ISSN: 2230-9926

*Available online at http://www.journalijdr.com*

IJDR

International Journal of DEVELOPMENT RESEARCH

*International Journal of Development Research*
*Vol. 07, Issue, 06, pp.13246-13251, June, 2017*

## *Full Length Research Article*

# PRECLINICAL STUDY SHOWED THEMAIN PREDICTORS OF ENAMEL EROSION AND DEMINERALIZATION AND TREATMENT ANALYSIS

**[1*]Juliana dos Reis Derceli, [2]Juliana Jendiroba Faraoni, [3]Idiberto José Zotarelli Filho and [2]Regina Guenka Palma-Dibb**

[1]University Center of the Educational Foundation of Barretos/ SP/Brazil- UNIFEB
[2]Department of Restorative Dentistry, School of Dentistry of RibeirãoPreto, University of São Paulo-FORP/USP, RibeirãoPreto, São Paulo, Brazil
[3]São Paulo State University (Unesp), Institute of Biosciences,Humanities and Exact Sciences (Ibilce), Campus São José do Rio Preto

**ARTICLE INFO**

***Article History:***
Received 14th March, 2017
Received in revised form
09th April, 2017
Accepted 27th May, 2017
Published online 30th June, 2017

***Key Words:***
Acidualated Phosphate Fluoride,
Lasers,
Tooth Erosion,
Tooth Wear.

**ABSTRACT**

The aim of this study evaluated the fluoride retention, morphologic changes on enamel surface and the resistance to demineralization after Er: YAG laser and fluoride gel pretreatments. Ninety-five bovine enamel specimens were obtained, half of the enamel surface were coated with nail varnish and wax (reference area), leaving other half to pre-treatments and to erosive challenge. The specimens were randomly divided into the following 5 groups: L laser irradiation; L+F laser and posterior application of fluoride (APF gel); F+L fluoride and posterior laser irradiation; L/F laser/fluoride application simultaneously; F APF gel (control group). The laser was irradiated for 10s (60mJ/2Hz), APF gel was applied for 4 min, and was exposed to Coca-Cola® for 1 min, four times/day/5 days. Fluoride retention was performed using EDS, morphological changes by SEM and enamel demineralization by micro hardness. SEM and EDS were performed for descriptive analysis of the data and micro hardness was performed for Dunn test/ANOVA one criteria. L/F showed higher retention of fluoride (174.0 %) followed by the F(27.0 %) and F+L (10.0 %). L(39.0 %) and L+F(7.5%) showed a decrease in the fluoride content , there was decrease Ca and P content about 0.2 to 8.0 % and 6 to 14.0 %, respectively, without cause alteration on Ca/P proportion. All the irradiated groups were ablated. Micro hardness was not significantly different between groups. L/F promoted higher retention of fluoride in the enamel and any pretreatment caused alteration on Ca/P proportion, Er: YAG laser promoted superficial enamel microablation under low fluency and the pretreatments was not able cause micro hardness alteration.



## INTRODUCTION

Dental erosion is the localised chronic and irreversible loss of pathological mineral tissue that is chemically removed from the tooth surface by acid or chelating substances without the involvement of bacteria (Alves *et al*., 2004). It may have an intrinsic cause, such as the hydrochloric acid present in gastric juice, or an extrinsic cause related to ingestion of acidic foods, drinks, drugs and medicine (Bartlett *et al*., 2001). An acidic diet is reported as a potent cause of dental erosionand can be considered the main cause of this lesion (Zero, 1996). In today's society, drinks with a low pH are being consumed more frequently, which can cause the demineralisation of the dental substrate and the loss of structure (Luo *et al*., 2005).

****Corresponding author: Juliana dos Reis Derceli,***
University Center of the Educational Foundation of Barretos/ SP/Brazil- UNIFEB.

Thus, the high consumption of soft drinks has caused concern about the possibility of erosion anddamage to dental structures. The daily consumption of soft drinks leads to a four timeincrease in the risk of dental erosion (Järvinen *et al*., 1972) , i.e., approximately 1.0µm of dental structure is lost per day due to excessive consumption (Sognnaes *et al*., 1972). The low pH of Coca-Cola (pH 2.29), one of the most commonly consumed soft drinks, is one of several factors that contributes to erosive lesion formation. The critical pH of enamel is approximately 5.5; therefore, any solution with a lower pH can initiate the erosion process, especially with prolonged or repeated exposure to acid (Johansson *et al*., 2004). Methods forpreventing dental erosion have been studied. The topical application of fluoride is a traditional treatment for preventingdemineralisation of the dental substrate erosion (Ganss *et al*., 204) , and increasing the resistance of the tooth in relation to acidic substances, especially when used at high

concentrations (Rios *et al*., 2009) and daily (Stenhagen *et al*., 2013). The main mechanism of fluoride is its ability to interfere in the dental demineralisation and remineralisation dynamic process during pH decreasesby interacting with demineralised hydroxyapatite to form of a new compound, fluorapatite (Ganss *et al*., 2004). The use of laser irradiation as an auxiliary preventive method or as an enhancer of topical fluoride application has been studied,in general, laser irradiation has been shown to reduce the critical pH of dental enamel dissolution (Fox *et al*., 1992) and has more satisfactory results when combined with fluoride (Bevilácqua *et al*., 2008).Recent studies suggest that laser and fluoride association decrease dental demineralization (Derceli *et al*., 2015; Altinok *et al*., 2011; Fornaini *et al*., 2014) and provide greater retention of fluoride through chemical and morphological changes in the enamel (Nammour *et al*., 2003) , for both laser irradiation must be absorbed and converted into heat without causing thermal damage to adjacent tissues (Featherstone, 1987 ). Therefore, the aim of this study was to evaluate the fluoride retention,morphologic changes on enamel surface and, investigate the preventive treatments, which could improve enamel demineralization.

## METHODOLOGY

### Experimental design

In this study,it was examined the effects of Er:YAG laser irradiation with or without fluoride. Ninety-five enamel specimens were obtained from bovine incisive teethand randomly assigned into the 5 groups: L, laser irradiation; L+F, laser and posterior application of fluoride; F+L, fluoride and posterior laser irradiation; L/F, fluoride/laser application simultaneously; and F(control group), topical fluoride application. The fluoride retention and morphologic changes on dental enamel were performed using EDS (X-Ray Energy-Dispersive Spectroscopy) and SEM (Scanning Electron Microscopy) and, enamel demineralization was performed by knoopmicrohardness test.

### Specimen Preparation

The bovineteeth were sectioned at the cement enamel junction. The crowns were bisected longitudinally, and the labial and lingual fragments were cut apart using a water-cooled diamond saw in a sectioning machine (Minitom, Struers A/S, Copenhagen, DK-2610, Denmark). The lingual fragment was discarded, resulting in enamel blocks of labial specimens (4x4mm), which were delineated and polished with water sandpaper (#600 and #1200), a felt disc and alumina suspension at 0.30 and 0.05 µm. After polishing, the specimens were sectioned through initial microhardness (Knoop, 25g/F and 10 seconds). All specimens with means above and below than 20.0 % were discarded and with standard deviation higher than 10.0 %. Ninety-nine specimens weresectioned and coated with nail varnish and wax (reference area) leaving half of the enamel surface without protection (8.0 $mm^2$) for the applicationof preventive treatments and erosive challenge. Afterwards, it was randomly divided into 5 groups according to the preventive treatments performed.

### Preventive Treatments

The experimental groups, L, L+F, F+L and L/F, were subjected to the Er:YAG laser treatment (Kavo Dental GmbH & Co. KG, Biberach, Germany) with a wave length emission of 2.94 µm without using air/water cooling. The parameter settings used with a wavelength emission of 2.94 µm and without using air/water cooling, 60.0mJ and 2.0 Hz for all specimens; the pulse duration was 250.0µs, and the total irradiation duration of 10.0 s with energy density was 3.92 $Jcm^{-2}$. A 2051 handpiece with a removable tip attached to a flexible fibre delivery system was used.The laser beam was delivered on a non-contact, pre-focus mode and the distance of the laser emission point from the target was 4.0 mm(13). The terms focused, unfocused, and prefocused refer to the focus point position in relation to the irradiated tissue plane.When we work over the target tissue, the laser can be used with the focal point positioned on the tissue surface (focused), positioned far from the tissue surface (unfocused or out of focus), or with the focal point below the surface of the enamel or in the tissue (prefocused).

The irradiation distance was standardised by using a custom-designed apparatus comprising two parts: a holder to fix the laser headpiece so that the laser beam was delivered perpendicular to the specimen surface at a constant working distance from the target site and a semi-adjustable base, to which the plexiglass plate with the fragment was firmly attached with wax. Two operators manipulated the apparatus' micrometer screws, so that the semi-adjustable base was moved by alternating right-to-left and forward-to-back directions, thus allowing the laser beam to act on the entire specimen.

The irradiation distance was checked with a ruler in every specimen. For groups L+F, F+L, L/F and F, an acidulated 1.23% phosphate fluoride (Sultan Topex, DFL Indústria e Comércio Ltda, Brazil) was applied on the enamel surface with a microbrush for 4 minutes and later removed with gauze. Group F (control group), received only fluoride and group L received only Er:YAG laser irradiation. Group L/F the fluoride was applied on the enamel surface, after 1 minute the Er:YAG laser was irradiate for 10 seconds. Then,the fluoride was kept on the surface until completion in 4 minutes (Derceli *et al*., 2015). To induce an erosive challenge, the specimens were immersed in Coca-Cola® four times a day for 1 min, under agitation, for a period of 5 days. Between these intervals, the specimens were washed in deionized water and stored in artificial saliva, which were renewed daily.

### Scanning Electron Microscopy (SEM) and X-Ray Energy-Dispersive Spectroscopy (EDS)

Twenty-five specimens (n=5) were used for the SEM and EDS analyses, which were subjected to preventive treatments but were not part of the erosion challenge. After the treatments, the specimens were washed and the nail varnish andwax were carefully removed, exposing the reference area.For cleaning, the specimens were placed in an ultrasonic cleaner (T1440D, Odontobrás Ltda., RibeirãoPreto, SP, Brazil) with distilled water for 10 min, and gauze and EDTA were used. The specimens were dehydrated with ascending concentrations of ethanol (25.0 % for 20 min, 50.0 % for 20 min, 75.0 % for 20 min, 90.0 % for 30 min and 100.0 % for 1 h), then they were fixed on stubsand coated with a thin film of Au in a vacuum evaporator The specimens were then observed under SEM,and the most representative area of each group was photographed in increasingmagnifications of 500X, 1000X and 1500X. The EDS analysis was performed at a magnification of 500X.

### Final Microhardness Analysis

After pretreatments and erosive challenges, cross-sectional enamel demineralization was analysed by microhardness test in 70 specimens (n=14). Which were washed and the nail varnish and wax were carefully removed, exposing the reference area and longitudinally sectioned through the center of the exposed enamel. Microhardness test was performed with a Knoop diamond, $25gF^{-1}$ load for 10 seconds. Three column of 5 indentations each were made in each specimen side (reference and eroded area), distance between indentations of 30, 60, 120.0 and 300.0 µm below the surface enamel.

### Statistical Analysis

SEM and EDS were performed as descriptive analyses of the data and microhardness was performed for Dunn test and ANOVA one criteria.

## RESULTS

### X-Ray Energy-Dispersive Spectroscopy (EDS)

Table 1 illustrates the descriptive analysis of EDS data. There was a 174.0 % increase in the fluoride content of the irradiated group L/F (simultaneous laser and fluoride treatment). In group F, there was a 27.0 % increase. Group L had a 39.0 % reduction in fluoride content. Additionally, in the areas irradiated with the laser, there was also a 6.0 to 14.0 % decrease in phosphorus and a 0.2 to 8.0 % decrease in calcium in all the groups except for group L+F. However, this changes were insufficient to cause alteration on $CaP^{-1}$ proportion.

### Scanning Electron Microscopy

SEM images revealed that ablation in all specimens irradiated with the Er: YAG laser, independent of the use of APF gel (Figure 1). Specimens treated with the acidulated fluoride gel showed no changes in the enamel surface compared to reference areas (Figure 2).

### Microhardnessanalysis

The micro hardness was performed to analysed the effect of the treatment using laser associate or not associate to APF. This way, laser isolated or associated with APF gel and APF gel isolated werenot able to reduce the loss of hardness when compared to reference area (RA)i.e. not significantly different ($p>0.05$). Table 2 show means and standard deviation (SD) of experimental groups.

**Table 1: Descriptive analysis of EDS, % variation of the fluoride content relation of enamel before and after superficial treatment**

| | L | L+F | F+L | L/F | F |
|---|---|---|---|---|---|
| F | 39.4±69.7 | 7.5±6.3 | *-10.3±10.7 | *-74.3±132.6 | *-27.0±57.4 |
| Ca | 1.6±13.9 | *-1.1±7.6 | 7.3±3.1 | 8.2±4.6 | 0.2±2.8 |
| P | 6.4±9.9 | 8.1±6.2 | 12.2±4.6 | 14.4±6.8 | 0.7±3.3 |

*Negative sign indicates that there was an increase in the amount of the element in the treated area, the positive number indicates a decrease occurred.

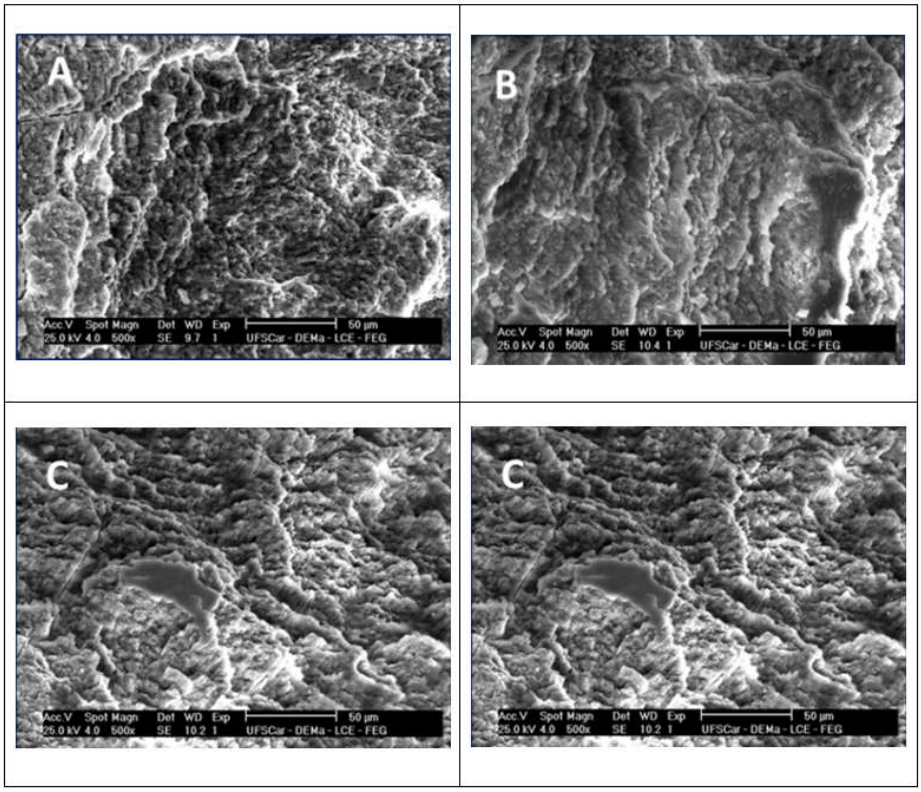


**Figure 1: Image of the ablated areas analyzed by SEM, in magnifications of 500X. A- L group. B- L+F group. C- F+L group. D- L/F group**

**Table 2: Mean and standard deviation (SD) in relation to microhardness test, comparing reference area (RA) with the groups**

| Groups | Mean ± SD |
|---|---|
| RA | 89.9±6.0a |
| L | 102.96±4.7a |
| L+F | 97.49±4.0a |
| F+L | 96.72±4.7a |
| F/L | 93.98±4.2a |
| F | 95.02±4.2a |

The same letters indicate a statistical similarity between pre-treatments.

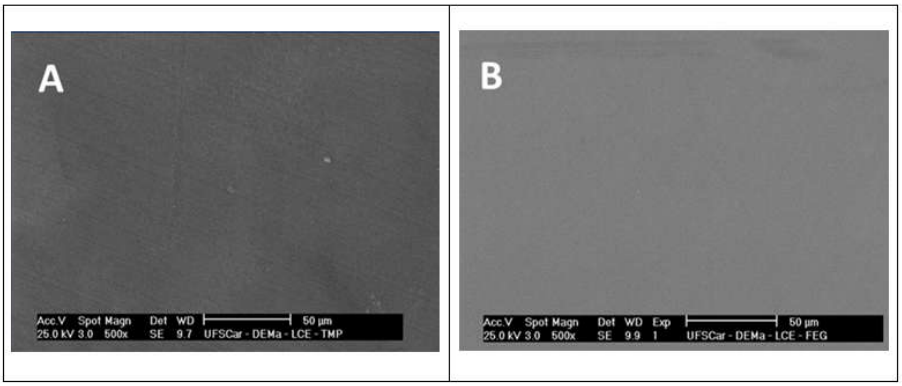


**Figure 2: Image of the ablated areas analyzed by SEM, in magnifications of 500X. A- F group. B- control area.**

## DISCUSSION

The fluoride treatments are used to prevent demineralisation caused by bacterial or dietary acids (Ganss *et al*., 2004).Fluoride can be present in the enamel in two forms: weakly linked fluoride ($CaF_2$ crystals) and strongly linked fluoride, such asfluorapatite. The mechanism of fluoride in the demineralisation process dependson the presence of calcium fluoride ($CaF_2$) on the dental surface when the pH decreases (18).The calcium ions of the carbonated hydroxyapatite crystals partially dissolve and attract fluoride ions resulting in the formationof fluorapatite crystals, which are larger crystals that are less soluble and more stable than hydroxyapatite (LeGeros *et al*., 1999). Studies involving laser and fluoride treatments have been performed for years, and it is believed that there is a synergistic interaction between them, resulting in greater fluoride retention and incorporation by the dental substrate and the establishment of more effective links between fluoride and enamel, promoting a long-term cariostatic effect (Ana *et al*., 2012).

This interaction demonstrateshow to increase the enamel acid resistance compared to treatment with fluoride or laser irradiation alone (Anaraki *et al*., 2012). Study performedby Fox *et al*. (1992) suggest that the critical pH for dissolution of enamel is 5.5, after laser irradiation the pH decreases to 4.3, and in the presence at least 0.1 ppm of fluoride, the critical pH can be even lower. Studies have evaluated the enamel fluoride content after laser irradiation associated with fluoride treatment (Bevilácqua *et al*., 2008) and verified that the combination promotes fluoride retention in the enamel. In this study the fluoride retention in dental enamelwas evaluated by EDS analyses, and observed that after laser irradiation there was fluoride content decrease in the L group (decrease of 39.4) and L+F group (decrease of 7.5%). L/Fgroup considerably increases the proportion of fluoride ions present in the enamel (increase of 174.0%) followed to F group (increase of 27.0 %) and F+L group (increase of 10.3%). Possibly the laser irradiation resulting in chemical changes on enamel surface what decrease fluoride uptake. This findings can be explained by some studies which suggest that the laser irradiation promoted a decrease in the fluoride content of enamel, once laser irradiation remove calcium ions, carbonated apatite, calcium phosphates, organic substances and water, which are necessary for $CaF_2$ formation (Oho, 1990; Corrêa-Afonso *et al*., 2015). Recent study demonstrated that after laser irradiation with Er,Cr YSGG and APF gel there was not increase enamel fluoride content, but after erosive challenge there was a significant increase in fluoride retention and laser alone was not able to increase fluoride content (Ana *et al*., 2012). This way, in the groups L/F and F+L it can be suggested that the fluoride used simultaneously or before laser irradiation decreased the chemical changes on enamel surface caused by laser, promotion fluoride content increase. Considering the importance of fluoride ionsfor the prevention of dental erosion, our data indicated that the simultaneous application of laser Er:YAG and fluoride provided a greater retention of fluoride ions, which may prevent dental erosion. Derceli *et al*. realized a study comparing different association forms between Er:YAg laser irradiation and APF gel, and concluded that the Er:YAG laser applied simultaneously to APF gel was better than others association forms (Derceli, 2015). The chemical changes on enamel surface caused by laser irradiation (Oho, 1990; Corrêa-Afonso *et al*., 2015) can also explain the decrease in calcium and phosphorus content in all the groups subjected to laser irradiation, except for group L+F, which showed a 1.0 % increase in calcium content.Although there has been decrease in calcium and phosfhorus content, there was not change in ratio between them.Ca/P proportion define the calcium phosphate of the hydroxyapatite, which is important to evaluated the calcium and phosphorus distribution in structure and, is associated with solubility of this material in acid environment, i.e, as lower the

Ca/P ratio higher the solubility of enamel. Contrary, previous studies have demonstrated that irradiation with the Er:YAG laser results in increased calcium and phosphorous in the dental enamel without modifying the ratio of these minerals (Hossain *et al*., 2003). However, this increase is attributed to the decrease in organic content and not to a real increase in the respective minerals (Liu, 2007). Based on images from scanning electron microscopy, it was noted that the groups irradiated with Er:YAG laser had irregular areas of structural loss and exposure of enamel prisms similar to the ablated areas. In contrast, the control area of these groups and group F (fluoride alone) did not have any surface changes. Er:YAG laser irradiation was performed manually, favouring the overlapping laser pulses, which may amplify the effect on the irradiated surface and increase the potential ablation at some points, however, it can also generate non-irradiated areas on the surface.

This micro-ablation sites increase the roughness, exposing a higher number of hydroxyapatite crystals that can reaction with fluoride, promoting enamel uptake. Study performed using Er,Cr:YSGG laser demonstrate that micro-ablation sites show higher quantity of $CaF_2$-like material globules formation (Ana *et al*., 2012). Another hypothesis proposes that laser irradiation forms micropores in the enamel surface due to the loss of water and carbonate, which act as a reservoir for $CaF_2$ and are available when the pH decreases (Oho, 1990). It is also believed that greater fluoride retention occurs and is incorporated in irradiated tissue in addition to the formation of more effective connections between fluoride and the enamel (Tepper *et al*., 1994). However, morphological changes resulting from laser irradiation are clinically unwanted because the ablated areas show higher roughness and are considered potential sites of plaque retention,in addition, they may lead to aesthetic problems (Quirynen, 1995).

Microhardnessanalysis have been used to evaluate mineral loss or gain by enamel, once mineral content is associated with length of indentation (Arends *et al*., 1980). Previous studies using micro hardness analysis showed that Er: YAG laser irradiations able to promote superficial changes on enamel , promoting increase of the acid resistance(20). Fornani *et al*. (2014) evaluated the effectiveness of demineralization decrease in enamel treated with Er:YAG laser irradiation followed by fluoride varnish application and observed that there was micro hardness increase of enamel. However, in the present study the hardness was similar in all groups, including reference area. It can be suggested that softening mineral was removed during the erosive challenge over agitation and during the specimens cleaning, leaving a mineralized surface with hardness similar to reference area. Altinok *et al*. (2011) evaluated the effect of Er:YAG laser irradiation and APF gel on enamel submitted to erosive solution. The experimental groups were: 1- control group (no treatment); 2- only APF gel; 3- laser irradiation; 4- laser irradiation followed to APF gel; 5- APF gel followed to laser irradiation. Observed that no statistical difference was found between groups.

The data of this study also are in agreement with a study realized using Er:YAG laser and CPP-ACPF in white spot, which showed not be able to increase remineralization of enamel (292). Using $CO_2$ laser irradiated 1, 2, 3 and 4 times, concluded that repeated applications decrease the demineralization (2015). It may be interesting to increase the number of laser applications to enhance the effect of the same.

The treatments are superficial, laser Er:YAG (1.0 μm) and fluoride application act on enamel surface, with a minimum of alterations to the sub-adjacent tissue. In this context, the objective of the present study was to evaluate the fluoride retention, the morphological alterations on the enamel surface and the resistance to demineralization after the pre-treatments with Er: YAG and fluoride gel laser. Ninety-five specimens of bovine enamel were obtained, half of the enamel surface was covered with nail polish and wax (reference area), leaving the other half to receive pre-treatments and erosive challenges. The specimens were randomized and divided according to 5 groups: L laser irradiation; L + F laser and subsequent application of fluoride (APF gel); F + L fluoride and subsequent laser irradiation; L / F laser / fluoride simultaneous application; F APF gel (control group).

The laser was irradiated for 10s (60mJ / 2Hz), APF gel was applied for 4 min and exposed in Coca-Cola® for 1 min, 4 times / day / 5 days. The retention of fluoride was evaluated by means of EDS, the morphological changes by SEM and the demineralization of the enamel by microhardness. SEM and EDS were analyzed by means of the descriptive data analysis and microhardness using the Dunn / ANOVA test at one criterion. L / F showed higher fluoride retention (174.0 %) followed by F (27.0 %) and F + L (10.0 %). L (39.0 %) and L + F (7.5%) had decreased fluoride content, there was a decrease in the content of Ca and P in about 0.2% to 8.0 % and 6.0 % to 14.0 %, respectively, with no change in Ca / P ratio. All irradiated groups were ablated.

**Conclusion**

Microhardness did not differ significantly between groups. L / F promoted greater retention of fluoride in the enamel and no pretreatment caused a change in the Ca / P ratio, the Er: YAG laser promoted microablation on the enamel surface when used at low creep and pre-treatments were not able to cause alteration in microhardness.

**Acknowledgements**

This study was supported in part by CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and FAPESP, process 2010 / 19531-8.

**Conflict of Interest**

I certify that I have no affiliation with or financial involvement in any organization or entity with a direct financial interest in the subject matter or materials discussed in the manuscript entitle “Analysis of fluoride retention after enamel erosion treatments”.

## REFERENCES

Altinok B, Tanboga I, Peker S, Eren F, Bakkal M, Peker F. 2011. The effect of laser-activated Acidulated Phosphate Fluoride on enamel submitted to erosive solution only: na in vitro preliminar evaluation. *Eur J PaediatrDent*;12:13-16.

Alves MB, MottaACF, Messina WC, Migliari DA. 2004. Saliva substitute in xerostomic patients with primary Sögrens Syndrome: A single-blind Trial Quintessence Int;35:392-396.

Ana PA, Tabchoury CPM, Cury JA, Zezell DM. 2012. Effect of Er,Cr:YSGG laser and professional fluoride application in enamel demineralization and on fluoride retention. *Caries Res:*46:441-451.

Anaraki SN, Serajzadeh M, Fekrazad R. 2012. Effects of laser-assisted fluoride therapy with a $CO_2$ laser and Er, Cr:YSGG laser on enamel demineralization. *Pediatr Dent*., 34:92-96.

Arends J, Schuthof J, Jongebloed WG. 1980. Lesions depth and microhardness indentations on artificial white spot lesions. *Caries Res*14:190-195.

Bartlett DW, Coward PY. 2001. Comparison of erosive potential of gastric juice and a carbonated drink *in vitro*. *J Oral Rehabil*;28:1045-1047.

Bevilácqua FM, Zezell DM, Magnani R, Ana PA, Eduardo CP. 2008. Fluoride uptake and acid resistance of enamel irradiated with Er:YAG laser. Lasers Med Sci;23:141-147.

Corrêa-Afonso AM, Bachman L, Palma-Dibb RG. 2015. Loss of structural water and carbonate of Nd:YAG laser-irradiated human enamel. *Lasers Med Sci*., 30:1183-1187.

Derceli JR, Faraoni-RomanoJJ, Azevedo DT, Wang L, Bataglion C, Palma-Dibb RG. 2015. Effect of pretreatment with an Er:YAG laser and fluoride on the prevention of dental enamel erosion. *Lasers Med Sci*;30:857-862.

Featherstone JDB, Nelson DGA. 1987. Laser effects on dental hard tissues. *Adv Dent Res*;1:21-26.

Featherstone JDB. 2000. The science and practice of caries prevention*. J Am Dent Assoc*;131:887-899.

Fornaini C, Brulat N, Milia G, Rockl A, Rocca J-P. 2014. The use of sub-ablative Er:YAG laser irradiation in prevention of dental caries during orthodontic treatment. Laser Therapy;23:173-181.

Fox JL, Yu D, Otsuka M, Higuchi WI, Wong J, Powell GL. 1992. The combined effects of laser irradiation and chemical inhibitors on the dissolution of dental enamel. *Caries Res*;26:333-339.

Ganss C, Klimek J, Brune V, Schürmann A. 2004. Effects of two fluoridation measures on erosion progression in human enamel and dentine *in situ*. *Caries Res*;38:561-566.

Heravi F, Ahrari F, Mahdavi M, Basafa S. 2014. Comparative evaluation of the effect of Er:YAG laser and low level laser irradiation combined with CPP-ACP cream on treatment of enamel caries. *J ClinExp Dent.,* 6:121-126.

Hossain M, Nakamura Y, Murakami Y, Yamada Y, Matsumoto K. 2003. A comparative study on compositional changes and Knoop hardness measurement of the cavity floor prepared by Er: YAG laser irradiation and mechanical bur cavity. *J Clin Laser Med Surg* \;21:29-33.

Järvinen VK, Rytömaa II, Heinonen OP. 1991. Risk factors in dental erosion. *J Dent Res*;70:742-747.

Johansson AK, Lingstrom P, Imfeld T, Birkhed D. 2004. Influence of drinking method on tooth-surface pH in relation to dental erosion. *Eur J Oral Sci*;112:484-489.

Le Geros RZ. 1999. Calcium phosphates in demineralization/ remineralization processes. *J Clin Dent*;10:65-73.

Liu Y, Hsu CY. 2007. Laser-induced compositional changes on enamel: a FT-Raman study. *J Dent*., 35:226-230.

Luo Y, Zeng XJ, Du MQ, Bedi R. 2005. The prevalence of dental erosion in preschool children in China. *J Dent*;33:115-121.

Nammour S, Demortier G, Florio P, Delhaye Y, Pireauxd JJ, Morciaux Y, Powell L. 2003. Increase of enamel fluoride retention by low fluence argon laser *in vivo*. Lasers Surg Med., 33:260-263.

Oho T, Morioka T. 1990. A possible mechanism of acquired acid resistance of human dental enamel by laser irradiation. *Caries Res*., 24:86-92.

Quirynen M, Bollen CML. 1995. The influence of surface roughness and surface-free energy on supra and subgingival plaque formation in man – a review of the literature. *J ClinPeriodontol*;22:1-14.

Rios D, Magalhães AC, Machado MAAM, da Silva SM, Lizarelli R de L, Bagnato VS, Buzalaf MA. 2009. *In vitro* evaluation erosion after Nd:YAG laser irradiation and fluoride application. *Photomed Laser Surg*;27:743-747.

Sognnaes RF, Wolcott RB, Xonga FA. 1972. Erosion-like patterns occurring in association with other dental conditions. J Am Dent Assoc;84571-576.

Stenhagen KR, Hove LH, Holme B, Tveit AB. 2013. The effect of daily fluoride mouth rinsing on enamel erosive/abrasive wear *in situ*. *Caries Res*;47:2–8.

Tepper AS, Zehnder M, Pajarola GF, 2004. Schmidlin PR.Increased fluoride uptake and acid resistance by $CO_2$ laser-irradiation through topically applied fluoride on human enamel in vitro. *J Dent*;32:635-641

Vieira KA, Steiner-Oliveira C, Soares LES, Rodrigues LKA, Nobre-dos-Santos M. 2015. *In vitro* evaluation of enamel demineralization after several overlapping $CO_2$ laser application. *Lasers Med Sci.,* 30:901-907.

Zero DT. 1996. Etiology of dental erosion – extrinsic factors. *Eur J Oral Sci*;104:162-177.

*******