ORIGINAL ARTICLE

Comparison of effects of diode laser and CO_2 laser on human teeth and their usefulness in topical fluoridation

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Abstract Various authors have reported more effective fluoridation from the use of lasers combined with topical fluoride than from conventional topical fluoridation. Besides the beneficial effect of lasers in reducing the acid solubility of an enamel surface, they can also increase the uptake of fluoride. The study objectives were to compare the action of CO_2 and GaAlAs diode lasers on dental enamel and their effects on pulp temperature and enamel fluoride uptake. Different groups of selected enamel surfaces were treated with amine fluoride and irradiated with CO_2 laser at an energy power of 1 or 2 W or with diode laser at 5 or 7 W for 15 s each and compared to enamel surfaces without treatment or topical fluoridated. Samples were examined by means of environmental scanning electron microscopy (ESEM). Surfaces of all enamel samples were then acid-etched, measuring the amount of fluoride deposited on the enamel by using a selective ion electrode. Other enamel surfaces selected under the same conditions were irradiated as described above, measuring the increase in pulp temperature with a thermocouple wire. Fluorination with CO_2 laser at 1 W and diode laser at 7 W produced a significantly greater fluoride uptake on enamel (89 ± 18 mg/l) and (77 ± 17 mg/l) versus topical fluoridation alone (58 ± 7 mg/l) and no treatment (20 ± 1 mg/l). Diode laser at 5 W produced a lesser alteration of the enamel surface compared to CO_2 laser at 1 W, but greater pulp safety was provided by CO_2 laser ($\Delta T° 1.60°\pm0.5$) than by diode laser ($\Delta T° 3.16°\pm0.6$). Diode laser at 7 W and CO_2 laser at 2 W both caused alterations on enamel surfaces, but great pulp safety was again obtained with CO_2 ($\Delta T° 4.44°\pm0.60$) than with diode ($\Delta T° 5.25°\pm0.55$). Our study demonstrates that CO_2 and diode laser irradiation of the enamel surface can both increase fluoride uptake; however, laser energy parameters must be carefully controlled in order to limit increases in pulpal temperature and alterations to the enamel surface.

Keywords CO_2 laser · Diode laser · Enamel · ESEM · Fluoride uptake · Pulpal temperature

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Introduction

The inhibition rather than mere treatment of dental caries is an important goal of modern dentistry. The mainstay of action against its development is fluoride [1], applied to dental tissue as pastes, mouthwashes, and pit-and-fissure sealants, among others. The laser now offers dentists a new procedure against the formation of dental caries. Various investigations over the past few years have reported the beneficial effects of laser-produced infrared radiation on enamel, either used alone [2–4] or in combination with fluoride [5–7], increasing its resistance to acids or favoring fluoride uptake, making it more resistant to caries.

Studies on the use of carbon dioxide (CO_2) lasers with wavelengths (λ) of 9–11 μm reported structural and ultrastructural changes on the enamel surface, including decomposition of organic matrix, carbonate loss, and recrystallization of hydroxyapatite in less acid-soluble

phases [8–10]. In vitro studies have shown the effectiveness of this laser in combination with fluoride to increase the enamel fluoride uptake and reduce its acid solubility [11, 12]. However, CO_2 laser can produce undesirable alterations to the enamel surface, including melted areas, fracture lines, and craters [13], and it can even thermally damage dentin or pulp [14].

Only a few studies have addressed the effect on dental enamel of diode laser with a λ of 809–960 nm. A low percentage of this λ is absorbed by hydroxyapatite, and the rest is reflected or transmitted in the form of heat on the surface of the enamel and adjacent structures [15]. However, as with the CO_2 laser, this increase in enamel temperature also produces structural and ultrastructural changes that reduce the acid dissolution of the enamel. These include the decomposition of organic matrix, loss of water and carbonates, and formation of acid-insoluble hydroxyapatite phases, such as calcium pyrophosphate or calcium metaphosphate, found in enamel treated with different lasers or heated at 200–400°C [16–18]. Our group previously reported that diode laser in combination with sodium fluoride (NaF) was effective in increasing fluoride uptake by enamel [19]. Other researchers demonstrated a significant decrease in enamel acid solubility [20] and the inhibition of carious lesions in in vitro studies [21]. The diode laser was not designed with this purpose in mind but is much more affordable and easier to use in routine dental practice.

The objective of this study was to compare the effectiveness of CO_2 laser versus diode laser, examining their effects on fluoride uptake and on dental enamel and pulp.

Materials and methods

Dental material

This study used third molars that required extraction at the School of Dentistry of the University of Granada (Granada, Spain) from patients not subjected to water fluoridation programs. All patients signed informed consent to participate in this study, which was approved by the ethical committee of the university.

For the first experiment, 45 healthy third molars with no visible defects on enamel surface were selected. Teeth were stored in bidistilled water with 0.02% thymol at 4°C to prevent changes in the optical or mineral properties of the enamel or dentin [22]. The root of each molar was sectioned and the crown was divided longitudinally into two halves, obtaining 90 study specimens. A 4-mm^2 enamel window was marked on the surface to be treated, coating the rest of the enamel with an acid-resistant varnish.

Laser treatment

The following lasers were used in the experiments.

– Gallium aluminum arsenide (GaAlAs) diode laser (Laser Smile, Biolase, San Clemente, CA, USA) at 809 nm λ, beam diameter of 0.6 mm and pulse duration of 30 ms. Two settings were used, designated settings **a** and **b** (see Table 1)
– CO_2 laser (Lasersat 15tm, Satelec, Merignac, France) at 10.6 μm λ, beam diameter of 0.6 mm, and pulse duration of 15 ms. Two settings were used, designated **c** and **d** (Table 1).

Study method

Selected molars were randomly assigned to one of the following six groups ($n=15$): Group 1, enamel window treated for 15 s with 0.1 mg amine fluoride (AmF) (ELMEX Fluid, GABA International AG, Münchenstein, Switzerland); Group 2, enamel window treated for 15 s with 0.1 mg AmF plus application of diode laser at setting (a). Group 3, enamel window treated as above plus diode laser at setting (b); Group 4, enamel window treated as above plus application of CO_2 laser at setting (c); Group 5, enamel window treated as above plus CO_2 laser at setting (d); Group 6; control group, enamel surface window with no fluoridation treatment.

The enamel window was always exposed to the laser by the same operator in the same manner, at an approximate irradiation distance of 5 mm and in scanning mode, moving the hand-piece longitudinally and uniformly over the entire surface. The aim was to simulate an in vivo experiment, in which the irradiation distance cannot always be precisely maintained. After each treatment, specimens were cleaned for 2 min under distilled water, using an electric toothbrush with standard head (ORAL-B Vitality; Braun, Hesse, Germany).

Environmental scanning electron microscopy (ESEM)

After the treatments, samples were studied with ESEM to detect possible surface defects or alterations, with a

Table 1 Technical settings of diode (a;b) and CO_2 laser (c;d)

Settings	Average power	Exposure time	Repetition rate	Energy density per pulse
a	5 W	15 s	16 Hz	53 J/cm^2
b	7 W	15 s	16 Hz	74 J/cm^2
c	1 W	15 s	30 Hz	5 J/cm2
d	2 W	15 s	30 Hz	10.5 J/cm2

minimum of five observations for each treated area. Magnifications ranged from 1000x to 4000x, using a Quanta 400 microscope (Philips, Eindhoven, The Netherlands) equipped with a secondary electron gas detector under operating conditions of 20 kV and 2–3 Torr.

Chemical analysis of fluoride ions

The amount of fluoride ion (F^-) on the treated enamel surface was measured with a selective ion electrode (ISE25F, Crison SA, Barcelona, Spain), which is an accurate method to measure small concentrations of fluoride >10 mg/l [23]. The surface was etched with 10 µl of 2 M HCl for 5 s to dissolve the outer enamel surface. The acid with the dissolved enamel elements was gathered on blotting paper disks and transferred to a 2-ml TISAB III solution for F^- measurement.

The potential difference obtained in each sample was compared to a reference curve to quantify the fluoride on each treated surface. The reference curve was prepared under the same environmental conditions (21–23°C) as the experiments and with known fluoride concentrations.

Measurement of intrapulpal temperature

This experiment used 20 third molars obtained from patients treated in our university clinic, extracted and stored in the same manner as the samples for fluoride ion quantification and microscopic analysis (see above). Four groups of 5 molars each were randomly formed (A, B, C, D). The pulp was removed from all molars by apico-coronal endodontic preparation, using conventional 25-mm-long k files (DENTSPLY, Maillefer, Tulsa, Ok, USA) and irrigation with 5.25% sodium hypochlorite. A small apical portion of each root was removed so that the apical foramen was typically 2–3 mm wide in diameter in order to facilitate subsequent instrumentation of the pulp chamber. The endodontic preparation sequence was from ISO file #8 to #60. A 4-mm^2 window was drawn on the free vestibular or lingual surface as the study area but there was no need to cover any part of tooth with varnish.

In this experiment, we used the same laser settings as in the fluoride ion test, i.e., laser setting a) for group A, b) for B, c) for C and d) for D. The enamel window was exposed to the laser in the same manner as in the ion test (irradiation distance of 5 mm and performed in scanning mode, moving the hand-piece longitudinally and uniformly over the entire surface for 15 s).

Thermal changes during laser irradiation were monitored by using a 0.5-mm k-type thermocouple probe (Omega Engineering Ltd, Manchester, UK) connected to a digital thermometer (Omega Engineering Ltd, Manchester, UK). Adhesive was used to attach the teeth to millimetric glass microscope slides, with the root axis parallel to the slide surface. The temperature-sensitive tip of the probe was positioned at the center of the study area, confirming the localization with radiography. Cement sealing was used to secure the thermocouple in the root opening (Cavit, ESPE, Seefield, Germany). Each molar was filled with physiological saline using a syringe, and the slides with the attached teeth and probe were immersed in a physiological saline thermostatic bath (Microterm, J.P. Selecta SA, Barcelona, Spain) at a constant temperature of 37°C, leaving exposed only the surface to be treated. All experimental designs are depicted in Fig. 1.

Before each measurement, and with the thermocouple placed inside the pulp chamber and connected to a thermometer, we verified that the baseline temperature was 37°C in order to simulate the real thermal diffusion and conductivity conditions of a molar in the oral cavity.

Each group (A, B, C, and D) comprised five molars and five temperature measurements were repeated for each molar, obtaining a total of 25 maximum temperature measurements per group.

Statistical analysis

Means, standard deviations, and ranges were calculated for the study groups. One-way analysis of variance was used to
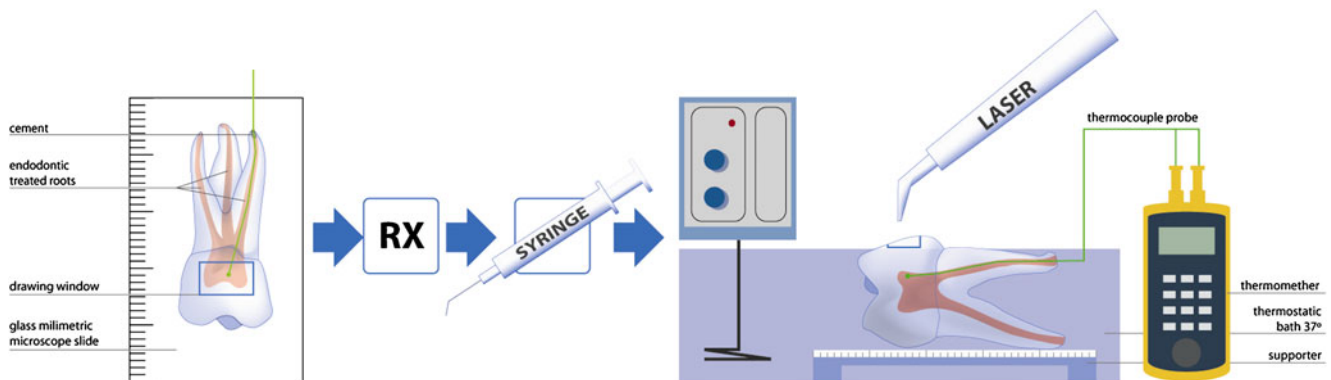

Fig. 1 Design of methodology performed

compare means among treatment groups. When the result was significant, paired comparisons were performed between treatment groups using the Bonferroni correction. Treatment groups were also compared with the control group by means of the Dunnett correction. When variances differed among groups, neperian logarithmic transformation was performed. $p < 0.05$ was considered significant. SPSS for Windows version 15.0 was used for the data analyses.

Results

Analysis of dental surface by ESEM

Figures 2, 3, 4, 5, 6, 7, 8, 9, 10 show the results of the ESEM observations of the surfaces of untreated control molars and of molars treated with diode or CO_2 laser under the different conditions tested. Figure 2 shows normal untreated enamel surface (control). Figure 3 depicts the surface treated with AmF 15 s without laser radiation, showing a surface with an apparently normal structure and morphology similar to untreated enamel. Figures 4 and 5 show surfaces treated with 5-W diode laser in the presence of AmF; the enamel appears normal, although there is a small globular agglomerate in the center of the image that is morphologically similar to an agglomerate of calcium fluoride (CaF_2). Figure 6 depicts the surface treated with 7-W diode laser; the enamel appears thermally altered, and a slight roughness and some craters are observed at the center of the image, compatible with heat fusion of the surface. Frequent globular precipitates appear on the treated surface, to a greater extent than in specimens treated with 5-W
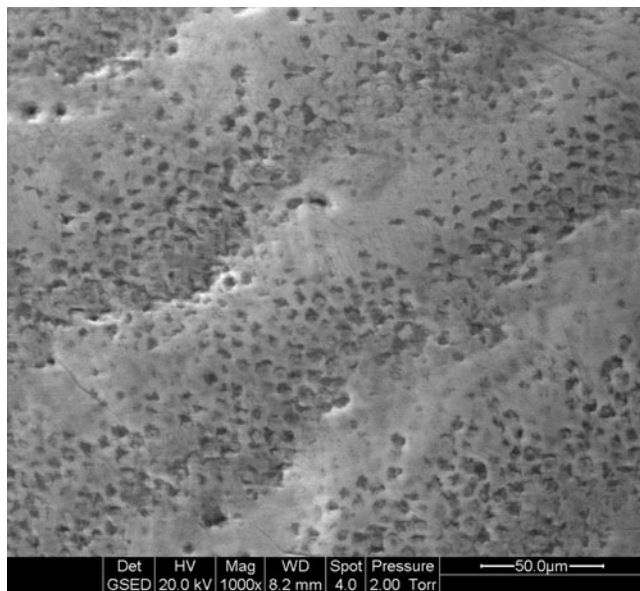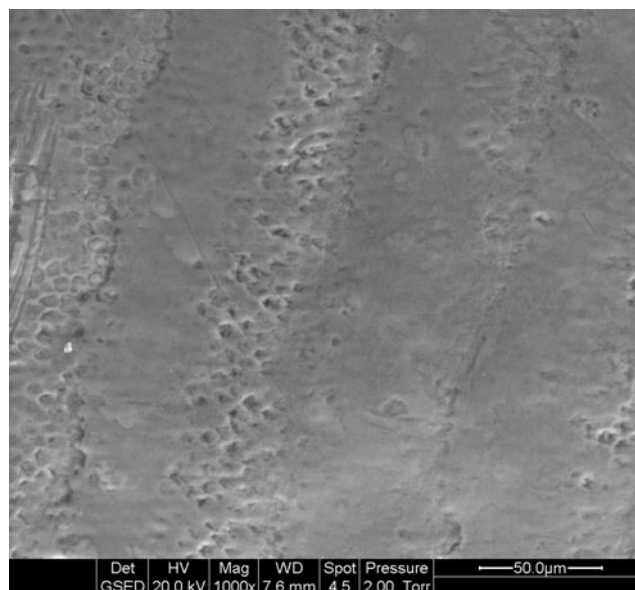
diode laser. Surface exfoliation phenomena can also be seen, as depicted in Fig. 7. Figure 8 shows surfaces treated with 1-W CO_2 laser in the presence of AmF. Most of the surface is altered, although without cracks or craters. The globular agglomerates (CaF_2) produced by diode laser are not observed. Figure 9 depicts a 2-W CO_2 laser-treated surface, evidencing wider thermal alteration and a localized fracture line close to the melted area; craters or exfoliation areas can also be seen with the same treatment in Fig. 10.

Fig. 3 Surface treated with AmF for 15 s (1000x)
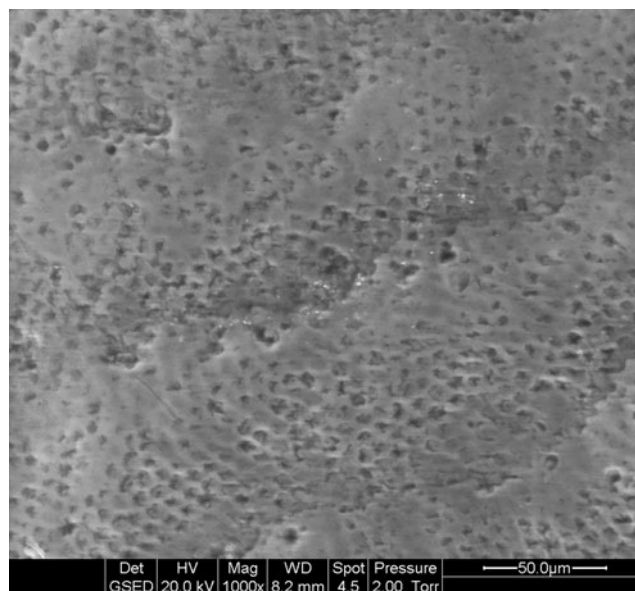

Fig. 2 Control (1000x)

Fig. 4 Surface treated with 5-W diode laser in the presence of AmF (1000x). In the center of the image there is a small globular agglomerate morphologically similar to an agglomerate of CaF_2
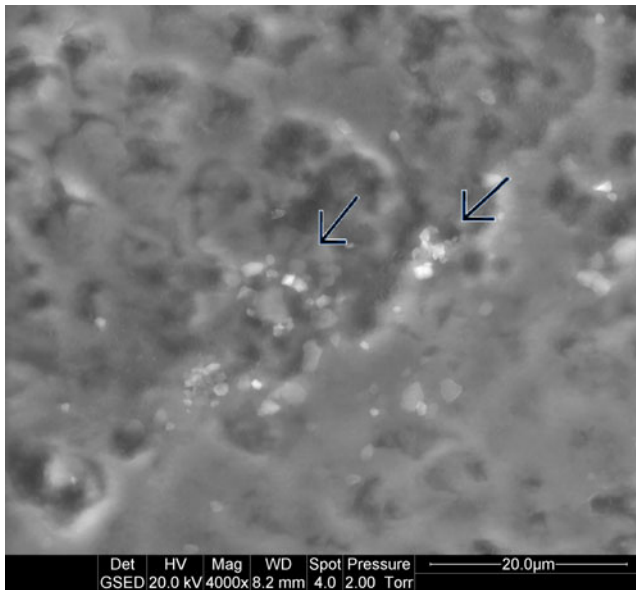
Fig. 5 CaF_2 agglomerates on surface treated with 5-W diode laser in the presence of AmF (4000x). *Arrows* show the CaF_2 agglomerates

Chemical analysis of fluoride ions

Figure 11 depicts the concentrations of fluoride ion obtained in the different treatments, expressed as maximum, minimum, and mean values with standard deviations. We discarded samples treated with 2-W CO_2 laser at 30 Hz and 15 s of exposure (setting d), due to the presence of numerous defects in the enamel structure under these conditions.

The global ANOVA test rendered significant results ($F_{exp}=$ 81.9 (5;84) d.f. $p<0.001$), therefore paired comparisons
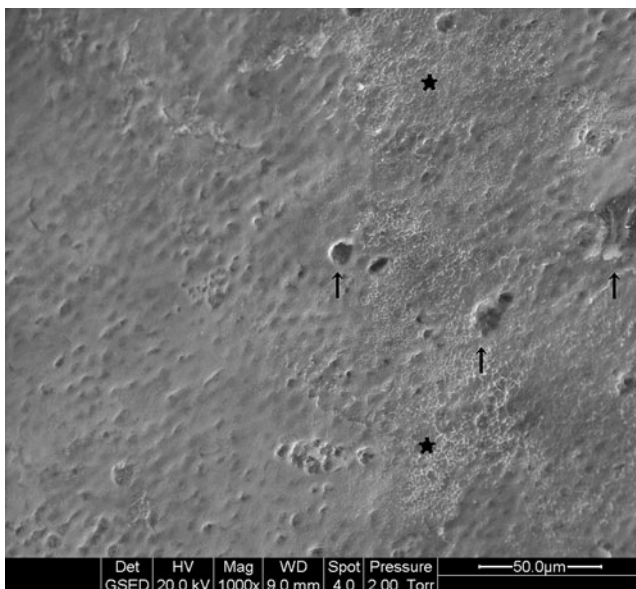

Fig. 6 Surface treated with 7-W diode laser in the presence of AmF (1000x). Slight roughness (*asterisks*) and craters (*arrows*) can be observed in the center of the image
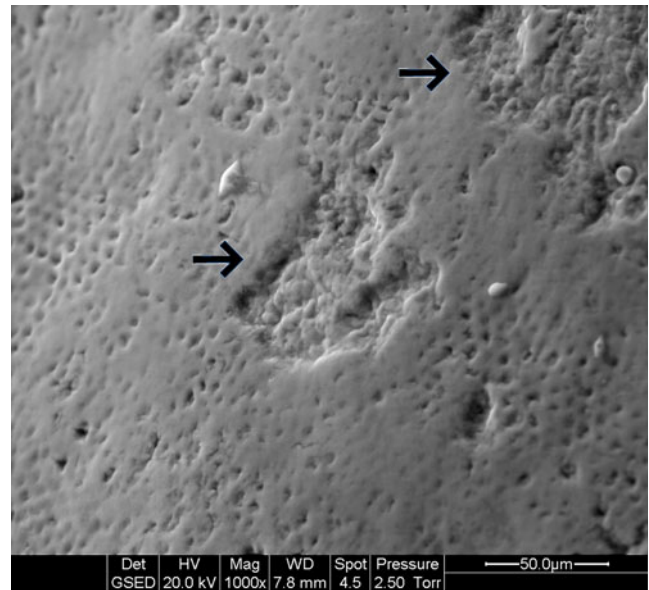

Fig. 7 Exfoliation phenomena (*arrows*) on surface treated with 7-W diode laser in the presence of AmF (1000x)

(Bonferroni method) and comparisons with the control group (Dunnett method) were performed. The lower part of Fig. 11 depicts the results obtained: lines that join two or more groups indicate absence of significant differences ($p>0.05$). As can be seen, the mean F^- concentration was significantly lower in the control group than in the other groups. Likewise, the mean F^- concentration was significantly lower in the unradiated AmF group than in the 7-W diode or 1-W CO_2 groups. Finally, the mean F^- concentration was significantly lower in the 5-W diode group than in the 1-W CO_2 group.
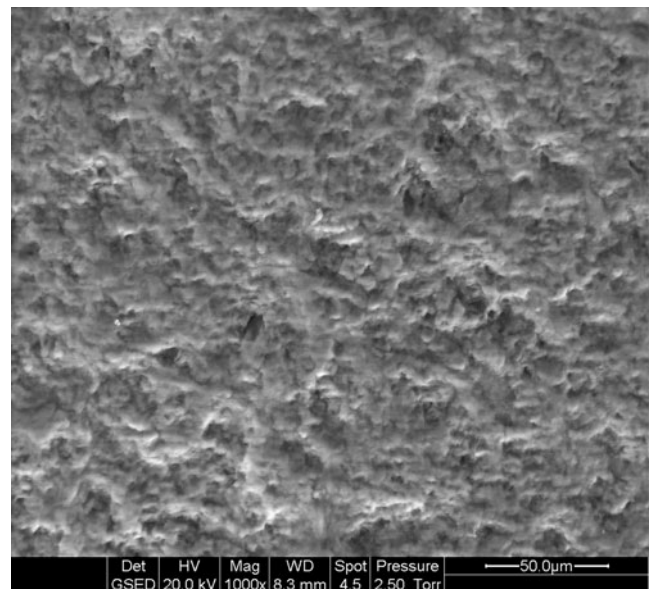

Fig. 8 Surface treated with 1-W CO_2 laser in the presence of AmF (1000x); most of the surface is altered, although without cracks or craters
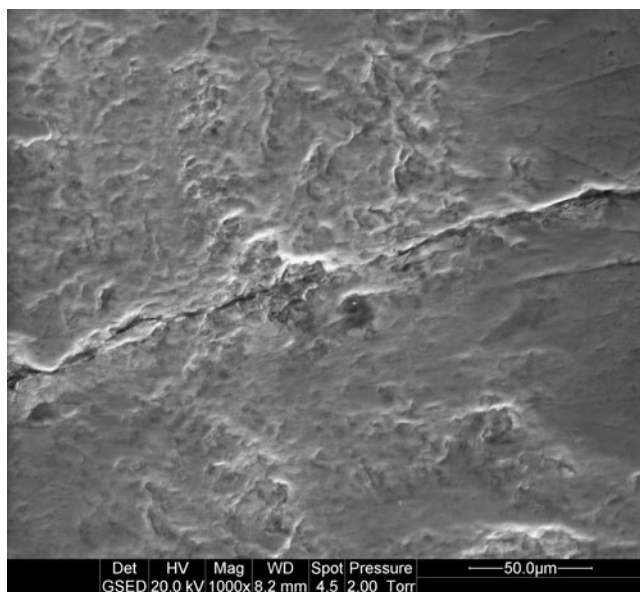
Fig. 9 Surface treated with 2-W CO_2 laser in the presence of AmF (1000x), evidencing wider thermal alteration and a localized fracture line close to the melted area

Intrapulpal temperature

Table 2 shows the intrapulpal temperatures obtained according to laser type and power, including initial and final temperatures and the temperature increase in each group, with standard deviations. Data were obtained during laser irradiation, and the internal pulp temperature increase was determined by calculating the difference between initial and maximum temperature values. Results indicate that: (1) the critical temperature increase of 5.5C° was not reached
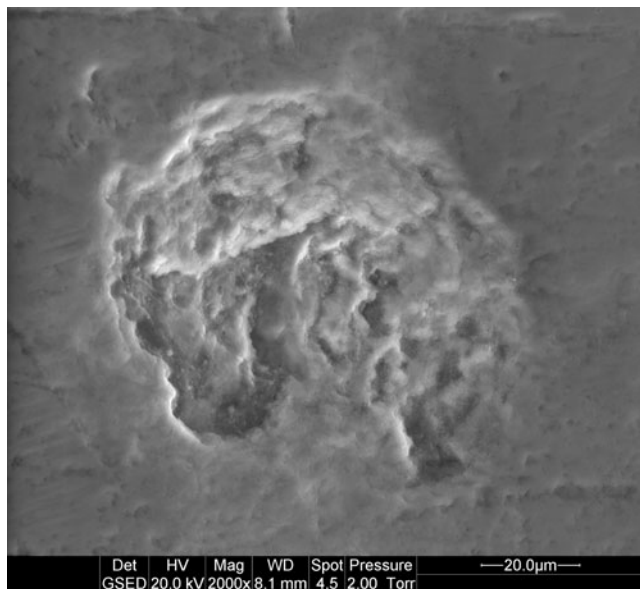

Fig. 10 Surface treated with 2-W CO_2 laser in the presence of AmF (2000x); an exfoliation area can be seen
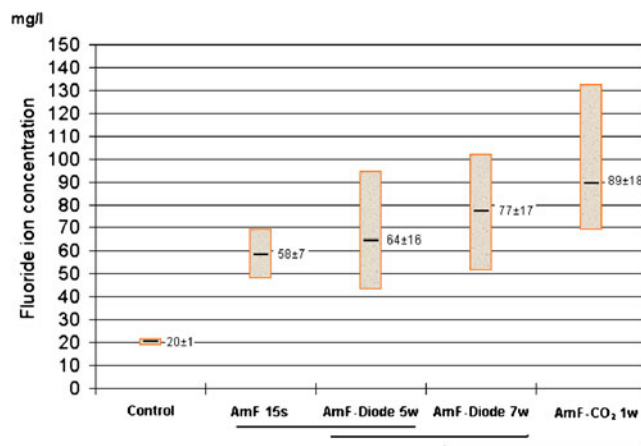
Fig. 11 Box diagram depicting maximum, minimum, and mean fluoride concentrations with corresponding standard deviations ($n=15$)

using the 5-W diode laser or the CO_2 laser at 1 or 2-W; (2) A temperature above 5.5C° was reached using the 7-W diode laser, although the mean value obtained was below this critical temperature; (3) CO_2 laser at 1-W produced the lowest intrapulpal temperature increases.

Discussion

This study evaluated the ability of CO_2 and diode laser applications to increase fluoride uptake by enamel. CO_2 laser effectively reduces the acid solubility of enamel and, when used in combination with fluoride, increases its fluoride uptake [24]. However, despite the wide utilization of diode lasers in dental practice, their usefulness as coadjuvant in topical fluoridation has been less well studied [15].

In the present study, the fluoride uptake by enamel was higher in groups treated with fluoride and irradiated with CO_2 laser than in the control group. Even at a very low power (1-W), a concentration of 89 ± 18 mg/l of fluoride was obtained on the study window, consistent with previous studies, although they reported a larger amount of fluoride on their samples [12, 25]. This discrepancy may be

Table 2 Intrapulpal temperature increment

Group/laser power	Initial temperature (°C)	Final temperature (°C)	ΔTemperature (°C)
A/Diode 5 W	37	40.16±0.68	3.16±0.68
B/Diode 7 W	37	42.25±0.55	5.25±0.55
C/CO_2 1 W	37	38.60±0.53	1.60±0.53
D/CO_2 2 W	37	41.44±0.60	4.44±0.60

explained by our use of laser in pulsed mode and at a lower power in comparison to the other authors, which would favor thermal relaxation of the enamel and a lower frequency of undesirable effects [26]. In addition, the teeth in the present study were from patients with no experience of a fluoridation program, which might have an influence on the amount of fluoride on their enamel, although this characteristic of the study population was not reported in the other studies. Finally, the meticulous post-treatment cleaning of the surfaces would facilitate detachment of almost all of the fluoride not integrated into the enamel.

The treatment of dental surfaces with diode laser at 5 and 7 W also significantly increased the fluoride uptake (64±16 and 77±17 mg/l) in comparison to the control group (20± 1 mg/l). The above laser treatment fluoride concentrations are greater than the group treated with amine fluoride alone (58±7 mg/l), but are not statistically relevant. Although the increase achieved with CO_2 laser (89±18 mg/l) was significantly higher than the results obtained with diode laser and amine fluoride alone, they were not statistically different with 7-W treatment. These results are consistent with a previous report by our group [19] of an increase in sodium fluoride (NaF) uptake using the same diode laser settings; although the concentration of fluoride on enamel was lower than in the present study. This may be explained by the more effective fluoridation achieved with AmF than with NaF [27, 28]. At any rate, both the present and the previous investigations demonstrate that the use of laser in this treatment is more effective than conventional fluoridation. Nevertheless, further studies in this field are warranted, since few data are available on the effect of diode laser radiation on dental enamel [20, 21, 29].

Microscopic study of the CO_2 laser-treated samples revealed a "frosted glass" type of melting at 1-W power, with signs of heat stress (e.g., exfoliation and cracks) appearing at 2 W. Hence, the objective of effective fluoridation without morphological damage is not achieved with CO_2 laser at 2 W. Study of the enamel surfaces treated with diode laser showed no changes to the enamel at 5 W but morphological changes and small areas of surface exfoliation at 7 W, although to a lesser extent than observed with 2-W CO_2 laser.

ESEM results were in agreement with the absorption data in hydroxyapatite for laser radiation at the assayed wavelengths. The wavelength of the CO_2 laser has a higher absorption coefficient on enamel surface in comparison to that of the diode laser, and it is therefore more likely to affect the surface, even at relatively low powers [30]. This is confirmed by the thermal alteration of specimen surfaces found when CO_2 laser was used, with melting at 1 W and exfoliation at 2 W.

The proposition that enamel melting by laser is desirable in order to increase resistance to acid attack has been supported by some authors [9, 31] and opposed by others [13, 32]. We feel closer to the latter group, since the effect does not appear to be uniform and there is a risk of fracture or exfoliation, leaving inner areas of enamel uncovered and unprotected against acids. The fractures and exfoliation areas observed with 2-W CO_2 and, to a lesser extent, with 7-W diode laser treatments can facilitate deeper penetration of acids [29], in direct opposition to the objectives of the fluoridation treatment. These defects are caused by the rapid expansion of water vapor in the organic matrix of enamel during laser treatment [33].

Microscopic studies of diode laser-treated surfaces showed small globular agglomerates that may be calcium fluoride (CaF_2), according to some authors [24, 34, 35]. This finding was previously reported by our group using NaF [19] but in lower amounts, which may be explained the deeper penetration of amine fluoride into the dental structure. The CaF_2 deposited on the enamel may correspond to fluoride that is not integrated into the enamel matrix. However, the deposit does not appear to influence caries prevention, since in vivo studies [36] reported its disappearance after a short time period due to the exchange of ions with oral fluids.

With our experimental laser settings (pulse duration, repetition rate, and exposure time), intra-pulpal temperature measurements indicated that no pulp damage is produced by CO_2 laser at a power below 2 W or by diode laser at 5 W or below. However the critical limit of 5.5°C [37] is exceeded with the use of diode laser at 7 W, ruling out its use in clinical practice with these experimental laser settings. However, other laser settings or exposure times could be used with 7 W without increasing the temperature.

In conclusion, CO_2 laser at power of 1 W and diode laser at power of 5 W, may both be used as effective methods of dental fluoridation that offer safety in terms of pulpal temperature and enamel surface integrity. However, they must be applied with due caution and at the appropriate laser settings, as in the present experiment. Diode laser, at both experimental settings used, has a lower capacity to integrate fluoride into enamel but also a lower capacity to alter the enamel in comparison to CO_2 laser. CO_2 laser has a higher absorption coefficient on enamel surface and can cause ablation effects, hence greater caution must be taken with its use in comparison to the diode laser. Fluoride can be integrated into hydroxyapatite with the use of lasers, unlike in conventional topical fluoridation, and further research is warranted on their use.

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