Effect of Er:YAG Laser on CaF$_2$ Formation and Its Anti-Cariogenic Action on Human Enamel: An *in Vitro* Study


**ABSTRACT**

*Objective:* The objective of this study was to evaluate the effect of Er:YAG laser on the formation of CaF$_2$, after the application of acidulated phosphate fluoride (APF), and its influence on the anti-cariogenic action in human dental enamel. *Background Data:* Er:YAG laser was designed to promote ablation of the enamel. However, the possibility of using this energy to increase the enamel’s resistance to caries has hardly been explored, and neither has its interaction with the use of fluorides. *Materials and Methods:* One hundred and twenty blocks of enamel were allocated to four groups of 30 blocks each: (1) C, control group; (2) Er:YAG, laser; (3) APF; and (4) Er:YAG+APF. Of these, 80 blocks were submitted to pH cycling for 14 days. In the other 40 blocks, fluoride (CaF$_2$) was measured before cycling. After pH cycling, surface microhardness (SMH), microhardness in cross-section (converted to mineral contents % vol. min.), and fluoride after cycling (40 blocks) were also determined. *Results:* SMH decreased in all groups. The control group showed the highest decrease, and Er:YAG+APF showed the lowest decrease ($p < 0.05$). Groups APF and Er:YAG showed the same results ($p > 0.05$). Mineral content at depths 10, 20, and 40 µm was lower in the control and Er:YAG groups, and higher in groups APF and Er:YAG+APF. CaF$_2$ (µgF/cm$^2$) deposited before pH cycling was higher in the APF group when compared to the Er:YAG+APF group. Control and Er:YAG groups showed the lowest values ($p > 0.05$). *Conclusion:* It was concluded that Er:YAG laser influenced the deposition of CaF$_2$ on the enamel and showed a superficial anti-cariogenic action, but not in depth.

**INTRODUCTION**

In dentistry, CO$_2$, argon, and Nd:YAG lasers are the most frequently used lasers in research, as their energy is more readily absorbed by the calcium-phosphate-based minerals that compose dental enamel.$^1-3$ However, the indiscriminate use of lasers, with the use of high energy, may diminish the irradiated enamel’s resistance to acids,$^4,5$ due to the transformation of hydroxyapatite in tri-calcium phosphate-$\alpha$ and -$\beta$(TCP), which are highly soluble in an acidic environment.$^4-6$

Applications of laser to dental enamel, at appropriate energy levels, using the lasers described above, promote an increase in resistance to acids and, followed by the application of topical fluoridated products, increase the fluoride levels in dental enamel, increasing its anti-cariogenic effect by the formation of fluorapatite.$^1$ However, the material of greatest importance that is responsible for the cariostatic potential of topically used fluoridated products is calcium fluoride.$^7$ Calcium ions, carbonated apatites, and calcium phosphates are good substrates and are necessary for the formation of CaF$_2$,$^8$ but are reduced in irradiated enamel.$^3,9$

Of the lasers indicated for promoting the ablation of enamel, the Er:YAG laser is absorbed more by water than by hydroxyapatite.$^10$ Thus, its thermal effect on mineralized tissues occurs through explosive ablation, due to the high absorption of irradiation by the water molecules contained in these tissues. However, the possibility of using this energy to increase the enamel’s resistance to caries or interaction with the use of fluorides has hardly been explored.

The aim of this study was (1) to investigate *in vitro* the effect of Er:YAG laser irradiation on the formation of calcium fluoride to human dental enamel, when followed by the topical ap-
lication of acidulated phosphate fluoride, and (2) to evaluate the resulting anti-cariogenic action and the pH cycling model, by means of microhardness analysis and fluoride measurement.

**MATERIALS AND METHODS**

**Experimental design**

Enamel blocks (3 × 3 mm) were obtained from impacted human third molar teeth that were stored in 2% formaldehyde solution pH 7.0 at room temperature. The enamel surface of the blocks was then serially polished, and those with a hardness (baseline) of 326–372 KHN units were selected and randomized in four groups of 30 specimens each. One group was used as control (C). Of the three other groups of 30 blocks each, one was treated with Er:YAG laser (Er:YAG), one was treated with acidulated fluoride gel (APF), and the last one was treated with Er:YAG+APF. Twenty blocks of each group were submitted to pH cycling solutions, and the other blocks were kept in a humid environment until the analysis. After the pH cycling, surface and cross-sectional enamel microhardness was determined in half of the blocks, and in the other half, fluoride (CaF<sub>2</sub>) in enamel was determined. These analyses were also made on the sound blocks and on the blocks submitted to fluoride application, but not on the blocks that were pH cycled.

**Treatments and pH cycling**

The enamel blocks of groups Er:YAG and Er:YAG+APF were irradiated with Er:YAG laser (2.94 μm). An unfocused laser beam, without spray, was used to scan the blocks at a distance of 40 mm from the surface of the sample at 60-mJ energy and 1-Hz pulse frequency. The exhibition time was 10 sec, and the outlet area of the spot was 0.63 mm. Total fluency was 0.95 J/cm<sup>2</sup>. The blocks of groups APF and Er:YAG+APF were submitted to APF treatment. A gel (Cristal, SS White, Rio de Janeiro, Brazil) containing 1.23% fluoride (NaF) at pH of 3.6–3.9 was applied for 4 min using a cotton swab. Then, the blocks were washed with deionized water for 1 min.

The laser equipment used was the Er:YAG (KaVo, Biberach, Germany), 2.94-μm wavelength, pulsed emission mode, fiber optic delivery system, pulse duration of 250–500 μsec, applied with a no. 2051 hand piece (0.63 mm in diameter).

Twenty blocks from each group were submitted to a pH cycling model simulating a high caries challenge for 10 days, according to Featherstone et al. The blocks were kept in a demineralizing solution (2.0 mM calcium, 2.0 mM phosphate in 0.075 M acetate buffer, pH 4.3) for 3 h (20 mL per block), and in a remineralizing solution (1.5 mM calcium, 0.9 mM phosphate, 150 mM of KCl in 0.1 M Tris buffer, pH 7.0) for 21 h (10 mL per block). During the weekend, the enamel blocks were stored in the remineralizing solution, and both the solutions were changed before starting another cycle of 5 days.

**Microhardness analysis**

After pH cycling, the surface microhardness of the enamel blocks from groups C, APF, Er:YAG, and Er:YAG+APF was measured again. Five indentations were made from the base-line, spaced 100 μm from each other. A Shimadzu HMV-2000 microhardness tester with a Knoop diamond was used at a 50-g force load for 10 sec. The mean values of the five baseline indentations and the five measurements after treatments were then averaged within each treatment group, and the percentage of surface microhardness change (%SMHC) was calculated.

After surface microhardness analysis (SMH), the blocks were longitudinally sectioned in two halves. Half of each block was used for cross-sectional microhardness determination and the other half for fluoride enamel analysis. To perform cross-sectional microhardness (CSMH) tests, one of the halves of each block was embedded in acrylic resin so that the cut section was exposed and could be polished. Three rows of six indentations at 10, 20, 40, 60, 80, and 100 μm from the outer dentine surface were made as previously described. The distance between the rows was 100 μm. The mean values at all five measuring points at each distance from the surface were then averaged. CSMH values were converted to mineral contents (% vol. min.) using the following relation: mineral content = 4.3 (KHN) + 11.3.<sup>16</sup>

**Analysis of fluoride concentration in enamel**

Fluoride in the form of CaF<sub>2</sub> was determined before and after pH cycling according to Caslavska et al. and removed from each block by immersion in 0.5 mL of 0.5 M KOH for 24 h under continuous agitation. An equal volume of TISAB II pH 5.0 modified with M HCl was added to each solution containing the dissolved fluoride. Fluoride measurements were performed using an ion-selective electrode Orion 96-09 and an ion analyzer Orion 720 A, and were expressed in μg F/cm<sup>2</sup> of enamel.

**Statistical analysis**

The Kruskal Wallis test was then used, and the nonparametric test of multiple comparisons was applied to distinguish significant differences among the treatments at 5%.<sup>15</sup>

**RESULTS**

Percentage of surface microhardness change (%SMHC) showed significant reduction in all experimental groups (Table 1). The control group showed a higher decrease in SMH (p < 0.05) when compared to the other treatments. Groups Er:YAG and APF showed the same SMH reduction (p > 0.05); however, it was different from group Er:YAG+APF, which showed the smallest SMH alteration (p < 0.05). Final SMH results showed a difference between control and the treated groups. Groups Er:YAG and APF showed the same values (p > 0.05), and group Er:YAG+APF showed the highest final SMH value (p < 0.05). In all experimental groups, final SMH values were always smaller (p < 0.05) when compared to initial SMH, which were the same (p > 0.05), regardless of the experimental group.

The results of mineral volume percentage (% vol. min.) in enamel, in each depth and group, and their respective standard deviation are shown in Figure 1. At the 10-μm depth, the blocks treated with Er:YAG+APF showed the highest mineral contents (p < 0.05), followed by APF. Blocks treated with Er:YAG and the control group showed similar mineral contents (p > 0.05),
At 20 and 40 μm, the control group and the Er:YAG group showed significantly lower mineral contents when compared to groups APF and Er:YAG+APF. However, the comparison between the control and Er:YAG, and between APF and Er:YAG+APF showed similar mineral contents (p > 0.05). Finally, at 60-, 80-, and 100-μm depths, no treatments differed significantly. With regard to fluoride in the form of CaF$_2$ before pH cycling, the data (Table 1) show a higher concentration (p < 0.05) in the enamel blocks treated with APF than those in the control, Er:YAG, and Er:YAG+APF groups. The blocks in the control and Er:YAG groups showed differences that were not significant (p > 0.05), but their values were lower than those of the blocks treated with Er:YAG+APF (p < 0.05). The data also show the same fluoride concentration (p < 0.05) after pH cycling in the enamel blocks from all the groups/treatments other than the baseline (group C).

### DISCUSSION

Many studies have reported that lasers can improve human dental enamel in order to resist acid attacks.$^{16,19}$ These are high-energy lasers that are absorbed by the calcium phosphates that compose the enamel structure and of greater diffraction in the enamel.$^{4,5,20}$ In this study, the hypothesis that specific infrared laser irradiation used for ablation can also inhibit caries progression was tested. The Er:YAG laser used for the preparation of cavities, cleaning fissure and fissures, and condition-

![FIG. 1. Enamel percentage volume mineral according to the treatments and the distance (μm) from the surface. Different letters show statistical significance (p < 0.05) among treatments at each distance from the enamel surface, and bars denote standard errors.](image-url)
ing of enamel was investigated. The effect on the deposition of calcium fluoride (CaF₂) was also observed.

The results of this study (Table 1) show the superficial action of Er:YAG laser promoting smaller superficial mineral loss. Surface microhardness (SMH) has been accepted for evaluating mineral loss or gain by enamel, because there is a correlation between the mineral contents of surface enamel and the length of indentations. Although it was possible to show a difference among the treatments by using SMH measurements, this method is considered more appropriate for evaluating dental caries initiation than progression, as it was done in this study, with lesion depth in the 20–40-μm range. Treatment with laser was capable of reducing the superficial mineral loss to a level similar to that of treatment with topical fluoride and, when compared, the percentage of loss was much lower (p < 0.05). These findings may indicate an alteration in the superficial enamel structure caused by the laser making it more resistant to mineral loss. Included in these changes are reduction of water and carbonate contents, increase in the hydroxyl ion contents, formation of pyrophosphates and the decomposition of proteins are alterations that promote a decrease in the solubility of enamel. The Er:YAG laser uses a wavelength of 2.94 μm, which coincides with the water absorption spectrum, and which results in maximum absorption and complete transformation into thermal energy, called the state of "sub-ablation." The low density of energy used (0.95 J/cm²) and the exposure time (10 sec) may have caused only the heating of water, steam, and pressure in the interior of the enamel, without promoting transformations, re-crystallization, or fusion of calcium phosphates in the structure of the enamel crystal. The laser may not have caused the formation of soluble calcium phosphates or altered the ratio of Ca/P of the enamel crystals, as the Er:YAG laser is highly absorbed, acting on the surface. These alterations are observed with a wavelength of around 9.3 μm, close to the absorption spectrum of the phosphate and carbonate groups, and the energy density close to 60 J/cm².

These alterations may be confined through the results presented in Table 1, by the smaller deposition of calcium fluoride (CaF₂) on the enamel surface observed in the group Er:YAG + APF when compared to the group treated with acidulated phosphate fluoride gel. With regard to the application of acidulated phosphate fluoride on the surface of the enamel, the dissolution of more soluble apatite crystals occurs, as the deposition of CaF₂ varies according to the surface conditions of the enamel. CaF₂ deposition has shown to be greater in enamel with caries lesion when compared to sound enamel. However, these substrates were eliminated in the irradiated enamel, as well as organic substances and water, which, supposedly, reduced the formation of CaF₂. In spite of this, we may observe the synergism of the association of Er:YAG and APF with a lower surface mineral loss when compared to group APF (p < 0.05), which is in agreement with data in the literature.

With respect to deep caries lesions, demineralization effect of the treatments was also evaluated by cross-sectional hardness and converted to mineral contents (% vol.min.). It is a recommended technique and has been useful for different purposes. Longitudinal section microhardness shows a reliable correlation coefficient (r² = 0.915) when compared to transversal microradiography. The data (Fig. 1) do not confirm the SMH results relative to the effect of laser in reducing demineralization. Better results are obtained with the laser treatment associated with topical fluoride on the surface of the enamel. However, when we consider mineral contents and observe the data of group Er:YAG, we notice that the Er:YAG+APF result similar to that of treatment with APF was propitiated by the deposition of CaF₂ causing a lower volume of mineral loss. One of the properties of Er:YAG laser is its lower diffraction in hard tissue, showing a superficial effect (1 μm) with a minimum of alterations to the sub-adjacent tissues, as it presents a wavelength of 2.94 μm, highly absorbed by the water molecules.

The results clearly show that the effect obtained was only on the surface of the irradiated enamel, without influencing the formation of sub-superficial caries lesions, contrary to what has been observed with the use of CO₂ argon, and Nd:YAG lasers, which show a reduction in the progression of caries in depth. Further investigations are necessary to study the effect of Er:YAG on the process of de-remineralization.

**CONCLUSION**

The Er:YAG laser–irradiated enamel showed lower superficial mineral loss and better results when the topical application of acidulated phosphate fluoride was employed. However, Er:YAG laser irradiation did not prevent the formation of sub-superficial caries lesions.

**REFERENCES**


Address reprint requests to:
Dr. Alberto C.B. Delbem
R. José Bonifácio, 1193
Araçatuba, SP, 16015-050, Brazil
E-mail: adelbem@foa.unesp.br