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Chemical, Morphological and Thermal Effects of 10.6-μm CO_2 Laser on the Inhibition of Enamel Demineralization

Carolina STEINER-OLIVEIRA[1], Lidiany K. A. RODRIGUES[2], Luís E. S. SOARES[3], Airton A. MARTIN[3], Denise M. ZEZELL[4] and Marinês NOBRE-DOS-SANTOS[1]

[1]Department of Pediatric Dentistry, Faculty of Dentistry of Piracicaba, State University of Campinas, Piracicaba, São Paulo, Brazil

[2]Department of Restorative Dentistry, Faculty of Dentistry of Federal University of Ceará, Fortaleza, Ceará, Brazil

[3]Faculty of Dentistry and Laboratory of Biomedical Vibrational Spectroscopy, Research and Development Institute, IP&D, UniVap, São José dos Campos, SP, Brazil

[4]Nuclear and Energetic Research Institute (IPEN/CNEN-SP), São Paulo, SP, Brazil

Corresponding author, Marinês Nobre-Dos-Santos E-mail: nobre@fop.unicamp.br

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Studies have shown that enamel can be modified by pulsed CO_2 laser to form a more acid-resistant substrate. This study evaluated the effects of a 10.6-μm CO_2 laser on enamel surface morphology and chemical composition as well as monitored intrapulpal temperature changes during irradiation. Human teeth were irradiated with fluences of 1.5-11.5 J/cm^2, and pulpal thermal as well as chemical and morphological modifications on enamel were assessed. The teeth were submitted to a pH-cycling model, and the mineral loss was determined by means of cross-sectional microhardness. For all irradiated groups, intrapulpal temperature changes were below 3℃. FT-Raman spectroscopy and scanning electron microscopy indicated that fluences as low as 6.0 J/cm^2 were sufficient to induce chemical and morphological changes in enamel. Then, for fluences reaching or exceeding 10.0 J/cm^2, laser-induced inhibitory effects on demineralization were observed. It was thus concluded that laser energy density in the range of 10.0 and 11.5 J/cm^2 could be applied to dental enamel in order to produce chemical and morphological changes and reduce the acid reactivity of enamel without compromising the pulp vitality.

Key words: FT-Raman spectroscopy, SEM, Cross-sectional microhardness

INTRODUCTION

Since the invention of ruby crystal laser by Maiman in 1960[1], different lasers have been studied for use in dentistry. Then, with the intent of employing lasers in various dental applications and treatments (such as using CO_2 laser in dental caries prevention), many studies were performed to examine the effects of lasers on dental hard substrates[2]. As a result, in the last 30 years, many studies have demonstrated the potential of laser pretreatment on dental tissues to inhibit enamel dissolution or artificial caries-like formation and progression in the laboratory[3-12]. With lasers, the chief effect on enamel substrate lies in the temperature changes which can be extremely high at the interaction site even for a short action time. This quick local temperature rise on enamel prompts melting and cooling of apatite crystals up to a 5-μm depth[7], and can cause undesirable effects such as cracking, pitting, and pulpal damage[13]. However, CO_2 laser proved to be effective without any significant damaging side effects, provided that care was taken to maintain temperature at a safe level. Temperature changes exceeding 5℃ within the pulp chamber could result in permanent damage to the dental pulp[13]. There have been reports of degenerative pulp changes and necrosis following accidental or intentional laser irradiation of teeth during oral surgical procedures, suggesting a potential hazard of laser use in the oral cavity.

There is still no consensus about the exact action mechanism of CO_2 laser in the inhibition of enamel demineralization. Most theories focus on enamel mineral phase changes, such as surface melting and hydroxyapatite crystal recrystallization. It is well known that irradiation of dental hard tissue with lasers of sufficient power leads to a variety of structural and ultrastructural changes in the tissue near the surface[14,15], and several studies have shown that irradiation by CO_2 laser at 10.6μm can produce surface changes in enamel[7,15,16]. In view of the uncertain mechanism of interaction between CO_2 laser and enamel, a more sensitive analysis should be performed to clarify the modification on enamel induced by laser irradiation, especially in the chemical aspect. Raman spectroscopy is a non-destructive and highly selective technique that can provide information about molecular species[17]. Indeed, the Raman spectrum of any mineral structure, such as human teeth, can reveal the chemical composition and structure of mineral and organic contents.

The successful use of lasers for caries prevention depends not only on the application of specific laser power levels that do not cause pulpal harm, but also on the promotion of demineralization inhibitory effects on dental enamel. However, to date, no studies in published literature have taken a holistic approach − which comprises the chemical, morphological, and thermal aspects − in investigating the inhibitory effects of pulsed 10.6-μm CO_2 laser on enamel demineralization.

Against this background, the two-pronged objectives of this study were: (1) to establish the lowest CO_2 laser energy power that can be applied to dental enamel to reduce its acid reactivity without causing pulpal damage; and (2) to investigate physical and chemical changes caused by a pulsed CO_2 laser at 10.6-μm wavelength using different energy densities.

MATERIALS AND METHODS

Tooth selection and sample preparation

Ninety extracted impacted human third molars were used to perform this *in vitro* study, in conformity to the norms of the Research and Ethics Committee of the Dental School of Piracicaba (protocol no. 102/2005). The teeth were then stored in a supersaturated 0.1% thymol solution for one month. After which, their pulpal contents were removed with a Kerr type file (Dentsply-Maillefer Instruments, Ballaigues, Switzerland) and their apical foramens enlarged by a Gates-Glidden rotary instrument (Dentsply-Maillefer) with the purpose of receiving a thermocouple inside their pulp chambers. In order to know the proportion between the pH cycling solutions and the exposed enamel, the teeth were coated with an acid-resistant varnish leaving a window (16 mm^2) of enamel including the central fissure.

Experimental design

The experiment involved six groups (n=15), which differed from each other in terms of laser irradiation power. The teeth of each group were randomly selected by lottery method[18], and the groups were named as follows: Control, 1.5 J/cm^2, 3.0 J/cm^2, 6.0 J/cm^2, 10.0 J/cm^2, and 11.5 J/cm^2. During laser irradiation, thermal changes were monitored by a thermocouple. To verify the chemical and morphological effects of CO_2 laser irradiation, five teeth from each group were kept for Fourier transformed Raman spectroscopy evaluation followed by scanning electron microscopy (SEM) analysis. The remaining teeth (n=10) were submitted to a pH-cycling model.

Laser irradiation

At a distance of 10 mm from the tip of the handpiece to the tooth, irradiation was carried out by scanning the occlusal central fissure of the exposed enamel of each tooth for approximately 10 seconds by manual movement of the laser tip. Scanning speed was approximately 1 mm/s. As for irradiation parameters, pulsed CO_2 laser at 10.6μm wavelength (Model UM-L30, Union Medical Engineering Co., Yangju-si, Gyeonggi-Do, Korea) was used with 10 ms pulse duration, 10 ms of time off, 50 Hz repetition rate, 0.3 mm beam diameter, and laser power of 2 W, 4 W, 6 W, 8 W, and 10 W according to the treatment groups. Using a power meter (Scientech 373 Model-37-3002, Scientech Inc., Boulder, CO, USA), average power output was measured and found to be 0.1 W, 0.2 W, 0.4 W, 0.7 W, and 0.8 W for the corresponding lased groups. Thus, the laser fluences applied on enamel were approximately 1.5, 3.0, 6.0, 10.0, and 11.5 J/cm^2.

Thermal analysis

During CO_2 laser irradiation, thermal changes were monitored by means of a cromel-alumel (K-type) thermocouple (Omega Engineer Inc., Stanford, CT, USA). A 125-μm thick tip was put in contact with the dentinal tissue inside the pulp chamber, and the teeth were kept in a 37℃ hot water bath. A thermal paste was used to enable good thermal contact between the thermocouple tip and the internal pulp wall. Contact between the thermocouple tip and ceiling of the pulp chamber was then assessed by radiography. Data were obtained during laser irradiation, and internal pulp temperature increase was determined by calculating the difference between the initial and maximum temperature values.

Raman spectroscopy and scanning electron microscopy

Before and after laser treatment, five teeth were evaluated by Fourier transformed Raman spectroscopy. Spectra of the teeth were obtained using a FT-Raman spectrometer (RFS 100/S, Bruker Inc., Karlsruhe, Germany) both before and after irradiation with one Ge diode detector cooled by liquid N_2. To excite the spectra, 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 100 mW and spectrum resolution was 4 cm^{-1}. The teeth were positioned in a sample holder, and an IR354 lens collected radiation scattered over 180° on the exposed occlusal surface. The FT-Raman spectra were obtained using 100 scans. Frequency ranged from 300 to 4,000 cm^{-1}, thereby allowing a characterization of both mineral (hydroxyapatite) and organic (essentially collagen) constituents.

After irradiation, the same five teeth were also analyzed by SEM. For this analysis, the teeth were longitudinally fractured through the enamel window and the surface, and the cut side then coated with a thin layer of gold (approximately 10-12 nm in thickness). Observations were performed using a scanning electron microscope (JSM-5600 LV, JEOL, Tokyo, Japan) at 15 kV and magnification of 800×.

pH-cycling process

The pH-cycling model used in this study was based on the one described by Featherstone *et al.*[19] and modified by Argenta *et al.*[20]. Every day, each tooth was kept three hours in a demineralizing solution (1.25 mL/mm^2 exposed enamel) containing 2.0 mmol/L calcium, 2.0 mmol/L phosphate in 75 mmol/L acetate buffer (pH 4.6), and then 21 hours in a remineralizing solution (0.62 mL/mm^2 exposed enamel) containing 1.5 mmol/L calcium, 0.9 mmol/L phosphate, 150 mmol/L KCl in 20 mmol/L cacodylic buffer (pH 7.0). This pH-cycling process was carried out for 10 days and after which, the teeth remained in the remineralizing solution for two days (37℃). Between immersions in demineralizing and remineralizing solutions and at the end of the pH-cycling regime, the teeth were rinsed with deionized distilled water for 10 seconds and wiped with tissue paper. The de- and remineralizing solutions were changed after five cycles, both containing thymol to prevent microorganism growth.

Cross-sectional microhardness test (CSMH)

After pH-cycling, the teeth were longitudinally sectioned through the border of the exposed enamel. Each cut section was embedded in acrylic resin, and then serially flattened and polished up to the central fissure area of the occlusal surface. Hardness profile was determined using a hardness tester (FM-ARS, Future-Tech, Tokyo, Japan) and a Knoop diamond indenter under a 25-g load for five seconds.

Mineral loss (ΔZ) calculation was then performed in the following way:

(a) Knoop hardness numbers (KHNs) at distances of 10, 20, 30, 40, 50, 60, 80, 100, 120, 140, 160, 180, and 200 μm from the outer enamel were obtained.

(b) KHN values were converted into volume percent mineral by using the equation proposed by Featherstone *et al.*[21]:

$$\text{Vol\% mineral} = 4.3(\text{KHN})^{1/2} + 11.3$$

(c) Volume percent mineral was plotted against depth for each slab, and the integrated mineral content of the treated enamel was calculated. For depths greater than 80 μm, a mean value of volume percent mineral was used as a measure of the integrated mineral content of inner sound enamel.

(d) To compute mineral loss (ΔZ), integrated mineral content of treated enamel was subtracted from that obtained for sound enamel[22], as shown in Fig. 1.

Statistical analysis

For Raman data analysis, average spectra were obtained from each treatment group before and after irradiation. Fluorescence spectrum was removed with a polynomial fitting with varying degrees from the spectra, by means of Origin5.0® software (Microcal Software, Northampton, USA). Relative peak areas were then calculated using the Microcal Origin5.0® software. Changes in mineral and organic components were evaluated by comparing the relative peak areas in enamel both before and after irradiation. Statistical analysis of the Raman results was performed by the paired t-test at a 95% confidence level using the BioEstat 3.0® software. The Kolmorogov and Smirnov tests verified the normal distribution of the sample data.

To assess the effect of CO_2 laser treatment, data of the dependent variable, ΔZ, were transformed (linear transformation a+bx) and tested by analysis of variance (ANOVA). Next, Tukey's test using the SAS software (SAS Institute Inc., 2001) was chosen to evaluate the statistical significance of all pairwise comparisons. Values of $p < 0.05$ were accepted as statistically significant. Comparison of temperature variations (*i.e.*, dependent variable ΔT) was also carried out and tested with analysis of variance (ANOVA) and Tukey's test with alpha set at 0.05.

RESULTS

A low temperature variation, below 3℃, can be observed for all groups, and the highest temperature variation were found in 10.0 and 11.5 J/cm^2 groups. Fig. 2 shows the mean temperature variations (ΔT in ℃) and error bars according to the laser fluences applied.

Fig. 3 shows the average Raman spectra of all groups, whereby spectral analysis revealed two characteristic parts. First, a region spanning from 300 to 1,100 cm^{-1} – with an intense broad band at 962

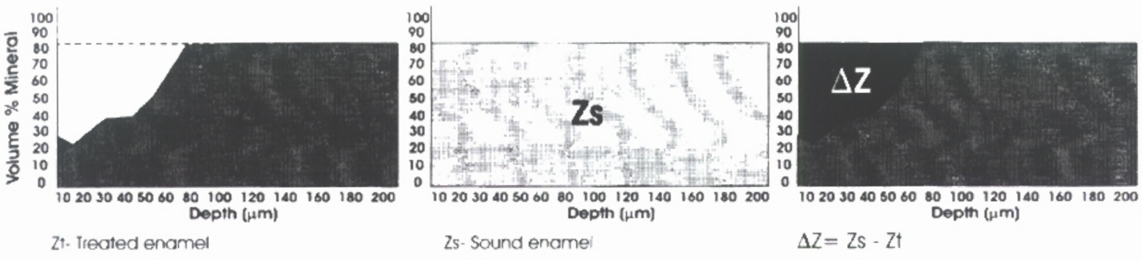

Fig. 1 Schematic representation of the calculations of the integrated mineral contents of the lesion and sound enamel (ΔZ).

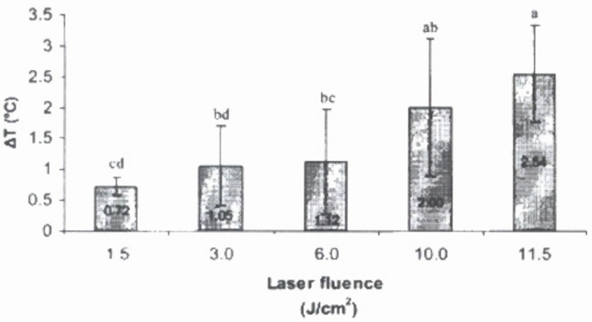

Fig. 2 Averaged intrapulpal temperature variations (ΔT) and error bars according to the laser fluences (J/cm^2) applied on enamel ($n=10$). Different letters indicate statistically significant differences by the Tukey's test ($p<0.05$).

cm^{-1} – was characteristic of phosphate groups and representative of the mineral phase of enamel. The other region, spanning from 1,200 to 3,000 cm^{-1}, was representative of the collagen phase and showed characteristic vibrational modes of the organic groups (amide and CH). The FT-Raman bands at υ_2 (430-450 cm^{-1}), υ_4 (585-612 cm^{-1}), υ_1 (960 cm^{-1}), and υ_3 (1,026-1,072 cm^{-1}) represent the phosphate vibrations in hydroxyapatite. The band in the range of 1026-1072 cm^{-1} could also represent carbonate vibration. For energy densities of 1.5 and 3.0 J/cm^2, no statistically significant differences in enamel spectrum could be observed before and after irradiation ($p<0.05$), as shown in Table 1. But in the 6.0 J/cm^2 group, there was a statistically significant decrease ($p<0.05$) in the intensity of 585 and 1045 cm^{-1} bands. Similarly in the 10.0 J/cm^2 group, there was a statistically significant decrease ($p<0.05$) in the intensity of 585, 612, 960, and 1,072 cm^{-1} bands (Table 1), and peaks

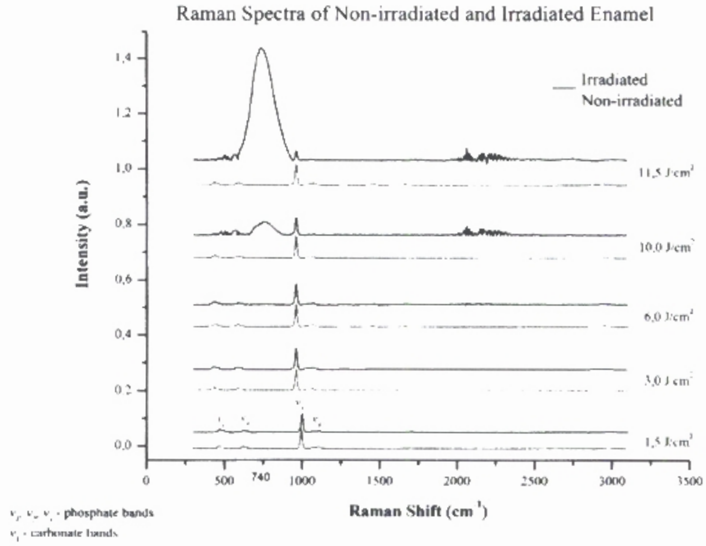

Fig. 3 Raman spectra of non-irradiated and irradiated enamel with energy densities of 1.5, 3.0, 6.0, 10.0, and 11.5 J/cm^2.

Table 1 Areas under the peak bands (mean±SD, $n=5$) for irradiated and non-irradiated groups

	Raman Peaks															
	430		450		582		612		960		1026		1045		1072	
Groups (J/cm^2)	NI	I	NI	I	NI	I	NI	I	NI	I	NI	I	NI	I	NI	I
1.5	0.22±0.02	0.22±0.05	0.20±0.03	0.20±0.04	0.30±0.04	0.29±0.05	0.04±0.05	0.04±0.01	1.46±0.18	1.38±0.33	0.06±0.02	0.07±0.02	0.14±0.05	0.14±0.05	0.17±0.04	0.18±0.04
3.0	0.23±0.03	0.24+0.05	0.22±0.02	0.21±0.01	0.27±0.13	0.31±0.04	0.04±0.00	0.05±0.00	1.49±0.17	1.50±0.14	0.08±0.04	0.06±0.01	0.13±0.06	0.14±0.02	0.16±0.03	0.20±0.06
6.0	0.24+0.02	0.28±0.13	0.20±0.10	0.44±0.22	0.36±0.03	0.45±0.03	0.05±0.00	0.18±0.16	1.62±0.13	1.64±0.38	0.07±0.02	0.12±0.11	0.15±0.04	0.08±0.05	0.20±0.04	0.24±0.07
10.0	0.24±0.03	0.00±0.00	0.24±0.04	0.00+0.00	0.33±0.04	0.00±0.00	0.05±0.01	0.00±0.00	1.61±0.27	1.47±0.66	0.06±0.00	0.06±0.01	0.17±0.04	0.14±0.08	0.14±0.03	0.26±0.04
11.5	0.25±0.10	0.00±0.00	0.21+0.08	0.00±0.00	0.33±0.11	0.00±0.00	0.04±0.02	0.00±0.00	1.59±0.63	0.73±0.42	0.08±0.05	0.00±0.00	0.20±0.11	0.00±0.00	0.15±0.09	0.00±0.00

NI: Non-irradiated enamel

I: Irradiated enamel

*Statistically significant difference ($p<0.05$)

ranging from 430 to 612 cm^{-1} have disappeared after irradiation. For 11.5 J/cm^2 group, all peaks disappeared except for 960 cm^{-1} band. In particular, after irradiation with laser fluences of 10.0 and 11.5 J/cm^2, a new peak appeared in the region of 740 cm^{-1} band.

SEM observations showed evidences of cracking, melting, and fusion on slabs irradiated by power intensities of 6.0 J/cm^2, 10.0 J/cm^2, and 11.5 J/cm^2 (Fig. 4).

Mean ΔZ represented the severity of average caries-like lesions that developed in each group. The lower the mean ΔZ value, it meant a lower rate of caries lesion formation. Although there were no statistically significant differences in ΔZ value among the irradiated groups, fluences above 10.0 J/cm^2 showed statistically significant demineralization inhibition when compared to control group (Table 2).

DISCUSSION

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

Elevation of intrapulpal temperature through the production of surface heat has been reported to be the most severe stress imparted to the living pulp[13]. Therefore, pulpal response to thermal injury can be assumed to be directly proportional to the intensity of the damage[23]. This study examined thermal changes in the pulpal chamber during CO_2 laser exposure operated in a pulsed mode. However, few thermal investigations were conducted using pulsed CO_2 laser, making it difficult to compare with the existing reports concerning thermal effects on enamel. Nonetheless, the results of this study showed a low variation range in temperature, not exceeding 3℃, even when the highest laser fluence was applied (11.5 J/cm^2). This was in accordance with a study performed by Malmström *et al.*[24], showing that with low energy levels there should be no detrimental effect on the pulpal tissue[13,23].

Table 2 Enamel mineral loss (vol% x μm) of each laser treatment group (mean±SD, n=10)

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

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

In the FT-Raman spectroscopy results, the strongest bands of phosphate — namely at υ_1 (962 cm^{-1}), υ_2 (430-450 cm^{-1}), υ_4 (585-612 cm^{-1}), and υ_3 (1,070 cm^{-1}) vibrational modes — which have been previously reported[25,26] were immediately identified. For 6.0 J/cm^2 group, changes in enamel spectrum were observed. The decrease in carbonate content by CO_2 laser irradiation was in agreement with previous studies that correlated this carbonate loss in laser-treated dental enamel with a reduction in the rate of acid dissolution[27]. With regard to the absence of well-known Raman bands that are attributed to hydroxyapatite minerals and the appearance of 740 cm^{-1} band, similar results were found by Tudor *et al.*[28] in their analysis of a thermally sprayed hydroxyapatite. These authors assigned this band to an overlap of hydroxide bands and concluded that FT-Raman spectrum of hydroxyapatite arose mainly from the hydroxide part. In addition, Rehman *et al.*[29,30] assigned this band to asymmetric P—O stretching vibration and a dense hydroxyapatite Raman band. However, as reported by Aminzadeh[31], the appearance of a band at the 740 cm^{-1} Raman shift of the enamel spectrum, which in this study arose after irradiation with the highest laser fluences (10.0 and 11.5 J/cm^2), most likely reflected a fluorescence band. The latter author[31] has suggested that this fluorescence band might arise from the presence of tricalcium phosphate (TCP) when hydroxyapatite was heated[26,31]. On the other hand, Barnes[32] pointed out that the locality and chemical environment of a mineral affect its luminescence, indicating that this fluorescence could be related to the irradiation of rare earth impurities, rather than that of TCP. This

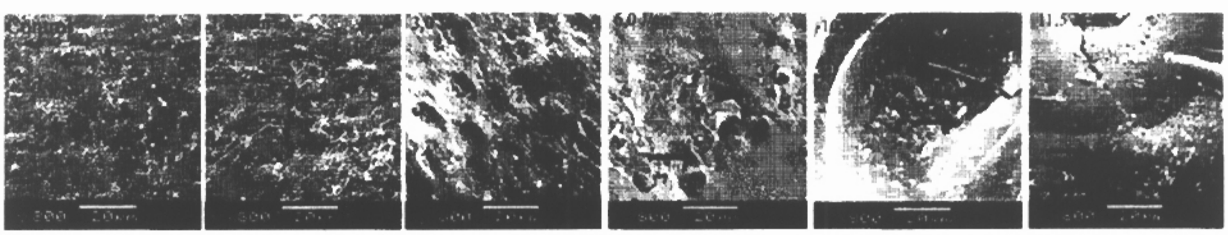

Fig. 4 SEM micrographs of non-irradiated enamel (control) and irradiated enamel of 1.5, 3.0, 6.0, 10.0, and 11.5 J/cm^2 groups at ×800 magnification. Arrows indicate areas of melted enamel. Bar=20μm.

calcium phosphate phase is formed when enamel is heated to temperatures of about 800℃. Moreover, it is considered to be a more soluble phosphate form, therefore increasing the surface solubility of enamel[33]. In our view, the hypothesis stating that fluorescence could be related to the irradiation of rare earth impurities would appear to be most appropriate for explaining the results, since a reduction in demineralization was found, as evidenced by microhardness analysis. However, further studies are necessary to identify the 740 cm^{-1} band and to explain the disappearance of the characteristic hydroxyapatite bands.

As for the mechanism of laser action, it remains unclear although reports of several related studies have been published. The most frequently mentioned hypothesis for laser effects states that caries inhibition is due to the melting and fusion of hydroxyapatite crystals[34,35]. In the present study, the SEM images suggested that the fusion and melting phenomena (Fig. 4) were correlated to the inhibition of demineralization found in the irradiated groups. These findings were in agreement with reports by Nelson *et al.*[6], Kantorowitz *et al.*[36], McCormack *et al.*[37], and Klein *et al.*[38], whereby energy densities similar to or higher than those used in the present study were used. Moreover, the surface crater formation after laser irradiation, as observed by SEM, was in agreement with the results of Stern *et al.*[15] and Malmstrom *et al.*[24].

Previous studies have shown that with CO_2 laser pretreatment, there was significant inhibition of enamel demineralization by 17 to 98% — depending on laser beam type, wavelength, operational mode, and energy output[6,11,39-41]. In the present study, the maximum percentage of demineralization inhibition achieved was 67%, which was lower than the 87% reported by Kantorowitz *et al.*[36] whereby similar parameters and laser wavelength were used. This discrepancy might be due to the higher susceptibility of occlusal surfaces to caries development than the smooth enamel surface, hence making caries inhibition more difficult[42,43]. On the other hand, in absolute contrast to the results of the present study were the findings of Hsu *et al.*[44]. In the latter study[44], almost complete inhibition of enamel demineralization was achieved with a very low energy density (0.3 J/cm^2). However, it should be noted that Hsu *et al.*[44] 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). On this note, Sakae *et al.*[45] demonstrated that some magnesium and carbonate ions were removed from the dental substrate upon NaClO treatment. Thus, it might be the NaClO effect, rather than the laser effect, that had resulted in caries inhibition as shown by Hsu *et al.*[44].

CONCLUSIONS

Findings of the present investigation indicated that 10.0 J/cm^2 was the lowest CO_2 laser energy fluence that should be applied to dental enamel in order to produce chemical and morphological changes that could reduce the acid reactivity of enamel without compromising pulp vitality. In addition, energy densities higher than 6.0 J/cm^2 were sufficient to induce chemical and morphological changes on enamel, as shown by decrease in Raman band intensity or disappearance of Raman band, or by fusion and melting of the enamel surface.

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