) A	368.74 (71.83) A	400.90 (79.71) A	389.33 (78.60) BA	394.87 (71.84) BA	393.49 (71.50) A	445.23 (44.14) A	452.21 (42.90) BA	449.87 (51.28) A	452.87 (36.09) A	451.28 (52.05) A	460.49 (42.24) BA
L2	344.62 (52.95) A	343.82 (56.67) A	367.46 (63.01) A	350.03 (53.86) BA	379.97 (77.32) BA	375.43 (88.27) A	439.54 (62.53) A	423.85 (69.59) BA	458.39 (55.72) A	447.54 (43.26) A	448.64 (41.53) A	458.77 (34.04) BA
L3	319.97 (79.25) A	370.85 (85.92) A	421.21 (80.53) A	424.46 (79.42) A	428.69 (61.73) A	425.33 (63.23) A	461.33 (56.23) A	474.54 (55.24) A	469.87 (60.14) A	483.23 (56.41) A	469.80 (55.17) A	481.13 (59.62) A
L4	332.59 (65.82) A	354.13 (66.87) A	352.95 (70.80) A	391.69 (87.05) BA	399.64 (56.40) A	378.00 (57.69) A	417.97 (58.81) BA	435.77 (71.26) BA	442.82 (59.80) BA	447.64 (51.03) A	435.42 (81.69) A	439.16 (70.95) BA
p value[1]	<0.0001	0.0003	0.0002	0.0083	0.0047	0.0515	0.0020	0.0091	0.0043	0.0002	0.0741	0.0186

[1]One way ANOVA, post-hoc test: Tukey ($p<0.05$).

Means followed by different letters within lines denote statistically significant differences.

Fig 2 (a,b,c,d,e). SEM micrographs (15 kV X2,500 Bar = 10 μm)

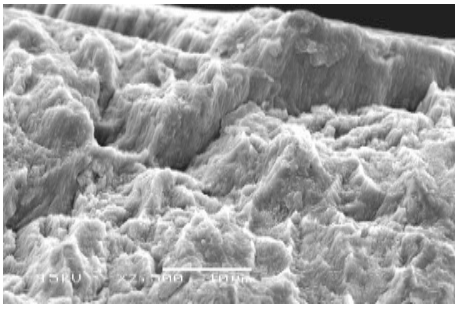


Fig. 2.a. Control Group. SEM micrograph of non-irradiated enamel and after pH cycling.

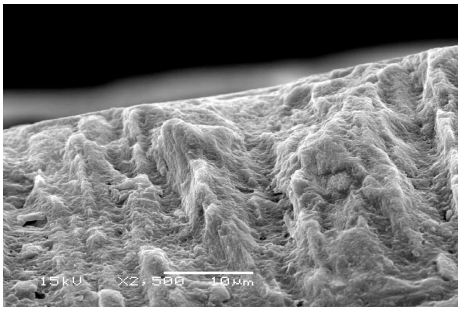


Fig. 2.b. L1 Group. SEM micrograph of 1 application of CO_2 laser on enamel and after pH cycling. Crystalline phase formation (melting of enamel crystallites). Enamel crystals fusion.

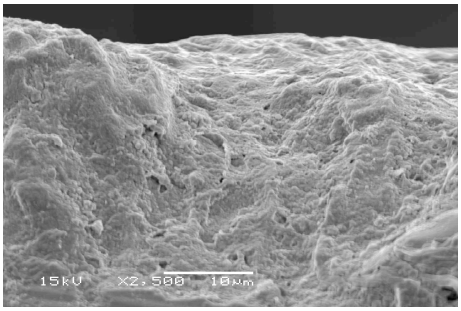


Fig. 2.c. L2 Group. SEM micrograph of 2 applications of CO_2 laser on enamel and after pH cycling. Surface alterations (melting and fusion). Enamel crystal fusion had progressed to the point that crystal definition was no longer visible.

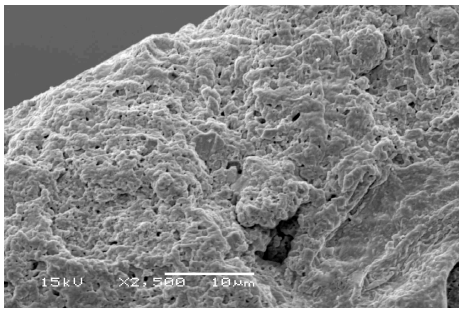


Fig. 2.d. L3 Group. SEM micrograph of 3 applications of CO_2 laser on enamel and after pH cycling. Crystal coalescence and alignment of crystals along the enamel rods. Droplets of recondensed enamel mineral in the irradiation area.

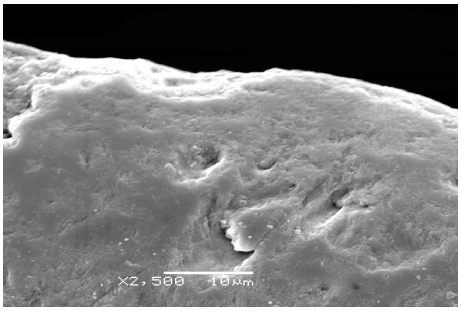


Fig. 2.e. L4 Group. SEM micrograph of 4 applications of CO_2 laser on enamel and after pH cycling. No enamel crystal boundaries are visible. Fusion. Solid Mass. Holes.

Discussion and Conclusion

The infrared spectrum of dental enamel contains bands due to each of the phosphate internal vibrational modes and carbonate modes, which have been previously reported[17,18] and immediately identified in this study.

No relevant differences were found between Raman spectra in respect to phosphate and carbonate bands, when irradiated groups were compared to the control one. However, except for group L2, the 1,045 cm^{-1} band, which represents carbonate vibration, was significantly decreased in all irradiated groups when compared to control group. It is possible that these effects of reduction in the carbonate content, which was previously reported by Zuerlein et al.[19] (1999), Steiner-Oliveira et al.[10] (2006) and Tagliaferro et al.[20] (2009), were responsible for the increased enamel acid resistance demonstrated in the present study.

Relating to phosphate and carbonate bands, Nishino et al.[21] (1981) reported that the peak intensities of CO_3^{-2}, and PO_4^{-3} bands are dependent of the CO_3^{-2} content of the specimens. The higher the CO_3^{-2} content, the higher the intensity of CO_3^{-2} bands, while the intensity of PO_4^{-3} bands shows a tendency to decrease. This suggests that if apatite had high concentrations of CO_3^{-2}, amounts of CO_3^{-2} substituted PO_4^{-3} in the lattice. Although without statistically significant differences in all cases, a numerical tendency of decreasing of the bands ascribed to phosphate vibrations in hydroxyapatite was found in irradiated enamel. This effect may be attributed to the lower degree of enamel crystallinity after irradiation. In this way, our results are in line with those reported by Steiner-Oliveira et al.[10] (2006) and Tagliaferro et al.[20] (2009).

In addition, it should be emphasized that no difference in carbonate content was found among the irradiated groups, thus demonstrating that the execution of several overlapping laser applications did not cause a higher carbonate reduction. Since carbonate fits less well in the lattice, causing distortions in the hydroxyapatite structure, generating a less stable apatite, its reduction may generate a more acid-resistant apatite phase[22,23]. Consequently, it can be

suggested that repeated laser applications are not relevant in augmenting enamel demineralization inhibition effect of CO_2 laser.

A broad band at around 740 cm^{-1} Raman shift of the enamel spectrum has been described in CO_2 laser irradiated enamel[13,24,25], but in this study such band was not observed in none of the irradiated groups as the enamel did not reach the high temperatures (>800-1100°C) required for identifying this band in Raman spectrum. Moreover, as a pulsed laser was used, and the thermal relaxation time for the enamel at a wavelength of 10.6 μm is around ~60-80 μs[26], the temperature increase caused by laser irradiation in enamel surface was not cumulative with more than one laser application.

The EDXRF analysis is based on bombarding the specimen with a beam of high voltage electrons that are refracted at different energy levels from individual minerals. The change in the energy returned from the specimen reflects the change in its mineral content[27]. This method allows the analysis of specimens accurately and non-invasively.[7] In our study, there was no statistically significant difference in mineral content between the lased and control groups. These findings are in agreement with the study of Moshonov et al.[7] (2005) and confirms that no ablation happened during the laser irradiation which was not desire as the laser was being used in a preventive way.

The control and irradiated groups differ statistically in the 10, 20, 30 μm depths. This is in agreement with Hirose et al.[28] (1996) and Zuerlein et al.[19] (1999) when they affirmed that the depth of laser impact in enamel could be estimated to be within 20-30 μm. The irradiated groups did not differ statistically, in the mean of HKN. Interesting was the group L3, which showed differences between the control group until 50 μm depth. Maybe, the reduced demineralization was correlated to permeability due to the sealing of enamel and its pores as reported by Nelson et al. [6] (1986). Furthermore, this melt layer presents reduced carbonate content when compared to normal surface enamel. As this phenomenon was not observed in the group L4, it is implied that this physical barrier has a limited effect on the enamel demineralization reduction. Moreover, it is possible that the organic blocking effect

reaches a maximum between 300 ~ 400 °C and decreases after complete decomposition of organic matrix above 400 °C[30].

The mechanism of laser action remains unclear although reports of several related studies have been published. One of the hypotheses for laser effects states that caries inhibition is due to the melting and fusing of hydroxyapatite crystals[27]. In the present study, the SEM images (Fig.2.a-e) suggested that the fusion and melting phenomena might be related to the inhibition of demineralization found in the irradiated groups. These results suggest that enamel surface was sealed by the laser irradiation and became less permeable to the subsequent diffusion of ions into and from the enamel[29]. These findings are in agreement with those found by Steiner-Oliveira et al.[10] (2006) and Klein et al.[9] (2005).

Rapid melting creates large hydrodynamic forces of expansion due to the volume change of the material upon melting and can eject material as liquid droplets[26]. SEM observations of irradiated enamel on group L3 (Fig.2.d.) showed this droplets and surface asperities, evidencing this hydrodynamic sputtering mechanism. Aside from that, holes (Fig.2.e.) were evidenced by SEM indicating that the pressure created by subsurface water vaporization was catastrophic.

In conclusion, our results showed that 3 repeated irradiations of dental enamel with a CO_2 laser at 10.6 μm, at an energy density of 10 J/cm^2, enhance the inhibition of enamel demineralization until 50 μm depth. The physical changes and also the organic blocking effect reduce enamel demineralization until a threshold is achieved (>400°C).

References

1. Micheelis W, Bauch J. Oral health of representative samples of Germans examined in 1989 and 1992. Community Dent Oral Epidemiol 1996; 24: 62-70.
2. Maiman TH. Stimulated optical radiation in ruby. Nature 1960; 187 (4736): 493-494.

3. Stern RH, Vahl J, Sonnaes RF. Lased enamel: ultrastructural observations of pulsed carbon dioxide laser effects. J Dent Res 1972; 51: 455-460.
4. Nelson DGA, Shariati M, Glena R, Shields CP, Featherstone JDB. Effect of pulsed low energy infrared laser irradiation on artificial caries-like lesion formation. Caries Res. 1986; 20: 289-299.
5. Patel CKN, McFarlane RA, Faust WL. Selective excitation through vibrational energy transfer and optical laser action in N_2-CO_2. Physiological Reviews 1964; 13: 617-619.
6. Nelson DGA, Jongedloed WI, Featherstone JBD. Laser irradiation of human dental enamel. New Zealand Dent J 1986; 82: 74-77.
7. Moshonov J, Stabholz A, Bar-Hilel R, Peretz B. Chemical analysis and surface morphology of enamel and dentin following 9.6 μm CO_2 laser irradiation versus high speed drilling. J Dent 2005; 33: 427-432.
8. Rodrigues LKA, Nobre-dos-Santos M, Pereira D, Assaf AV, Pardi V. Carbon dioxide laser in dental caries prevention. J Dent 2004; 32: 531-540.
9. Klein AL, Rodrigues LK, Eduardo CP, Nobre-dos-Santos M, Cury JA. Caries inhibition around composite restorations by pulsed carbon dioxide laser application. Eur J Oral Sci 2005; 113 (3): 239-244.
10. Steiner-Oliveira C, Rodrigues LK, Soares LE, Martin AA, Zezell DM, Nobre-dos-Santos M. Chemical, morphological and thermal effects of 10.6-microm CO_2 laser on the inhibition of enamel demineralization. Dent Mater J 2006; 25 (3): 455-462.
11. Hess JA. Scanning electron microscopy study of laser-induced morphologic changes of a coated enamel surface. Lasers Surg Med 1990; 10: 458-462.
12. Arcoria CJ, Lippas MG, Vitasek BA. Enamel surface roughness analysis after laser ablation and acid etching. J Oral Rehabil 1993; 20: 213-224.
13. Aminzadeh A, Shahabi S, Walsh LJ. Raman spectroscopic studies of CO_2 laser-irradiated human dental enamel. Spectrochim Acta A Mol Biomol Spectrosc 1999; 55: 1303-1308.

14. Takahashi K, Kimura Y, Matsumoto K. Morphological and atomic analytical changes after CO_2 laser irradiation emitted at 9.3 microns human dental hard tissue. J Clin Laser Med Surg 1998; 16: 167-173.
15. Rodrigues LK, Cury JA, Nobre-dos-Santos MN. The effect of gamma radiation on enamel hardness and its resistance to demineralization in vitro. J Oral Sci 2004; 46: 215-220.
16. Featherstone JDB, Shariati M, Brugler S, Fu J, White DJ. Effect of an anticalculus dentifrice on lesion progression under pH-cycling conditions in vitro. Caries Res 1988; 22: 337-341.
17. Steiner-Oliveira C, Rodrigues LKA, Lima EB, Nobre-dos-Santos MN. Effect of the CO_2 laser combined with fluoridated products on the inhibition of enamel demineralization. J Contemp Dent Pract 2008; 9 (2): 1-10.
18. Fattibene P, Carosi A, De Coste V, Sacchetti A, Nucara A, Postorino P et al. A comparative EPR, infrared and Raman study of natural and deproteinated tooth enamel and dentin. Phys Med Biol 2005; 50: 1095-1108.
19. Zuerlein MJ, Fried D, Featherstone JDB. Modeling the modification depth of carbon dioxide laser-treated dental enamel. Lasers Surg Med 1999; 25 (4): 335-347.
20. Tagliaferro EPS, Rodrigues LKA, Soares LES, Martin AA, Nobre-dos-Santos. Physical and Compositional changes on demineralized primary enamel induced by CO_2 laser. Photomed Laser Surg 2009; 27 (4): 585-590.
21. Nishino M, Yamashita S, Aoba T, Okazaki M, Moriwaki Y. The Laser-Raman spectroscopic studies on human enamel and precipitated carbonate-containing apatites. J Dent Res 1981; 60 (3): 751-755.
22. Rehman I, Smith R, Hench LL, Bonfield W. Structural evaluation of human and sheep bone and comparison with synthetic hydroxyapatite by FT-Raman spectroscopy. J Biomed Mater Res 1995; 29: 1287-1294.
23. Rehman I, Hench LL, Bonfield W, Smith R. Analysis of surface layers on bioactive glasses. Biomaterials 1994; 15: 865-870.

24. Nelson DG, Williamson BE. Raman spectra of phosphate and monofluorophosphate ions in several dentally-relevant materials. Caries Res 1985; 19: 113-121.
25. Fowler BO, Kuroda S. Changes in heated and laser-irradiated human tooth enamel and their probable effects on solubility. Calc Tiss Int 1986; 38: 197-208.
26. McCormack SM, Fried D, Featherstone JDB, Glena RE, Seka W. Scanning electron microscope observations of CO_2 laser effects on dental enamel. J Dent Res 1995; 74 (10): 1702-1708.
27. Ferreira JM, Palamara J, Phakey PP, Rachinger WA, Orams HJ. Effects of a countinuous-wave CO_2 laser on the ultrastructure of human dental enamel. Arch Oral Biol 1989; 34: 551-562.
28. Hirose M, Tange T, Igarashi S, Hirose Y, Nakagaki H. In vivo fluoride profiles at different sites of buccal and lingual enamel surfaces obtained by enamel biopsy of human maxillary first permanent molars in young adults. Arch Oral Biol 1996; 41 (12): 1187-1190.
29. Hossain M, Nakamura Y, Kimura Y, Mitsuhiro I, Yamada Y, Matsumoto K. Acquired acid resistance of dental hard tissues by CO_2 laser irradiation. J Clin Laser Med Surg 1999; 17 (5): 223-226.
30. Hsu CYS, Jordan TH, Dederich DN, Wefel JS. Effects of low-energy CO_2 laser irradiation on the organic matrix inhibition of enamel demineralization. J Dent Res 2000; 79 (9): 1725-1730.

CONCLUSÃO GERAL

1. O estudo do laser de CO_2 (10.6 μm) como um dos métodos alternativos na redução da desmineralização do esmalte dentário tem crescido ao longo de 40 anos. No entanto, ainda não há protocolos definidos que padronizem os parâmetros do laser, nem tão pouco, um número significante de estudos clínicos/ *in situ* randomizados que viabilizem seu uso na prática clínica.

2. Uma aplicação do laser de CO_2 (10.6 μm – 10 J/cm^2) sobre a superfície do esmalte dentário foi capaz de modificar a superfície do esmalte aumentando sua resistência à desmineralização. No entanto, 3 aplicações produziram uma redução da perda mineral até 50 μm.

REFERÊNCIAS BIBLIOGRÁFICAS*

1. Ana PA, Bachmann L, Zezell DM. Lasers effects on enamel for caries prevention. Laser Physics 2006; 16 (5): 865-75.
2. Bachmann L, Zezell DM. Estrutura e composição do esmalte e da dentina. Tratamento térmico e Irradiação Laser. São Paulo: Livraria da Física; 2005.
3. Coluzzi DJ. Fundamentals of dental lasers: a science and instruments. Dent Clin N Am 2004; 48 (4): 751-70.
4. Featherstone JDB, Nelson DGA. Laser effects on dental hard tissues. Adv Dent Res 1987; 1 (1): 21-6.
5. Featherstone JDB, Barret-Vespone NA, Fried D, Kantorowitz Z, Seka W. CO_2 laser inhibition of artificial caries-like lesion progression in dental enamel. J Dent Res 1998; 77 (6): 1397-403.
6. Featherstone JDB. Caries detection and prevention with laser energy. Dent Clin N Am 2000; 44 (4): 955-69.
7. Ferreira JM, Palamara J, Phakey PP, Rachinger WA, Orams HJ. Effects of a continuous-wave CO_2 laser on the ultrastructure of human dental enamel. Arch Oral Biol 1989; 34 (7): 551-62.
8. Fortes LAR, Villela LC. Efeitos do laser de Dióxido de Carbono (CO_2) sobre os tecidos dentários. Rev Biociênc 2000; 6 (1): 29-34.
9. Fowler BO, Kuroda S. Changes in heated and laser-irradiated human tooth enamel and their probable effects on solubility. Calc Tiss Int 1986; 38 (4): 197-208.
10. Fried D, Featherstone JDB, Visuri SR, Seka W, Walsh J. The caries inhibition potential of Er:YAG and Er: YSGG laser radiation. In: Lasers in Dentistry II. Vol. 2672. Denver, CO: SPIE 1996: 73-78.

* De acordo com a Norma da FOP/UNICAMP, baseada na norma do International Committee of Medical Journal Editors – Grupo de Vancouver. Abreviatura dos periódicos em conformidade com o Medline.

11. Fried D, Glena RE, Featherstone JDB, Seka W. Permanent and transient changes in the reflectance of CO_2 laser-irradiates dental hard tissues at λ= 9.3,9.6, 10.3 and 10.6 μm and at fluences of 1-20 J/cm^2. Lasers Surg Med 1997; 20 (1): 22-31.
12. Fried D, Ragadio J, Akrivou M, Featherstone JD, Murray MW, Dickenson KM. Dental hard tissue modification and removal using sealed transverse excited atmospheric-pressure lasers operating at lambda = 9.6 and 10.6 microm. *J Biomed Opt* 2001; 6 (2): 231-238.
13. Gimbel CB. Hard tissue laser procedures. Dent Clin N Am 2000; 44 (4): 931-53.
14. Hirota F, Furumoto K. Temperature rise caused by laser (CO_2, Nd:YAG, Er:YAG) irradiation of teeth. Inter Cong Series 2003; 1248: 301-304.
15. Hsu CY, Jordan TH, Dederich DN, Wefel JS. Effects of low-energy CO_2 laser irradiation and the organic matrix on inhibition of enamel demineralization. J Dent Res 2000; 79 (9): 1725-30.
16. Jeffrey IWM, Lawrenson B, Longbottom C and Saunders EM. CO_2 laser application to the mineralized dental tissues – the possibility of iatrogenic sequelae. J Dent 1990; 18 (1): 24-30.
17. Kantorowitz Z, Featherstone JDB, Fried D. Caries prevention by CO_2 laser treatment: dependency on the number of pulses used. J Am Dent Assoc 1998; 129 (5): 585-91.
18. Klein AL, Rodrigues LK, Eduardo CP, Nobre dos Santos M, Cury JA. Caries inhibition around composite restorations by pulsed carbon dioxide laser application. Eur J Oral Sci. 2005; 113 (3): 239-44.
19. Kuroda S, Fowler BO. Compositional, structural and phase changes *in vitro* laser-irradiated human tooth enamel. Calcif Tissue Int 1984; 36 (4): 361-9.
20. Longbottom C; Pitts NB. CO_2 laser and the diagnosis of occlusal caries: *in vitro* study. J Dent 1993; 21 (4): 234-9.

21. Nelson DGA, Shariati M, Glena R, Shields CP, Featherstone JD. Effect of a pulsed low energy infrared laser irradiation on artificial caries-like formation. Caries Res 1986; 20 (4): 289-99.
22. Nelson DGA, Wefel JS, Jongebloed WL, Featherstone JDB. Morphology, histology and crystallography of human dental enamel treated with pulsed low-energy infrared laser radiation. Caries Res 1987; 21 (5): 411-26.
23. Rodrigues LKA, Nobre-dos-Santos M, Pereira D, Assaf AV, Pardi V. Carbon dioxide laser in dental caries prevention. J Dent 2004; 32 (7): 531-40.
24. Steiner-Oliveira C, Rodrigues LKA, Soares LES, Martin AA, Zezell DM, Nobre-dos-Santos M. Chemical, morphological and thermal effects of 10.6 μm CO_2 laser on the inhibition of enamel demineralization. Dent Mat J 2006; 25 (3): 455-62.
25. Steiner-Oliveira C, Rodrigues LKA, Lima EB, Nobre-dos-Santos M. Effect of the CO_2 laser combined with fluoridated products on the inhibition of enamel demineralization. J Contemp Dent Pract 2008; 9 (2): 113-21.
26. Stern RH, Sognnaes RF. Laser beam effects on dental hard tissues (abstract). J Dent Res 1964; 43: 873.
27. Stern RH, Sognnaes RF, Goodman F. Laser effect on *in vitro* enamel permeability and solubility. J Am Dent Assoc 1966; 73 (4): 838-43.
28. Stern RH, Vahl J, Sognnaes RF. Lased enamel: ultrastructural observations of pulsed carbon dioxide laser effects. J Dent Res 1972; 51 (2): 455-60.
29. Tagliaferro EPS, Rodrigues LKA, Nobre-dos-Santos M, Soares LES, Martin AA. Combined effects of carbon dioxide laser and fluoride on demineralized primary enamel: an *in vitro* study. Caries Res 2007; 41 (1): 74-6.

ANEXO

Anexo 1 - Resolução CCPG/002/06 a qual dispõe a respeito do formato das teses de mestrado e doutorado aprovados pela UNICAMP (Parte I).

INFORMAÇÃO CCPG/OO2/06[6]

Tendo em vista a necessidade de revisão da regulamentação das normas sobre o formato e a impressão das dissertações de mestrado e teses de doutorado e com base no entendimento exarado no Parecer PG n° 1985/96, que trata da possibilidade do formato alternativo ao já estabelecido, a CCPG resolve:

Artigo 1º - O formato padrão das dissertações e teses de mestrado e doutorado da UNICAMP deverão obrigatoriamente conter:

I. Capa com formato único ou em formato alternativo que deverá conter informações relativas ao nível (mestrado ou doutorado) e à Unidade de defesa, fazendo referência à Universidade Estadual de Campinas, sendo o projeto gráfico das capas definido pela PRPG.
II. Primeira folha interna dando visibilidade à Universidade, a Unidade de defesa, ao nome do autor, ao título do trabalho, ao número de volumes (quando houver mais de um), ao nível (mestrado ou doutorado), a área de concentração, ao nome do orientador e co-orientador, ao local (cidade) e ao ano de depósito. No seu verso deve constar a ficha catalográfica.
III. Folha de aprovação, dando visibilidade à Comissão Julgadora com as respectivas assinaturas.
IV. Resumo em português e em inglês (ambos com no máximo 500 palavras).
V. Sumário.
VI. Corpo da dissertação ou tese dividido em tópicos estruturados de modo característico à área de conhecimento.
VII. Referências, formatadas segundo normas de referenciamento definidas pela CPG da Unidade ou por critério do orientador.
VIII. Todas as páginas deverão, obrigatoriamente, ser numeradas, inclusive páginas iniciais, divisões de capítulos, encartes, anexos, etc... As páginas iniciais poderão ser numeradas utilizando-se algarismos romanos em sua forma minúscula.
IX. Todas as páginas com numeração "impar" serão impressas como "frente" e todas as páginas com numeração "par" serão impressas como "verso".

§ 1º - A critério do autor e do orientador poderão ser incluídos: dedicatória; agradecimento; epígrafe; lista de: ilustrações, tabelas, abreviaturas e siglas, símbolos; glossário; apêndice; anexos.

§ 2º - A dissertação ou tese deverá ser apresentada na língua portuguesa, com exceção da possibilidade permitida no artigo 2° desta Informação.

§ 3º - As dissertações e teses cujo conteúdo versar sobre pesquisa envolvendo seres humanos, animais ou biossegurança, deverão apresentar anexos os respectivos documentos de aprovação.

Artigo 2º - A critério do orientador e com aprovação da CPG da Unidade, 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.

[6] Disponível em: http://www.prpg.unicamp.br/ccpg_inf002_06.pdf

Anexo 2 - Resolução CCPG/002/06 a qual dispõe a respeito do formato das teses de mestrado e doutorado aprovados pela UNICAMP (Parte II).

§ único - O orientador e o candidato deverão verificar junto às editoras a possibilidade de inclusão dos artigos na dissertação ou tese, em atendimento à legislação que rege o direito autoral, obtendo, se necessária, a competente autorização, deverão assinar declaração de que não estão infringindo o direito autoral transferido à editora.

Artigo 3º - Dependendo da área do conhecimento, a critério do orientador e com aprovação da CPG da Unidade, a dissertação ou tese poderá ser apresentada em formato alternativo, desde que observados os incisos I, II, III IV, V e VII do artigo 1º.

Artigo 4º - Para impressão, na gráfica da Unicamp, dos exemplares definitivos de dissertações e teses defendidas, deverão ser adotados os seguintes procedimentos:

§ 1º - A solicitação para impressão dos exemplares de dissertações e teses poderá ser encaminhada à gráfica da Unicamp pelas Unidades, que se responsabilizarão pelo pagamento correspondente.

§ 2º - Um original da dissertação ou tese, em versão definitiva, impresso em folha tamanho carta, em uma só face, deve ser encaminhado à gráfica da Unicamp acompanhado do formulário "Requisição de Serviços Gráficos", onde conste o número de exemplares solicitados.

§ 3º - A gráfica da Unicamp imprimirá os exemplares solicitados com capa padrão. Os exemplares solicitados serão encaminhados à Unidade em, no máximo, cinco dias úteis.

§ 4º - No formulário "Requisição de Serviços Gráficos" deverão estar indicadas as páginas cuja reprodução deva ser feita no padrão "cores" ou "foto", ficando entendido que as demais páginas devam ser reproduzidas no padrão preto/branco comum.

§ 5º - As dissertações e teses serão reproduzidas no padrão frente e verso, exceção feita às páginas iniciais e divisões de capítulos; dissertações e teses com até 100 páginas serão reproduzidas no padrão apenas frente, exceção feita à página que contém a ficha catalográfica.

§ 6º - As páginas fornecidas para inserção deverão ser impressas em sua forma definitiva, ou seja, apenas frente ou frente/verso.

§ 7º - O custo, em reais, de cada exemplar produzido pela gráfica será definido pela Administração Superior da Universidade.

Artigo 5º - É obrigatória a entrega de dois exemplares para homologação.

Artigo 6º - Esta Informação entrará em vigor na data de sua publicação, ficando revogadas as disposições em contrário, principalmente as Informações CCPG 001 e 002/98 e CCPG/001/00.

Campinas, 13 de setembro de 2006

Profa. Dra. Teresa Dib Zambon Atvars
Presidente
Comissão Central de Pós-Graduação

Anexo 3 – Comitê de Ética (Estudo *in vitro* – Capítulo 2).

COMITÊ DE ÉTICA EM PESQUISA
FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa **"Avaliação de diversas aplicações do laser de CO2 na redução da desmineralização do esmalte dentário humano in vitro"**, protocolo nº 017/2009, dos pesquisadores Marinês Nobre dos Santos Uchôa, Carolina Steiner Oliveira, Juliana Dias Dutra e Karlla Almeida Vieira, satisfaz as exigências do Conselho Nacional de Saúde - Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 08/04/2009.

The Ethics Committee in Research of the School of Dentistry of Piracicaba - State University of Campinas, certify that the project **"In vitro evaluation of CO2 laser irradiations on the inhibition of human dental enamel demineralization"**, register number 017/2009, of Marinês Nobre dos Santos Uchôa, Carolina Steiner Oliveira, Juliana Dias Dutra and Karlla Almeida Vieira, comply with the recommendations of the National Health Council - Ministry of Health of Brazil for research in human subjects and therefore was approved by this committee at .

Prof. Dr. Pablo Agustin Vargas
Secretário
CEP/FOP/UNICAMP

Prof. Dr. Jacks Jorge Junior
Coordenador
CEP/FOP/UNICAMP

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