

# Combined Effect of Holmium Laser and Fluoride in Prevention of Dental Caries *in vitro*

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## Abstract

The aim of this work was to investigate the possibility of using a holmium laser for preventions of dental caries. Changes in physical properties of dental enamel were observed by measuring microhardness, analyzing the relative concentration of calcium and phosphorous atoms after acid demineralization of enamel. It was observed increase in enamel microhardness, increase in enamel fluoride uptake, and a lower lost of calcium when samples were acid exposed, indicating that holmium laser can be useful for prevention of caries.

## Introduction

Benefits of holmium laser in dentistry are mainly associated with its wavelength emission, absorbed into water with shallow depth of tissue penetration. The use of laser irradiation in prevention of dental caries was first indicated by Stern et. al.<sup>1</sup> in 1972. Several investigators proposed mechanism for this effect, in which a decreased enamel permeability and decreased solubility of enamel resulting from an alteration in composition of the mineral phase<sup>2,3,4</sup>. A positive combination between laser irradiation and treatment of enamel with fluoride, dodecylamine HCl (DAC), or ethane-1-hydroxy-1, 1-diphosphonic acid (EHDP) was measured by Fox et al.<sup>5,6</sup>, where specimens had complete dissolution inhibition when exposed for 5 min., in 0,1 M acetate buffer (pH 4.5) containing no calcium or phosphate common ions. However the extent of the effectiveness of laser irradiation in those studies is limited to a very thin surface layer (1 µm) and a partial transformation of dissolution behavior throughout a thicker zone on the order of tenths of microns.

The aim of our group is to investigate the possibility of using a holmium laser, to change physical properties of enamel, measuring changes in microhardness, that can improve resistance against enamel demineralization caused by cariogenic bacteria. This can be possible because the emission in 2 µm penetrates deeper in enamel than the radiation of those lasers most frequently related in the literature.

## Materials and Methods

Premolar teeth were sectioned longitudinally in order to separate sections of enamel. These sections were then embedded under pressure in resin. Samples were light polished to assure plane surface for irradiation, cleaned under ultrasound and divided in four groups: I – control, attacked with 0,5 M perchloric acid for 10 minutes, II - coated for 10 minutes with acidulated phosphate fluoride APF (2% NaF, 0,68 M H<sub>3</sub>PO<sub>4</sub>, pH 5.3), III - laser irradiated and IV - APF for 10 min. and laser irradiated.

X-ray Fluorescence Spectrometer (Rigaku RIX 3000, Japan) was used to measure the calcium and phosphorous contents before demineralization and irradiation. Samples were irradiated with a prototype of holmium laser developed at IPEN for biomedical applications. This is an Er:Tm:Ho:YLF<sub>4</sub> laser<sup>7</sup> emitting at  $\lambda = 2,065 \mu\text{m}$ , with 500 mJ/pulse, 250 ms of pulse width, one pulse per position with a focus diameter of 0,2 mm. The samples were automatically moved by a step motor.

All groups, except group I, were demineralized when 0.5 M perchloric acid was used for 10 minutes. In order to measure the microhardness a different group (V) was irradiated with the late conditions, and another group (VI) was used as the control. Samples were cut in parallel with the direction of irradiation, and perpendicular to teeth axis, then lapped and polished using 3  $\mu\text{m}$  alumina. Rhodamina 6G in 1% of ethanol was used to evidence irradiated areas. Hardness measurements were carried out using a HMW-2000 (Shimatsu-Japan) to obtain the Knoop Hardness Number (KHN), that is proportional to the loading mass in grams used to make an indentation, times the length of indentation. Loading time was 40 s.

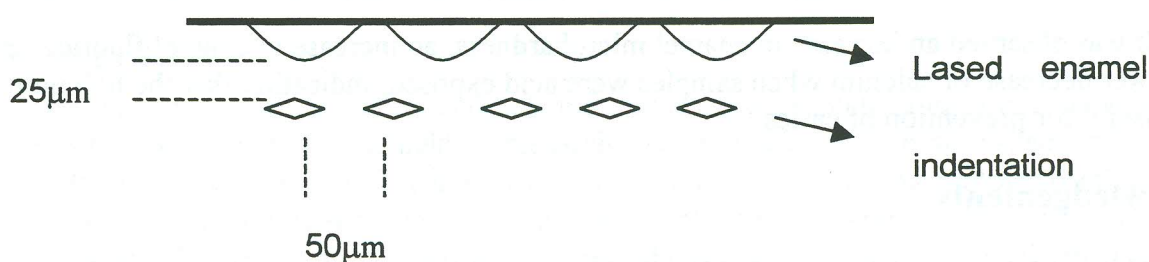


Figure 01 - Conditions of hardness measurements

## Results

Table 01 shows the relative concentrations in the four groups:

Table 01

Concentration % atoms	only HClO <sub>4</sub>	Fluoride plus HClO <sub>4</sub>	Laser plus HClO <sub>4</sub>	Laser plus fluoride plus HClO <sub>4</sub>
Ca	39.6	39.1	40.8	36.1
P	17.9	17.4	18.2	16.8
O	42.1	43.0	41.0	46.3
F	0.40	0.49	0.29	0.75
Ca	36.0	38.1	40.1	35.0
P	17.1	17.8	18.3	14.7
O	47.0	43.0	41.2	49.0
F	1.1	0.9	0.40	1.6

It was observed that there was much less demineralization in group IV than in group I, and a significant fluoride uptake in group IV. The mean value for KHN of enamel in irradiated group V was 381.7 KHN (standard deviation 66.5) and 268 KHN (standard deviation 9.79) for the control group VI. At the level of 0.05 the obtained averages are significantly different using the single factor randomized ANOVA test.

## Discussion

Enamel and dentin contain about 90% and 69% of inorganic components like carbonate hydroxyapatite. The literature shows thermal induced structural and chemical changes in accordance to temperature ranges<sup>8,9</sup>; 1) 100°C to 650°C loss of water and carbonate, rearrangement of phosphate and hydroxide ions, formation of pyrophosphate from hydrogenophosphates, and decomposition and denaturation of proteins occurs. This reduces the hydroxyapatite dissolution<sup>8</sup>; 2) between 650°C and 1100°C, recrystallization and crystal growth of  $\beta$ -Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> occurs, hydroxide decrease, and a loss of water and carbonate take place in tooth enamel; 3) above 1100°C,  $\beta$ -Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> is converted into  $\alpha$ -Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>, modifying the crystalline structure. It may actually increase the susceptibility of dental enamel to acid dissolution because the  $\beta$ -Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> phase formed at high temperatures is more soluble than hydroxyapatite<sup>8,9</sup>.

## Conclusion

It was observed an increase in enamel microhardness, an increase in enamel fluoride uptake, and a lower decrease of calcium when samples were acid exposed, indicating that the holmium laser can be useful for prevention of caries.

## Acknowledgements

This work was partially supported by CAPES and CNPq.

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