# Acid Resistance of Enamel Treated with Nd:YAG Laser Associated with Fluoride and Exposed to a *S. MUTANS* Culture Media

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## SUMARY

This study determined the effect on demineralization resistance in enamel using x-ray fluorescence and SEM as a function of pulsed Nd:YAG laser irradiation, fLuoride treatment and the S.mutans culture media. In the first part of experiments sixteen third molars were divided in four groups, each one receiving different treatment: 1) perchloric acid ($HClO_4$); 2) acidulated phosphate fluoride (APF) + $HClO_4$; 3) Nd:YAG laser irradiation (80 mJ, 25 Hz, 99.5 J/cm$^2$) + APF + $HClO_4$; 4) Nd:YAG laser (80 mJ, 25 Hz, 99.5 J/cm$^2$)+ $HClO_4$. Calcium, phosphorous and fluoride concentration were analysed before (control) and after the treatment using X-ray fluorescence.

In the second phase of the experiment forty two teeth, third molars and premolars, were divided in two groups that remained in the S. mutans culture media for 15 days and 21 days, respectively. Teeth were divided in other three groups, in a total of six groups, that received pulsed Nd:YAG laser irradiation at these parameters: I) 67 mJ;15 Hz; 83.75 J/cm$^2$; II) 133 mJ; 15 Hz; 166.25 J/cm$^2$ and III) 150 mJ; 20 Hz; 187.50 J/cm$^2$. The morphological changes were observed using SEM.

**Melting and recristalization of the enamel surface were observed by SEM from all laser treatment. X-ray fluorescence analysis demonstrated increase in acid resistance of the enamel surface when treated with Nd:YAG laser + APF. No cavitation and parcial melting were observed from all the Nd:YAG laser irradiated surfaces submeted to S. mutans culture media which can be related to a decrease in the acid solubility of the enamel.**

## INTRODUCTION

With the advent and development of new techniques, Preventive Dentistry is being seen as 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 demineralization. Consequently, the laser has come to represent a promising alternative concerning Preventive Dentistry. Because of their 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 early 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 first "in vitro" so they can later be analysed "in vivo". The first part of this work analysed the results obtained "in vitro" with the application of Nd:YAG laser, with and without the application of APF (acidulated phosphate fluoride) through a quantitative analysis of the elements calcium, phosphorous and fluoride. The second part of this study determined the effect on acid resistance in enamel as a function of Nd:YAG laser irradiation and the action of acid produced by Streptococcus mutans for 15 and 21 days on the enamel surface.

## MATERIALS AND METHODS

The Nd:YAG laser emitting a wavelength of 1.064 μm (10 to 100 Hz, up to 320 mJ, 100 μs - Pulse Master 1000 American Dental Technology-USA) was used with an energy of 80 mJ, 25 Hz 2.5 W and energy density of 99.5 J/cm$^2$. Glazing the surface of the specimen with a initiator (nankin) increased its absorption and the effect of laser. Using a 300 mm fibre, 16 enamel specimens included in acrilic resin were subjected to four different conditions: 1) four specimens were coated with acidulated phosphate fluoride APF (2% NaF; 0.68 M $H_3PO_4$; pH 5.3) for 10 minutes, after laser irradiation; 2) laser irradiation; 3) coated with APF 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 4 which had been previously treated) and the concentrations of Ca (calcium), P (phosphorous) and F (fluoride) were analysed to determine the level of demineralisation caused. All the specimens were analysed by X-ray fluorescence technique prior to and after the treatment.

The exposure time to the fluoride and the acid were determined in a pilot study based on the sensitivity level of the evaluation equipment used.

A second part of this study was carried on in order to complement and assure the Nd:YAG laser effects concerning the prevention of dental tooth decay. At this time a 0.32 mm fiber optic of a Nd:YAG laser-d lase 300-American Dental Laser-USA was used with three different parameters as follows: I)1.0W; 15 Hz; 67 mJ; 83.75 J/cm²; II) 2.0 W; 15 Hz; 133 mJ; 166.25 J/cm²; III) 3.0 W; 20 Hz; 150 mJ; 187.5 J/cm². They were observed using scanning electron microscopy(Phillips LX 30, Eindhover, Ho).

## DISCUSSION

Various authors such as Yamamoto & Ooya[10], 1974; Yamamoto & Sato11,1980; Morioka & Tagomori 5, 1988; Tagomori & Morioka[9], 1989; Kumasaki et al.[4], 1993, have been studying the effect of laser irradiation in the field of tooth decay prevention, with and without the application of fluoride, after 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 through the modifications in the crystalline structure (Sognnaes & Stern[8], 1965; Yamamoto & Ooya[10], 1974; Shirazuka et al.[7], 1990; Arcoria et al.[1], 1993; Featherstone et al.[2], 1995). 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 & Ooya[10], in 1974, analysed the effect of the Nd:YAG laser on the enamel permeability when exposed to a culture media, and they observed that when they used an energy density of 20 J/cm², demineralization was not found on the enamel surfaces, in contrast to the control surfaces that showed enamel demineralization.

Yamamoto and Sato11, 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[3] 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[9], 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/cm²; 20 Hz per 0.5 seconds. The amount of calcium dissolved in the acid solution was determined with atomic absorption spectrophotometry. There was a high increase of fluoride incorporated, little calcium increase and decrease of phosphate concentration.

Oho & Morioka[6] (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/cm²) was used 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 provided different calcium concentrations after the treatment. The Nd:YAG laser irradiation and posterior application of APF and perchloric acid maintained a higher amount 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+$HClO_4$ |
|---|---|---|---|
| 25.200 | 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 |

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 + HClO4) | Group III (laser +APF+HClO4) |
|---|---|
| 3.3 | 4.0 |
| 4.1 | 3.4 |
| 3.6 | 2.1 |
| 3.1 | 3.6 |

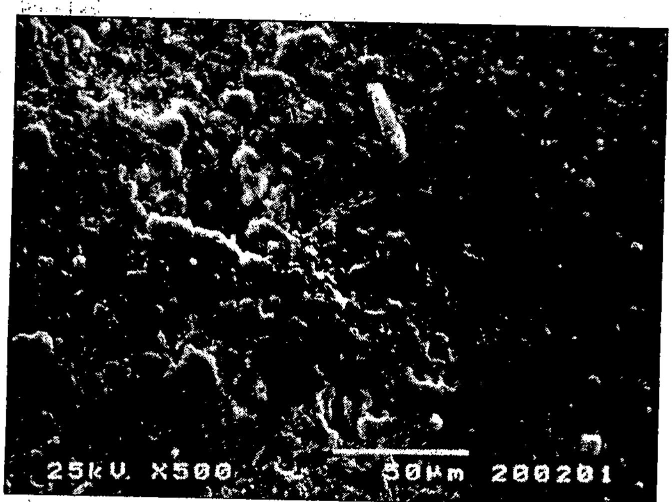
In spite of being data not taken into consideration in this essay, it was also noted that a quantitative analysis of the oxygen and the figures conferred the remaining percentage of the above charts.

As to the morphologic aspect, it was observed in scanning electron microscopy that the enamel became fused and recrystalized, resembling a mosaic. A large number of craters of diverse depth (arrows) could be observed, leaving a very irregular surface. In the second phase of this study we coud observe through the SEM analyses the enamel irradiated with Nd:YAG laser prior to remaining in the S. mutans culture media for 15 days showed reduction of those structures formed with the laser irradiation, thus not showing regular shapes of these referred structures. Besides, these surfaces have not shown demineralization in comparison to the control surfaces what showed cavitation.

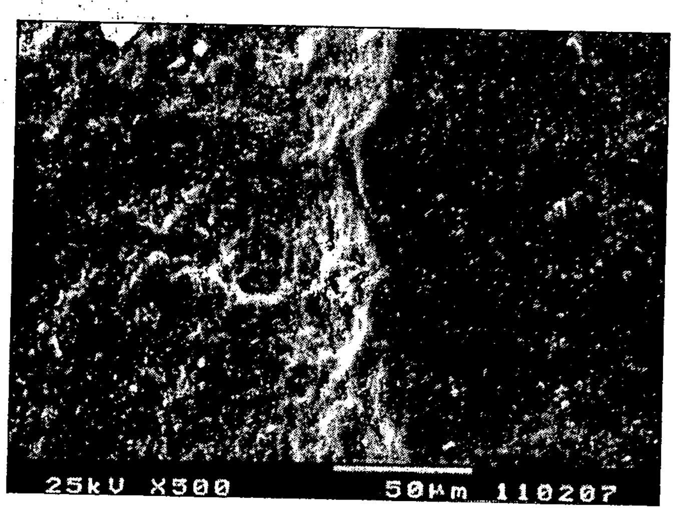
The same ocurred with the enamel surfaces irradiated with the Nd:YAG laser and after remained in the S. mutans culture media for 21 days. These structures formed by the laser irradiation reduced and no demineralization was seen. On the other hand, the control surfaces showed cavitation.

## CONCLUSIONS

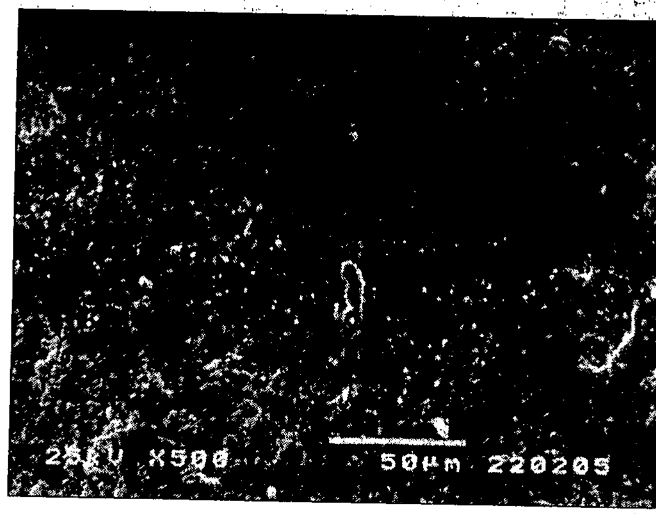
The results obtained by X-ray fluorescence analysis showed that there was a reduction in enamel surface demineralisation of the irradiated specimens with the Nd:YAG + APF laser (10 min.), under the conditions, when submitted to an acid environment ($HClO_4$– 0.5 M) for 10 minutes; making the enamel more 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 scanning electron microscopy showed areas of fusion and enamel recrystalization when irradiated with Nd:YAG laser. In the enamel surface treated with Nd:YAG laser we could observe by SEM an increase of the acid resistance, not showing demineralization of these surfaces, but in the control group demineralization was noted on all enamel specimens.




Picture I:166,25 J/cm². *S.mutans* culture media-15 days.




Picture II: no laser irradiation. S.mutans culture media-21 days.




Picture III:166,25 J/cm² *S.mutans* culture media-21days.

Picture IV: no laser irradiation. *S.mutans* culture media-21days

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