Evaluation of the anti-caries effect beyond the critical enamel pH of preventive treatment of fluoride associated with Nd:YAG laser irradiation

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***Abstract*— This study aimed to evaluate the anti-caries effect of fluoride associated with Nd:YAG laser irradiation in the treatment of enamel Eight groups (n = 5) were analyzed: Negative Control pH 4.5; Negative control pH 4; Fluoride pH 4.5; Fluoride pH 4; Nd-YAG pH 4.5; Nd-YAG pH 4; (Fluoride + Nd-YAG) pH 4.5 and (Fluoride + Nd-YAG) pH 4. All samples were analyzed by Scanning Electron Microscopy (SEM) before and after the cycle. Quantification of phosphorus in the cycling solutions was carried out using the colorimetric method, as an indication of enamel demineralization. The anti-caries effect of the Fluoride + Nd:YAG treatment in addition to the critical pH of the enamel can be observed in the morphological analysis, however in the analysis of the demineralization solutions, the amount of phosphorus showed a difference only in the Fluoride group compared to the others in the investigative pH.**

Keywords*— *laser, caries, prevention, Nd:YAG, fluoride, enamel

I INTRODUCTION

Dental caries is the result of an imbalance between the concentrations of dental ions and saliva that occur due to varied factors that influence bacterial metabolism and a cariogenic diet [1]. This imbalance is determined by a borderline value denominated critical pH, that is, when the oral environment is more acidic than this value, there will be demineralization of the tooth [2]. The critical pH of human dental enamel is presented as 5 5 [3]

The use of fluoride by topical means has been a great ally in the prevention of dental caries because it allows the formation of new elements on the enamel surface, such as: fluorapatite, calcium fluoride and fluoridated apatite. These elements are more acid resistant than the original hydroxyapatite of the tooth, thus altering the borderline pH The critical pH of enamel in the presence of fluorine is 4 5 [4]

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High intensity infra-red lasers have been used in prevention, such as Nd:YAG laser (neodymium doped with yttrium aluminum-garnet), and causes an increase acid resistance of dental tissues through the alteration of crystallinity, solubility and permeability of the structure. The light emitted in the tissues is absorbed and converted into heat, promoting a melting and resolidification of the surface, reducing the carbonate concentration and increasing the proportion of the mineral content due to water evaporation [5, 6, 7, 8, 9, 10]. The irradiation with high intensity lasers has the possibility of being associated with fluoridated products and allows a maximization of the fluoride effect due to the increase in the contact area [11, 12, 13, 14, 15, 16]

The objective of this research is to investigate whether the acid resistance achieved through the synergistic treatment of topical application of fluoride and Nd:YAG laser irradiation can promote an anti-caries effect in addition to the critical pH of enamel treated with fluoride.

II MATERIALS AND METHODS

A. Experimental design

40 human enamel dental slabs were prepared and were homogenized by Knoop hardness number. The microhardness surface (SMH) was determined on a microhardness tester (Shimadzu, HMZ-2000, Japan) producing 5 indentations on the enamel surface using a Knoop Diamond under 25-g load for 5 sec and the average of SMH were calculated Samples with SMH between 360 and 420 Knoop hardness were accepted. The slabs were randomly assigned to eight groups (n = 5):

G1: Negative Control pH 4.5;
G2: Negative Control pH 4;
G3: Fluoride pH 4.5;
G4: Fluoride pH 4;
G5: Nd:YAG pH 4.5;
G6: Nd:YAG pH 4;
G7: Fluoride + Nd:YAG pH 4.5;
G8: Fluoride + Nd:YAG pH 4.

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The variation between groups was made according to the proposed treatment and also with the pH used in pH cycling to simulate the critical pH of enamel in the presence of fluoride (pH 4.5) and an investigative pH (pH 4).

To ensure assuredness, all solutions had their pH measured after cycling, were tabulated and a statistical analysis was performed

The enamel morphological analysis was performed using scanning electron microscopy (SEM) and the solutions from pH cycling were analyzed for phosphorus quantification by the colorimetric method as an indication of demineralization. The statistical analysis was performed considering the level of significance of 5%

B. Treatments

The topical application professional with acidulated fluoride phosphate gel at a concentration of 12,300 µg F- / g (Biodinamica, Brazil) were realized with serial pipette (1 mL for slabs) and kept for 4 minutes.

For irradiation, it were used a Nd:YAG laser (Power Laser TM ST6, Lares Research, CA, USA), λ = 1.064 nm, 100 µs, 10 Hz, 0.6 W, 60 mJ, 84.9 J/cm^2. The irradiation laser were realized in contact mode with a high-precision motorized translator (ESP300, Newport Corporation) and the energy per pulse were measured by an energy/power meter (FieldMaster, Coherent, Santa Clara, CA, USA).

The pH cycling simulating the process of demineralization and continuous remineralization in the oral environment. The demineralization and remineralization solutions were used as artificial saliva It was performed with a demineralization solution for 4 hours and a remineralization solution for 20 hours each day, totaling 8 days of the cycle. The protocol used was the one proposed by Ortiz, Tenuta, Tabchoury & Cury (2016) but the pH was adapted for the purpose of this study using HCl for pH control (Dual Star, Thermo Scientific Orion, USA) The composition of the demineralization solution was: 0.03 µg F / mL, 0.05 mol / L acetate buffer, 0 74 mmol / L P and 1 28 mmol / L Ca. And the remineralizing solution was composed of: 0 1 mol / L of TRIS buffer, 0 05 µg F / mL, 0.9 mmol / L P, 1.5 mmol / L Ca and 150 mmol / L KCl [17]

C. Scanning Electron Microscopy (SEM)

All slabs (n=40) underwent morphological analysis by Scanning Electron Microscope (SEM; TM 3000 Tabletop Microscope Hitachi, Japan) at 15 kV The images were collected in the same slabs, before and after cycling at 4,000x in original magnification

D. Phophorus quantification (Colorimetric Method)

Phosphorus quantification were performed in all demineralization solutions used in cycling using colorimetric method proposed by Fiske & Subbarow [18]. The DU 800 spectrophotometer (Beckman Coulter, CA, USA) was used to obtain the absorbency of the solutions.

E. Statistical Analysis

The pH data were obtained through an electrode suitable for this type of analysis, the original data were grouped in days and according to the pH where the treatment was carried out, then an analysis of normality of the data was made, according to the D 'Agostino & Pearson Test, with a 95% confidence level, the samples have abnormality profile. Then, based on this information, an ANOVA test was applied, analysis of variance, which allows investigating the differences and similarities between samples, this test used the same software. The two different pH groups were compared with each other and with 95% confidence they are considered different from each other.

III RESULTS AND DISCUSSION

A. Scanning Electron Microscopy (SEM)

Before treatment and cycling (Fig 1) the images demonstrated the enamel integrity, being possible to verify a regular looking surface with small scratches resulting from the polishing of the samples and were possible to observe enamel prisms.

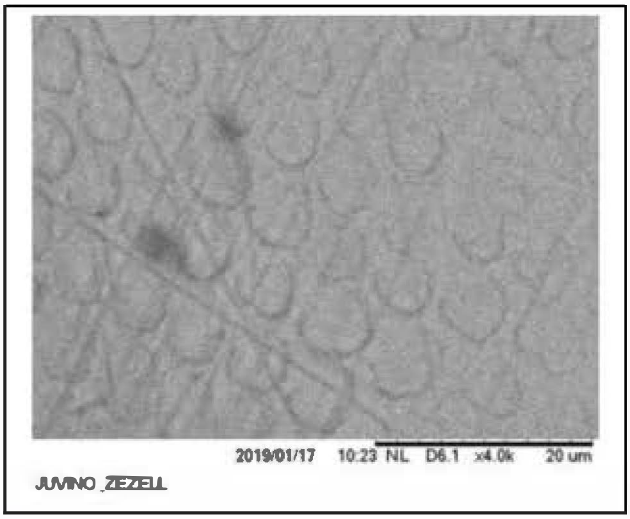

Fig.1.Electron micrographs of enamel untreated. Original magnification = 4.000x

Representative images (Fig 2) show the morphology of enamel after pH cycling 4.5. The Group Negative Control pH 4 5 (Fig 2A) has smoky enamel suggestive of demineraliation, loss of the central portion of the prisms and microcavitations. The Group Fluoride pH 4 5 shows a lower loss of structure than the Group Negative Control, with only the loss of the central portion of some prisms (Fig 2B) Group Nd:YAG pH 4.5 shows loss of conformation of the enamel prisms (Fig 2C) Fig 2D shows the Fluoride + Nd:YAG Group, presenting an integral enamel, however with alterations suggestive of coming from the alteration of the enamel crystallinity.

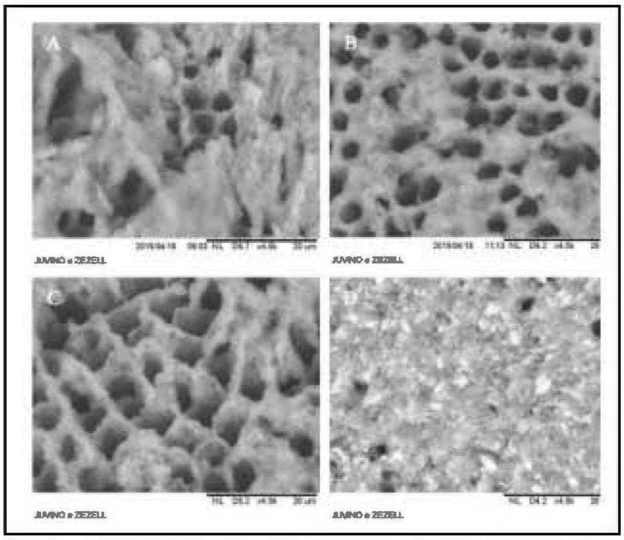

Fig. 2. Electron micrographs of groups after the pH cycling 4.5. (A) Negative Control. (B) Fluoride. (C) Nd:YAG. (D) Fluoride + Nd:YAG.

Before pH cycling pH 4, representative images of groups were tabulated (Fig 3) The images Fig 3A B C demonstrate that the Negative Control, Fluoride and Nd:YAG groups had enamel microcavitations The group Fluoride + Nd:YAG (Fig. 3D) showed light demineralization in the form of a small smudge on the surface, demonstrating that it started the loss of ions but the integrity of the surface suggests greater acid resistance compared to the other groups.

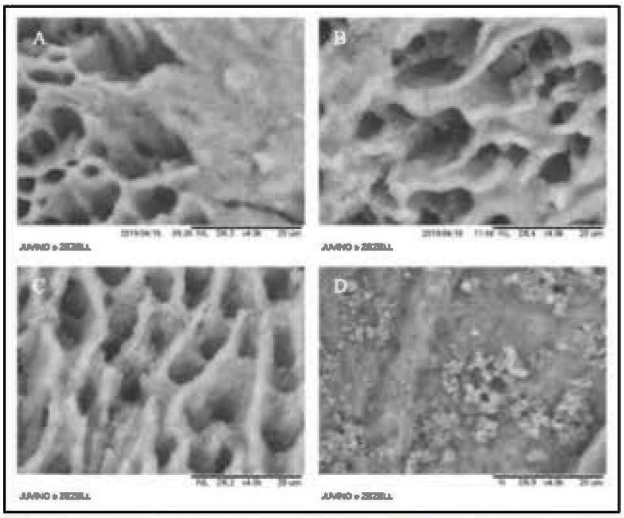

Fig 3 Electron micrographs of groups after the pH cycling 4 (A) Negative Control. (B) Fluoride. (C) Nd:YAG. (D) Fluoride + Nd:YAG

B. Phosphorus quantification (Colorimetric Method)

After the construction of the curve (R^2 = 0.9843) the sample data were quantified, and the final values expressed in mol/L. The data were according to the pH of pH cycling

In the static analysis, a normality test was performed, and the samples do not show a normal distribution profile according to D'Agostino & Pearson Test. Thus, the t-test were used

When analyzing the sample set referring to pH = 4.5 (Fig 4), most comparisons in pairs result with a significant difference between both analyzed, with the exception of comparisons between Negative Control and Nd:YAG and between groups Nd:YAG and Fluoride + Nd:YAG 4. And finally, when analyzing the set of pH = 4 0 (Fig 5), only the double comparisons involving the Group Fluoride were found to be significantly different

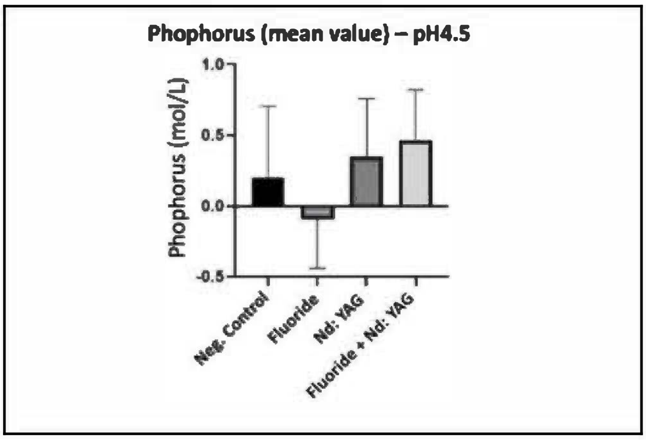

Fig 4 Mean of the amount of phosphate in the solutions after 8 days of pH cycling (pH = 4.5).

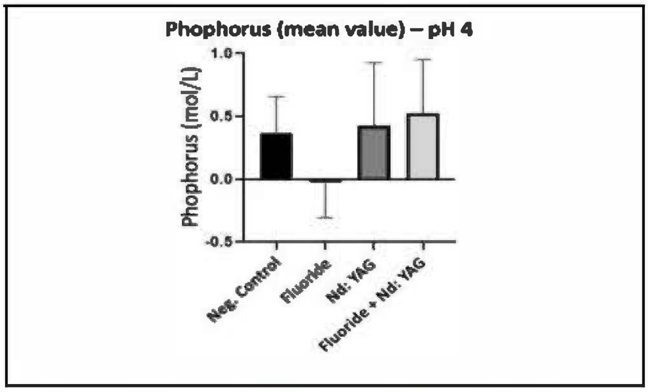

Fig 5 Mean of the amount of phosphate in the solutions after 8 days of pH cycling (pH = 4).

IV CONCLUSIONS

The anti-caries effect of the Fluoride + Nd:YAG treatment in addition to the critical pH of the enamel can be observed in the morphological analysis, however in the analysis of the demineralization solutions, the amount of phosphorus showed a difference only in the Fluoride group compared to the others in the investigative pH Further analysis must be carried out to confirm the hypothesis.

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