IN VITRO ANALYSIS OF THE Nd:YAG LASER EFFECT IN ENAMEL USING X-RAY FLUORESCENCE AND SCANNING ELECTRON MICROSCOPY

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This study determined the effect on acid resistance in enamel as a function of Nd:YAG laser irradiation and fuoride treatment.

Sixtheen molars were divided in four groups, each one receiving different treatment: 1) $HClO_4$; 2) acidulated phosphate fluoride (APF) + $HClO_4$; 3) Nd:YAG laser irradiation + APF + $HClO_4$; 4) Nd:YAG laser + $HClO_4$. Calcium, phosphorous and fluoride atoms concentration were analysed before and after treatment using X-ray fluorescence and specimens were observed using scanning electron microscopy. Morphological changes occured in Nd:YAG irradiated enamel with production of melted and solidified material which were not observed on the other specimens. Results show increase on acid resistance of the dental enamel when treated with Nd:YAG laser + APF.

INTRODUCTION

In a world where new diseases appear day after day, man still fights against diseases like dental tooth decay that is an infectious disease; multifactorial and of an enormous incidence in the human race.

With the advent and development of new techniques, Preventive Dentistry is being seen in a new light, opening the way for new methods that will come not just to increment the action of fluoride, but also to act on the resistance of tooth enamel. Consequently, the laser has come to represent a promising alternative in respect to Preventive Dentistry. Having a thermic effect, some high-powered lasers could promote fusion and recrystalization of the enamel, causing alterations in its permeability and solubility. It can act at the beginning of the tooth enamel demineralization contributing to the prevention of the tooth decay process as well. To really confirm the efficiency of these methods, it becomes necessary to carry out a considerable amount of research based on qualitative and quantitative analyses of samples treated firstly "in vitro" so they can later be analysed "in vivo". This work analysed the results obtained "in vitro" with the application of an Nd:YAG laser, with and without, the application of APF (acidulated phosphate fluoride) through a quantitative analysis of the elements calcium, phosphorous and fluoride, correlating them with the prevention of tooth decay.

DISCUSSION

The Nd:YAG laser emitting a wavelength of 1,064 μm (10 to 100 Hz, up to 320 mJ, 100 μs - model Pulse Master 1000 American Dental Technology Ca USA) was used with an energy of 80mJ, 25 Hz 2,5 W and energy density of 99,5J/cm². Glazing the surface of the specimen with a initiator (indian ink) increased its absorption and the effect of laser. Using a 300 μm diameter fiber, 16 enamel specimens were subjected to four different conditions : 1) four specimens were coated with acidulated phosphate fluoride (2% NaF; 0,68 M H_3PO_4; pH 5,3) for 10 minutes, after laser irradiation; 2) laser irradiation; 3) coated with fluoride for 10 minutes; 4) subjection to an attack of 0,5 M perchloric acid for 10 minutes.

Consequently the samples were attacked with the perchloric acid for 10 minutes (except the ones from group 2 which had been previously treated) and the concentrations of Ca , P and F atoms were analysed to determine the level of demineralisation caused. All the specimens were analysed by X-ray fluorescence technique prior to and after been treatment. They were also observed using scanning electron microscopy.

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Various authors such as Kumasaki, Sato, Yamamoto, Tagomori, Morioka, have been studying the effect of laser irradiation in the field of tooth decay prevention, with and without the application of fluoride, after neodimium:YAG laser irradiation. The inhibition of demineralisation in enamel, promoted by some lasers, is believed to be caused by the forming of a surface more resistant to tooth decay lesions (Featherstone et al., 1995) through the modifications in the crystalline structure. The results indicate a bigger absorption of the fluoride by the enamel surface, and the consequent alteration of the sub-surface; enabling an even bigger contribution to the preventive effect of it. Yamamoto and Sato, in 1980, demonstrated that the enamel surface exposed to Nd:YAG laser, after the application of $Ag(NH_3)_2F$, increases the absorption of fluoride in the enamel and reduces the sub-surface demineralisation. Fowler and Kuroda suggested, in 1986, that the pyrophosphate formation on the irradiated enamel could have had an effect in the reduction of enamel solubility levels. A very positive result was obtained with the experiment in dental enamel, performed by Tagomori and Morioka, in 1989, where the acid resistance of enamel was evaluated when exposed to Nd:YAG laser with an energy density varying between 0 and 100 J/cm^2; 20 Hz per 0,5 seconds. The quantity of calcium dissolved in the acid solution was determined with atomic absorption spectrophotometry. There was a high elevation of fluoride incorporated, little elevation of concentration of calcium atoms and an inclination of phosphate concentration. Morioka (in 1990) proposed a possible method for enamel to acquire acid resistance after laser irradiation. An argon laser (continuous emission, wavelength between 457.9 nm and 514.5 nm, 67 J/cm^2) was utilised in the studies in which the enamel specimens irradiated were immersed in different solutions with the intention of evaluating the enamel permeability and birefringence after the irradiation. Among them stands out the APF (acidulated phosphate fluoride) in which the enamel irradiated permeability to the fluoride were higher than the one not irradiated, enabling it to be attributed to the microspaces formed by irradiation. Those areas could act as places for the deposit of liberated ions through an acid attack. This hypothesis could explain the dental enamel acid resistance after laser irradiation.

RESULTS

The four techniques provide different calcium concentrations after the treatment. The Nd:YAG laser irradiation and posterior application of APF and perchloric acid maintained a higher quantity of calcium on the surface of the specimens when compared with the treatment with APF and perchloric acid; irradiation with Nd:YAG laser and perchloric acid; and attacked with perchloric acid alone, in decreasing order respectively (Table II).

Table I - [%] calcium levels before treatment

Group I	Group II	Group III	Group IV
25,200	24,899	24,399	24,700
24,700	25,000	24,399	23,100
24,600	24,399	23,700	24,600
24,899	25,399	24,700	24,799

Table II - [%] calcium levels after specimen treatment

Group I $HClO_4$	Group II APF+$HClO_4$	Group III L+APF+$HClO_4$	Group IV L+$HClO_4$
25.399	27.200	27.799	25.200
24,500	27,299	28,600	25,000
23,600	25,600	28,799	24,799
24,299	26,500	29,200	24,799

After treatment, the Nd:YAG laser irradiation technique and posterior application of acidulated phosphate fluoride and perchloric acid promoted less concentration of phosphorous (Table IV)

Table III-[%] phosphorous levels prior to treatment

Group I	Group II	Group III	Group IV
65,300	65,900	65,599	64,599
65,099	65,300	65,400	65,800
65,199	65,400	65,500	65,500
65,500	65,300	65,500	65,000

Table IV- [%] phosphorous levels after specimen treatment

Group I $HClO_4$	Group II $APF+HClO_4$	Group III $L+APF+HClO_4$	Group IV $L+HClO_4$
65,300	61,900	59,200	65,099
64,300	65,300	58,299	66,400
66,099	65,400	57,099	65,900
65,699	65,300	58,200	64,500

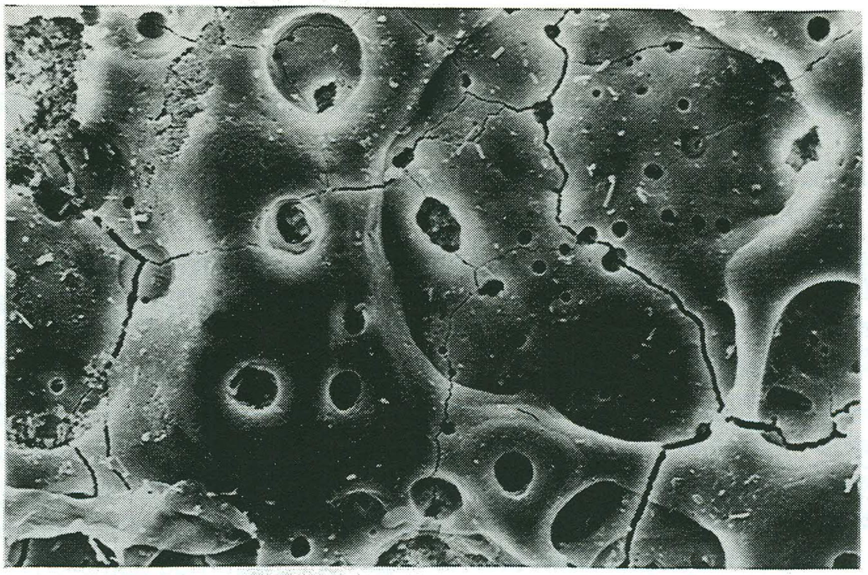
X-ray fluorescence analysis did not reveal significant signs of fluoride ($\alpha < 0,05$) in the specimens neither prior to, nor after treatment, even in those that were not subjected to applications of APF. Consequently, concentrations of fluoride were detected in the specimens that received just acidulated phosphate fluoride and perchloric acid applications, and for the ones which received Nd:YAG laser irradiation, posterior acidulated phosphate fluoride application and perchloric acid application (Table V)

Table V– [%] of fluoride of the 2 specimens treated with APF

Group II ($APF + HClO_4$)	Group III (laser $+APF+HClO_4$)
3,3	4,0
4,1	3,4
3,6	2,1
3,1	3,6

In spite of data not being taken into consideration in this essay, it was also noted that a quantitative analysis of the oxygen and the figures conferred the remaining percentage of the above table.

As to the morphologic aspect, it was observed in scanning electron microscopy that the enamel became fused and solidified, resembling a mosaic. A large number of craters of diverse depth (arrows) could be observed, leaving a very irregular surface.(Picture 1)



Picture 1: Melted and recrystalised enamel. Craters with different depths (1000X)

CONCLUSIONS

The results obtained by X-ray fluorescence analysis showed there was a reduction in enamel surface demineralisation of the irradiated specimens with the Nd:YAG + APF laser (10 min.), under the conditions, when submitted to an acid environment ($HClO_4$- 0,5 M) for 10 minutes; making the enamel more resistant. There was no significant difference noted, with the methodology used, in the absorption of fluoride when it was applied to the enamel in isolation, or with the association of the Nd:YAG laser. The by scanning electron microscopy showed areas of fusion and enamel recrystalization when irradiated with Nd:YAG laser.

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