

# Effect of Er:YAG Laser on Enamel Acid Resistance: Morphological and Atomic Spectrometry Analysis

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**Background and Objectives:** This study evaluated the effect of Er:YAG laser on enamel acid resistance.

**Study Design/Materials and Methods:** Seventy human enamel slabs were randomly divided into seven groups ( $n = 10$ ): G1, Er:YAG laser (Key Laser 2, KaVo, Germany) 60 mJ, 2 Hz, 33.3 J/cm<sup>2</sup> (handpiece no. 2051, non-contact); G2, Er:YAG laser 80 mJ, 2 Hz, 44.4 J/cm<sup>2</sup> (handpiece no. 2051, non-contact); G3, Er:YAG laser 120 mJ, 2 Hz, 66.6 J/cm<sup>2</sup> (handpiece no. 2051, non-contact); G4, Er:YAG laser 64 mJ, 2 Hz, 20 J/cm<sup>2</sup> (handpiece no. 2055, contact); G5, Er:YAG laser 86.4 mJ, 2 Hz, 26.9 J/cm<sup>2</sup> (handpiece no. 2055, contact); G6, Er:YAG laser 135 mJ, 2 Hz, 42.2 J/cm<sup>2</sup> (handpiece no. 2055, contact); G7, control. After laser irradiation, samples were submitted to an acid challenge. For both the nos. 2051 and 2055 handpieces, irradiation was performed with a water cooled spray (5.0 ml/minutes). The calcium and phosphorous ions delivered from the tooth surface were quantified by atomic emission spectrometry, and morphological analysis of the enamel surface was performed under scanning electron microscopy. Kruskal–Wallis and multiple comparisons tests were applied to distinguish significant differences among the treatments ( $\alpha = 5\%$ ).

**Results:** Groups G1, G2, and G4 presented decreased demineralization. The SEM evaluation revealed different surface alterations as a result of the different energies used.

**Conclusion:** Lower energies can decrease enamel solubility without severe alterations of the enamel. *Lasers Surg. Med.* 37:366–372, 2005. © 2005 Wiley-Liss, Inc.

**Key words:** atomic emission spectrometry; caries; demineralization; enamel; Erbium laser

## INTRODUCTION

Fluoride has been widely used and approved for the treatment of caries lesions due to its effectiveness and low cost [1]. However, in the last few years, new techniques for the prevention of this pathology have been studied; among them, laser irradiation seems to be very promising.

The effectiveness of laser on caries preventive treatment is closely related to the laser light interaction with dental hard tissues. Since 1965, when Sognaes and Stern [2] first

suggested their potential to decrease enamel solubility, some studies have been developed with the argon, Nd:YAG (1.06  $\mu\text{m}$ ), Er:YAG (2.9  $\mu\text{m}$ ), Er,Cr:YSGG (2.8  $\mu\text{m}$ ), and carbon dioxide (9.6  $\mu\text{m}$ ) lasers. Except for argon and Nd:YAG lasers, the other wavelengths referred to are rapidly adsorbed by the water. Both CO<sub>2</sub> and Er,Cr:YSGG lasers are also strongly absorbed by the hydroxiapatite present in the tooth structure [3–11].

One of the most critical regions that require efficient cleaning and that are considered risk areas for the development of caries lesions, are the pits and fissures. The possible use of laser as a coadjutant therapy in the preventive treatment of pit and fissure caries is based on its ability to reduce microorganisms [12], to remove hard tissues [4,9], and to change the chemical [10,13] and morphological [14,15] structure of the enamel. The most accepted hypothesis regarding the mechanism by which laser enhances enamel acid resistance assumes that heating the enamel surface in the range of 400–1,000°C reduces the amount of bound carbonate, resulting in increased resistance to acid [13].

In recent years, numerous studies have described the increased acid resistance of laser-irradiated enamel [10,14,16–21]. However, there are few studies revealing the potential of Er:YAG laser for decreasing enamel solubility.

The use of Er:YAG laser on dental hard tissue was first described by Hibst et al., in 1988 [22], and was introduced for cavity preparation. According to these authors, the ablation of enamel can occur without the thermal effects on adjacent tissues. However, for caries preventive treatment, laser irradiation is supposed to not ablate the surface, but to

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change the morphological or chemical composition of the enamel, instead. Therefore, the use of energy densities below the ablation threshold is recommended.

This study aimed to evaluate the mineral and morphological alterations occurring in enamel after Er:YAG laser irradiation with different parameters.

## MATERIALS AND METHODS

### Experimental Design

The surface treatment was performed under the following irradiation conditions: G1, Er:YAG laser (handpiece no. 2051) at 60 mJ, 2 Hz, 33.3 J/cm<sup>2</sup>; G2, Er:YAG laser (handpiece no. 2051) at 80 mJ, 2 Hz, 44.4 J/cm<sup>2</sup>; G3, Er:YAG laser (handpiece no. 2051) at 120 mJ, 2 Hz, 66.6 J/cm<sup>2</sup>; G4, Er:YAG laser (handpiece no. 2055) at 64 mJ, 2 Hz, 20 J/cm<sup>2</sup>; G5, Er:YAG laser (handpiece no. 2055) at 86.4 mJ, 2 Hz, 29.9 J/cm<sup>2</sup>; G6, Er:YAG laser (handpiece no. 2055) at 135 mJ, 2 Hz, 42.2 J/cm<sup>2</sup>; G7, control. This study used 70 human experimental samples (n=10) to evaluate the chemical alterations of the enamel and 30 samples (5 from each test group G1–G6) were used for the SEM evaluation. The atomic emission spectrometry analysis evaluated the calcium and phosphorous delivered from the laser enamel when submitted to an acid challenge. For the SEM evaluation, five samples were prepared for each group, except for the non-irradiated group, to show the effects of different parameters of laser irradiation on the enamel surface morphology. Figure 1 illustrates the experimental design.

### Specimen Preparation

The study protocol was reviewed and approved by the local ethical committee. Fifty-one extracted, non-erupted human third molars were collected and immediately stored in physiological saline solution (pH 7.0) until their use (1 week). The roots were removed approximately to the dentin–enamel junction and the crowns were longitudinally sectioned in the mesial to distal direction with diamond burs no. 1091 (KG Sorensen, Barueri, SP, Brazil) used with a high-speed motor with water-cooling (KaVo, Joinville, SC, Brazil) to obtain two different samples. After sectioning

was completed, the enamel slabs were stored in physiological saline solution (1% NaCl) in order to prevent drying-out. The samples with stains or cracks, observed under a stereomicroscope used at X30 (EMZ series, Meiji Techno, Saitama, Japan), were rejected.

Seventy enamel slabs from the buccal and lingual surfaces were individually embedded in a self-curing polyester resin in a polyvinyl-chloride ring mould, so that their external surfaces were exposed, and then left to polymerize. After the resin polymerization, the moulds were removed and the specimens were cut in a hard tissue microtome (LABCUT 1010, Extec Corp., Enfield, CT) to obtain samples with a 3×3 mm standardized area. Except for the exposed enamel surface area, the samples were subsequently covered with a thin coat of acid-resistant nail varnish and submitted to the following treatment. For the SEM evaluation, 30 samples were selected from the test groups G1–G6 (n=5) and submitted to the surface treatment with Er:YAG laser and acid challenge.

### Treatment of the Enamel Surface

The specimens were randomly assigned to seven groups (n=10), as described previously. The samples from the control group (G7, no treatment) were kept in a buffer solution while the samples from the experimental groups (G1–G6) were being irradiated.

These were then irradiated with the Er:YAG laser (Key Laser 2, KaVo, Joinville, SC, Brazil)<sup>1</sup> emitting photons at a wavelength of 2.94 μm. To ensure consistent spot size with the hand irradiation, an endodontic file was fixed at the handpiece and the distance of 12 mm from the surface was kept during all the procedure. The output power and repetition rate of this equipment range from 60 to 500 mJ and 1 to 15 Hz, respectively. The beam diameters at the focal areas for the handpiece no. 2051 (non-contact) and the handpiece no. 2055 (contact fiber 50/10) were 0.63 mm and 0.32 mm, respectively. The handpiece no. 2051 was positioned 12 mm from the enamel surface. The samples from groups G1, G2, and G3 (non-contact/12 mm) were irradiated with the energy depicted on the equipment display and corresponded to the energy delivered from the handpiece. The energies described for groups G4, G5, and G6 (contact) refer to the energy delivered at the end of the tip, taking into account the transmitting factor (0.54) for this handpiece (no. 2055). The transmitting factor is given by the manufacturer and is depicted in the user manual of the equipment<sup>2</sup>. The tips were positioned perpendicular to the enamel surface and the samples were irradiated by scanning once in each direction, horizontal and vertical, in order to promote homogeneous irradiation and to cover the entire sample area. The irradiation was performed by hand, screening the enamel surface with a uniform motion [23].

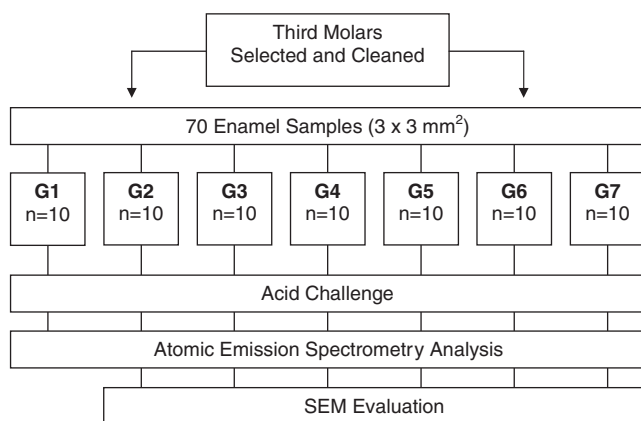


Fig. 1. Diagram of the experimental design.

<sup>1</sup>The Er:YAG Laser (Kavo Key II, KaVo) equipment was purchased by the Special Laboratory of Lasers in Dentistry (LELO/FOUSP) with financial support provided by FAPESP (grant no. 97/10823-0).

<sup>2</sup>User manual (Key Laser 2, KaVo Joinville/Sc., Brazil), Session Handstuck E 2055/Handstuck P 2056, page 14.

For both the nos. 2051 and 2055 handpieces, irradiation was performed with a water cooled spray (5.0 ml/minutes).

### SEM Evaluation

Five enamel samples from each test group (G1–G6), except the non-irradiated group (G7), were analyzed under SEM (Philips LX 30, Eindhoven, Holland), immediately after the acid challenge. The samples were dehydrated by an alcohol series of increasing grade (70%–100%) for a total of 24 hours. Then, they were sputter-coated using a carbon coating device and submitted to the SEM analysis.

### Acid Challenge

After irradiation, each sample was individually immersed in a plastic vial with a 2 ml acetate buffer solution (0.1 M acetate buffer, pH 4.5) for 8 hours [24–27]. All plastic vials were fixed onto a polystyrene board to prevent the recipients from falling. The polystyrene board was placed in a thermal bath at 37°C. After the acid challenge, the samples were removed from the vials and washed with distilled water.

### Atomic Emission Spectrometry

After the acid challenge, the acetate buffer solutions from each vial (2 ml) of both the experimental and control groups were collected and analyzed under atomic emission spectrometry (Spectro ceros CCD, Spectro Analytical Instruments, Kleve, Germany). Measurements of the calcium and phosphorous ions in the solution (mg/L) were obtained and compared within the experimental and control groups Table 1. In order to obtain a more accurate result of the effect of laser irradiation on the enamel chemical structure, two measurements of calcium and phosphorous present in the solution of each specimen were performed.

### Statistical Analysis

The statistical analysis of the data was made considering the average of the two measurements of the atomic emission spectrometry. The Kruskal–Wallis test was used and the non-parametric test of multiple comparisons was applied to distinguish significant differences among the treatments at the level of 5% of significance.

**TABLE 1. Mean Values and Standard Deviations of the Calcium and Phosphate Measurements for Each Group**

Group	N	Calcium (mg/L)	SD	Phosphate (mg/L)	SD
1	10	47.69	12.33	12.75	5.88
2	10	39.20	12.06	11.65	2.69
3	10	78.18	27.72	11.09	7.06
4	10	39.56	15.42	12.04	6.53
5	10	123.12	80.73	31.14	21.06
6	10	104.03	39.05	23.25	10.00
7	10	61.29	27.32	16.08	4.66

Kruskal–Wallis test ( $P = 0.0025$ ).

## RESULTS

### Atomic Emission Spectrometry

The data did not show normal distribution. Therefore, the measured results of the calcium and phosphorous content from the two measurement points on the irradiated and the control samples were recorded and statistically analyzed using the Kruskal–Wallis test ( $\alpha = 5\%$ ). Groups G1, G2, and G4 acid solutions exhibited a lower calcium concentration than group G7 (control). On the other hand, the treatments performed in groups G3, G5, and G6 lead to a higher loss of calcium from the enamel, compared to the other groups.

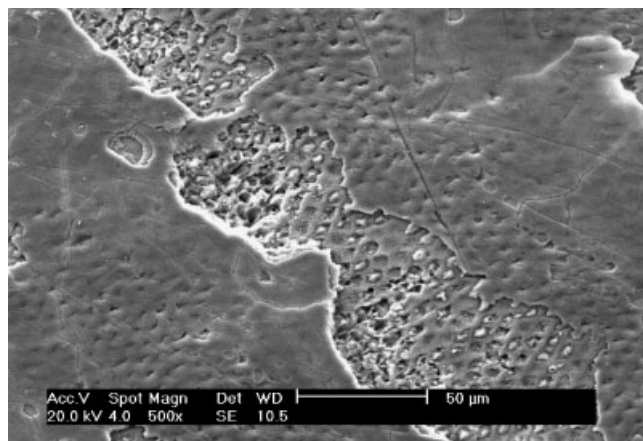
The phosphorous present in the solutions of the groups G1, G2, G3, and G4 were statistically different from the control group (G7), showing a lower phosphorous concentration. However, the acid solution from groups G5 and G6 presented the higher amount of phosphorous delivered from the enamel. Figures 8 and 9 show the mean values of the calcium and phosphorous concentrations for each solution of irradiated and non-irradiated samples.

### SEM Evaluation

All the irradiated samples presented morphological alterations, as shown in Figures 2–7. However, there was no evidence of denaturing or disruption of enamel structure, resulting from the increase in surface temperature during irradiation. Morphological evaluation also revealed exposed enamel prisms, a rough surface, and different crater sizes as a result of the different energies used. The enamel exposed to lower energies and with the non-contact handpiece (no. 2051) presented a more homogeneous irradiation pattern than those irradiated with higher energies.

## DISCUSSION

Recently studies have been concerned about the effects of this laser on enamel and dentin conditioning and on preventing caries lesions.



**Fig. 2.** Micrograph representative of samples from group G1, enamel irradiated with Er:YAG laser at 60 mJ, 2 Hz, 33.3 J/cm<sup>2</sup> (non-contact handpiece). The enamel presents a homogeneous surface with some irradiated areas in which the exposure of enamel prisms can be verified.

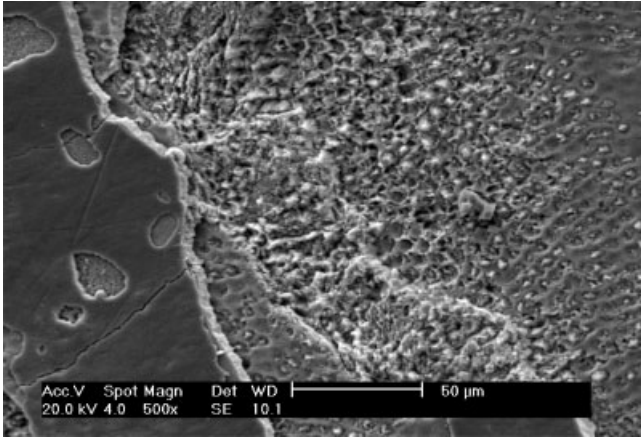


Fig. 3. Micrograph representative of samples from group G2, enamel irradiated with Er:YAG laser at 80 mJ, 2 Hz, 44.4 J/cm<sup>2</sup> (non-contact handpiece). The enamel surface presents areas of irradiation, showing exposed prisms. However, there are areas without irradiation.

Although many studies have shown the effect of laser irradiation on enamel acid resistance [10,14,16–21], there are still contradictions regarding the effect of Er:YAG laser on the decrease of enamel solubility. To enable better discussion of the possible relation between chemical and morphological alterations after the use of Er:YAG laser, this study evaluated the mineral loss after laser irradiation and also verified the irradiated enamel surface morphology under SEM.

Laser irradiation was performed with two different handpieces (nos. 2051 and 2055), so that the effect of either contact or no contact with the enamel could be evaluated. The handpiece no. 2055 (contact) is usually used for endodontic treatment (microbial reduction), while the non-contact handpiece (no. 2051) can be used for both soft

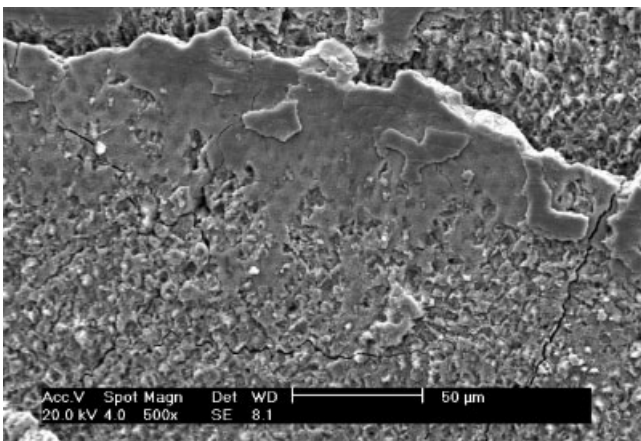


Fig. 4. Micrograph representative of samples from group G3, enamel irradiated with Er:YAG laser at 120 mJ, 2 Hz, 66.6 J/cm<sup>2</sup> (non-contact handpiece). The irradiated enamel reveals a homogeneous irradiation pattern. However, small cracks can be seen in the enamel.

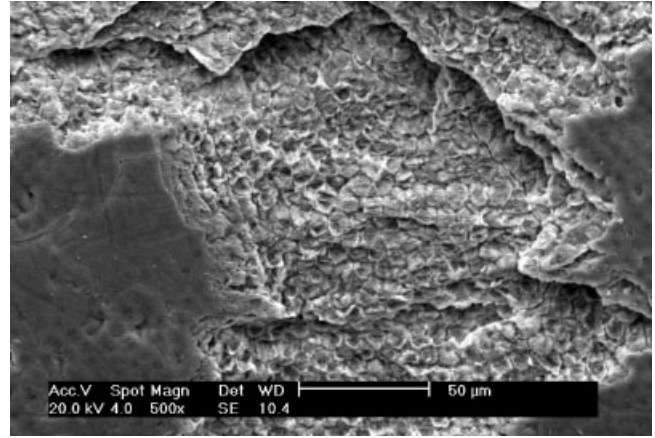


Fig. 5. Micrograph representative of samples from group G4, enamel irradiated with Er:YAG laser at 64 mJ, 2 Hz, 20 J/cm<sup>2</sup> (contact handpiece). The irradiation pattern is not homogeneous. A great number of exposed enamel prisms can be seen.

tissue procedures and removal/treatment of mineralized hard tissues. The findings of the present study have shown that both handpieces (with different parameters of irradiation) can be used on caries prevention and thus professionals do not have to purchase a specific handpiece for this purpose.

Even after scanning irradiation of the enamel surface in both directions (horizontal and vertical), it was possible, under SEM, to verify areas without irradiation (Figs. 2, 3, and 5), possibly due to the fact of Er:YAG laser being pulsed. Despite the irregular aspect of irradiation, the samples submitted to lower energies presented a decrease in demineralization. According to Hibst and Keller (1989) [4], the distribution of the laser light energy influences both the geometry of the crater and the extension of the lesion.

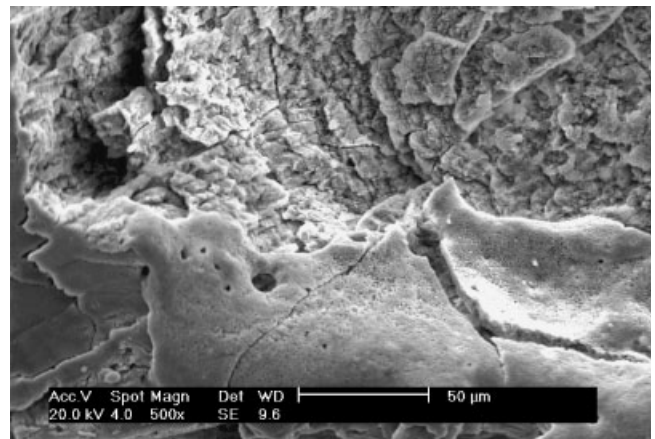


Fig. 6. Micrograph representative of samples from group G5, enamel irradiated with Er:YAG laser at 86.4 mJ, 2 Hz, 26.9 J/cm<sup>2</sup> (contact handpiece). There are cracks and deep craters on the irradiated enamel surface.

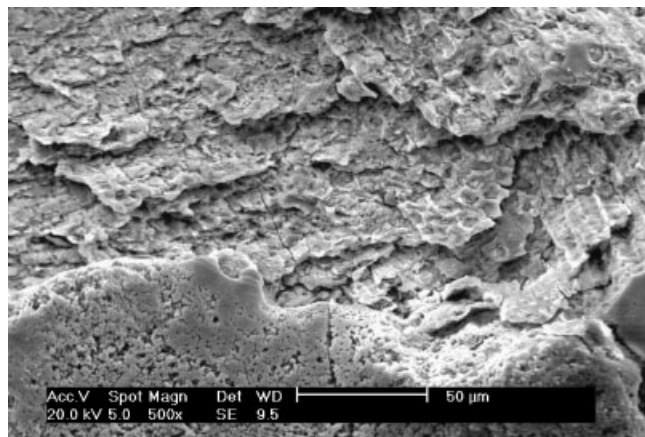


Fig. 7. Micrograph representative of samples from group G6, enamel irradiated with Er:YAG laser at 135 mJ, 2 Hz, 42.2 J/cm<sup>2</sup> (contact handpiece). There are cracks and deep craters on the irradiated enamel surface.

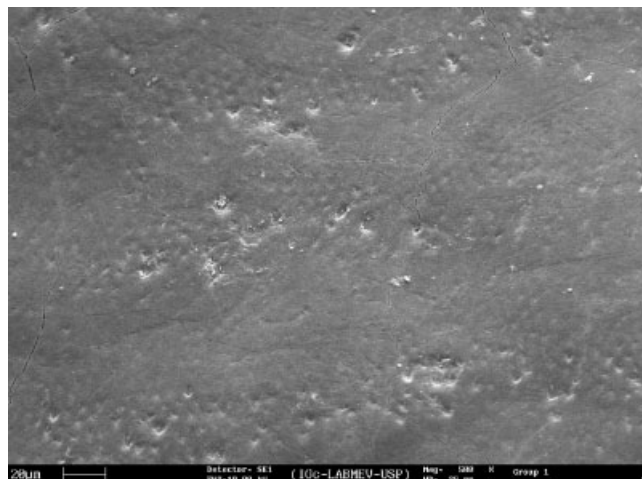


Fig. 8. Micrograph representative of samples from group G7, enamel without laser irradiation. The enamel surface presents a smooth surface without exposure of prisms.

The use of a scanning device to irradiate the enamel surface [16] could have lead to a more homogeneous irradiation by overlapping pulses. However, in the present study the irradiation was performed by hand [23] in order to reproduce the clinical condition. When higher energies were used, mainly with the handpiece no. 2055 (tip 50/10—contact), there was a greater change in the enamel surface morphology, showing deep craters and cracks (Figs. 5 and 6). When using this handpiece, the alterations were more

evident when compared to higher energies used with the non-contact handpiece (no. 2051) (Fig. 3).

Some studies suggest that enamel acid resistance is related to morphological changes [14,20,28,29]. However, it seems that the enamel surface does not necessarily need to be morphologically changed to reduce tooth solubility; possibly, the chemical alteration is more important than the changes in surface topography [10,15,30]. The increase in the enamel temperature (approximately up to 700°C) can

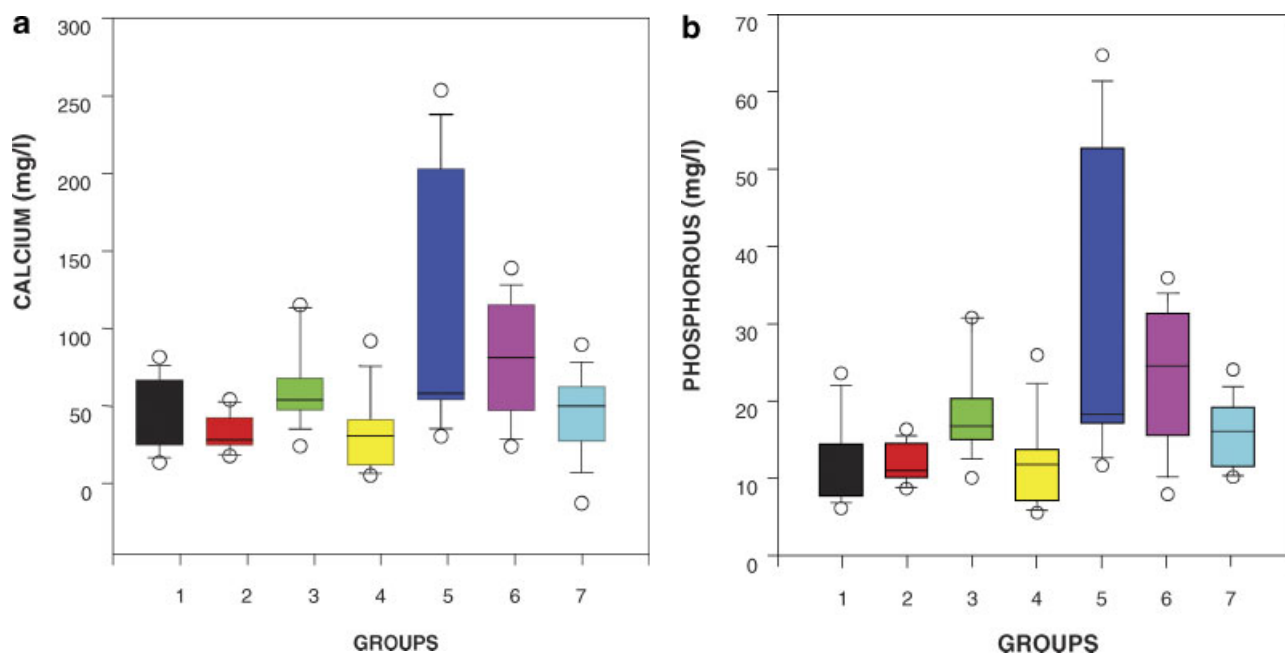


Fig. 9. Comparison of the calcium (a) and phosphorous (b) concentration in acid solution after irradiation with Er:YAG laser. Data correspond to minimum, maximum, and mean values ( $\pm$  SD). ( $P = 0.05$ ). [Figure can be viewed in color online via [www.interscience.wiley.com](http://www.interscience.wiley.com).]



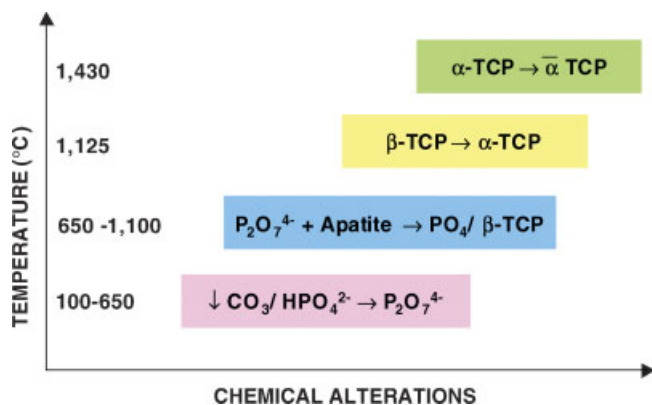


Fig. 10. Chemical changes occurred with the increase of enamel surface temperature. [Figure can be viewed in color online via [www.interscience.wiley.com](http://www.interscience.wiley.com).]

lead to the decrease of the amount of carbonate and water, resulting in a more acid resistant structure, as reported by Fowler and Kuroda [13] (Fig. 10). When the surface temperature increases between 100 and 650°C, the main chemical changes that occur in the tooth are that the major  $\text{CO}_3$  component in the phosphate position decreased and the acid phosphate ions condense to form pyrophosphate ions; at 650–1,100°C the main changes are thermal recrystallization and crystal size growth, and pyrophosphate react with apatite to form  $\text{PO}_4$  along with the formation of  $\beta$ -tri-calcium-phosphate ( $\beta$ -TCP); at  $> 1,100^\circ\text{C}$  the main change is that the  $\beta$ -TCP is converted to a  $\alpha$ -TCP and when the temperature reaches 1,430°C this compound changes into a high-temperature polymorph. Tri-calcium-phosphate  $\alpha$  and  $\beta$  are potentially soluble in acid environment [31–33].

In order to evaluate the influence of the energy on the enamel chemical structure, different radiation parameters were used. As expected, enamel demineralization was observed in the control group, since the samples were not previously treated with any remineralizing solution, such as fluoride, which could have contributed to the decrease of enamel solubility [1].

The observation that higher energies of Er:YAG laser irradiation at the enamel surface can induce more accentuated chemical alterations on the treated surface and not necessarily the enhancement of acid resistance [15] was also verified in this study. The groups irradiated with lower energies presented an increase in enamel acid resistance, with a lower amount of both calcium and phosphorous delivered in the solution compared to the control group. Under SEM evaluation, these groups presented a lava-like surface, with some areas without irradiation (Figs. 2, 3, and 4). Laser can promote microspaces on the enamel surface and during an acid challenge, calcium and phosphorous can be delivered from the tooth structure. Some authors suggest that these microspaces can act as an area of ions precipitation, contributing to the remineralization of the irradiated enamel [34–36]. In this study, the samples were not exposed to a remineralizing solution after the acid challenge; therefore, the effect of Er:YAG laser on the tooth

uptake of minerals was not verified. Higher energies might have led to ablation or the formation of deeper or more extensive spaces in the enamel structure, contributing to the loss of minerals during exposure to an acid solution.

The Er:YAG laser irradiation used for the prevention of caries seems to be a very promising treatment in Dentistry. Although further research is needed to assure the effect of this treatment not only on enamel acid resistance, but on other tooth physical properties that can be affected by laser irradiation, this study showed that lower energies can decrease enamel solubility without severe alterations of the tooth structure and also suggests that the non-contact handpiece may be more promising for caries preventive treatment.

## ACKNOWLEDGMENTS

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