Compositional changes promoted by Er,Cr:YSGG laser when used to inhibit dentin erosion

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Abstract— **Lasers are used for preventing demineralization, but there are no studies that report the compositional changes on root dentin irradiated with Er,Cr:YSGG (2.78µm) laser in an erosive process. In this *in vitro* study, fifty dentin slabs were distributed in 5 groups to be treated with Er,Cr:YSGG laser associated or not with application of acidulated phosphate fluoride gel (APF-gel); then, a 10-days erosion regimen was conducted and the composition changes were monitored by Fourier transform infrared spectroscopy. The data suggest a synergistic effect between laser irradiation and APF-gel, with a greater preventive effect when APF-gel was applied after irradiation.**

Keywords— dentin, erosion, Er,Cr:YSGG laser, fluoride, FTIR spectroscopy.

I. Introduction

The consumption of beverages and other acid products has augmented considerably in recent years due to new eating habits adopted by population. An increase in the frequency of dental erosion is detected mainly in young people, and it is related to the drinking of soft drinks, as well as the presence of gastroesophageal reflux [1]. Therefore, preventive strategies are necessary to control the development of erosion lesions, which can lead to dentin hypersensitivity and, in the most severe cases, the loss of tooth.

In this way, it is still necessary to look for alternatives that decrease the solubility of enamel or dentin, such as the use of high-intensity lasers [2]. Previous studies have shown that infrared emitting lasers, when well absorbed by water and hydroxyapatite, can change the chemical and crystalline composition of dental enamel [3, 5, 7], making it more resistant to demineralization. Among these lasers, the Er,Cr:YSGG (λ = 2.78 µm) stands out.

The literature also evidences that the use of the Er,Cr:YSGG laser can prevent the appearance of erosion lesions[4]. In this way, this work aims to highlight its effects on the chemical composition of root dentin when subjected to the erosive challenge, and when it is associated to the topical application of an acidulated phosphate fluoride gel (APF-gel). Still, it seeks to assess whether there is synergy between the agents and what would be the order of treatments to obtain a better effect.

II. Material and Methods

After approval by the Ethics Committee on Animal Use of UFABC, fifty cervical root dentin slabs were prepared from 25 lower bovine incisor teeth as reported before [5] and kept in humid environment until the beginning of the experiments.

The slabs were randomly assigned to 5 experimental groups of 10 samples each: **G1-** untreated slabs; **G2-** treatment with topical application of acidulated phosphate fluoride gel for 4 minutes (APF-gel, 1.23% F-, pH 3.6 - 3.9, Fluor Gel, Dentsply, Brazil); **G3-** treatment with Er,Cr:YSGG laser; **G4-** treatment with Er,Cr:YSGG laser followed by APF-gel application; and, **G5-** treatment with APF-gel application followed by Er,Cr:YSGG laser irradiation.

In groups 3, 4 and 5, it was used an Er,Cr:YSGG laser [5] (beam diameter of 750 µm, 2.78 µm, 0.25W, 20 Hz, 2.8 J/cm^2, Millenium, Biolase Technology Inc., USA). The irradiations were performed using a X-Y-Z motorized stage (ESP 300, CA, USA), at the speed of 10.3 mm/s. The distance from the laser tip (S75) to the dentin surface during irradiation was 1 mm, and the irradiations were performed without air-water spray. Before irradiations, the energy emitted was measured using a power/energy meter (FieldMaster, Coherent, USA).

After treatments, an erosion lesion was induced through an 10-days erosive cycling regimen [6]. For this purpose, each experimental group was submerged in 300 mL (30 mL/sample) of Sprite Light® (citric acid, Coca-Cola Co., Brazil; pH = 2.87), at room temperature, for 1 minute, followed by immersion in 300 mL (30 mL/sample) of artificial saliva (1.5 mM $Ca(NO_3)_2.4H_20$, 0.9 mM $NaH_2PO_4.2H_2O$, 150 mM KCl, 0.03 ppm F^-, Tris 0.1M, pH 7.0) for 59 minutes. This procedure was repeated 4 times a day and the samples were kept in artificial saliva for the remaining 20 hours of each day.

The compositional analysis was performed by Fourier transform infrared spectroscopy (FTIR, Varian FTIR 610, Agilent, USA), using the attenuated total reflection (ATR with a ZnSe crystal) method. The analyzes were performed at three different times: immediately after treatments, after 5 and 10 days of erosive cycling. The spectra were collected with a resolution of 4 cm^{-1} and 60 scans in the region of 4000 to 650 cm^{-1}. The absorption bands considered for this study were ν_3 asymmetric vibrations of phosphate (1300–900 cm^{-1}), amide I (1680-1600 cm^{-1}), amide II (1580-1480 cm^{-1}), amide III (1200-1300 cm^{-1}), the ν_2 vibration mode of carbonate (around 870 cm^{-1}) and the superposition of the stretching ν_3 and

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bending ν_4 vibration mode of carbonate (between 1600-1300 cm^{-1}) [7]. For the comparative intensity analysis, the vectorial normalization of the spectra was performed [8]. For semi-quantitative analysis, the areas under the considered bands were calculated after normalization by the area of phosphate band (1300–900 cm^{-1}) [7] using the OriginPro 8 software (Origin Lab, USA).

III. RESULTS AND DISCUSSION

Fig. 1 shows the average spectra of all experimental groups immediately after treatments. It is observed that the laser irradiation alone did not promote significant effects on the dentin composition, except in the intensities of amide 1 and amide 2, with a small decrease. This consequence may be related to the rise in temperature generated during irradiations, which can alter the organic matrix of dentin, such as protein denaturation and water evaporation [9].

The application of APF-gel, either alone or associated with laser irradiation, reduced the intensity of the phosphate peak, as well as carbonate ones. However, the treatments augmented the peak intensities corresponding to amides 1 and 2. These effects can be explained by the action of phosphoric acid from the APF-gel, which demineralizes the dentin surface and removes, therefore, carbonated hydroxyapatite [10]. This small loss of mineral was detected by the equipment's evanescent wave. Also, the rise in the intensity of amides 1 and 2 peaks can be explained by the permanence of the gel's thickener on the surface, whose composition overlaps the positions of these absorption bands [10].

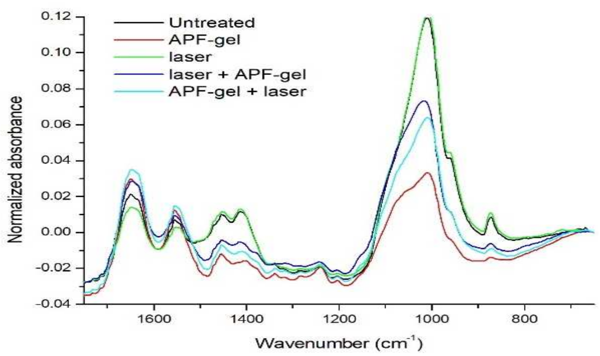

Fig. 1 Average infrared absorption spectra of root dentin immediatelly after the proposed treatments, in the region between 1700 – 650 cm^{-1}.

Considering the association of laser irradiation with the application of APF-gel, regardless of the order of application (G4 or G5 groups), a lower reduction in the intensity of the phosphate peak was also observed, when compared with the group treated only with the APF-gel (G2). When the irradiations were performed before the application of APF-gel (G4), the reduction in the phosphate content was smaller; in this way, we can infer that the thermal effects due to the laser irradiation may have increased the resistance of dentin to the action of acid phosphoric acid present in APF-gel. On the other hand, when laser irradiation was carried out after the application of APF-gel (G5), there was a greater loss in the intensity of the phosphate peak when compared to the G4 group, but this loss was fewer when compared to the G2 group (treated with APF-gel alone). In this case, it seems that the laser irradiation may have promoted a greater retention of the products formed because of the topical treatment and, perhaps, a greater incorporation of them. Future studies on the determination of formed and retained fluoride may further clarify this hypothesis.

After 5 days of erosion lesion induction (Fig. 2), an intense loss in the phosphate and carbonate peak intensities was observed in all experimental groups, except for the group treated with laser+APF-gel. This evidences a greatest preventive effect of this treatment and corroborates the literature [2,7,10]. Previous studies carried out with enamel [2,7] show that the micro-ablations promoted by laser irradiation increase the surface area for reaction with the APF-gel and, therefore, a greater amount of CaF_2-like material is formed. This greater amount of material provides, in turn, a larger amount of fluoride that reacts for a longer period and, for this reason, the cariostatic effect is more pronounced even in longer challenges. On the other hand, when the APF-gel is applied alone, the amount of CaF_2-like material formed can be lost in the erosive cycling solutions in the first days and, therefore, the loss of mineral occurs again. Laser irradiation after application of APF-gel did not result in any additional effects.

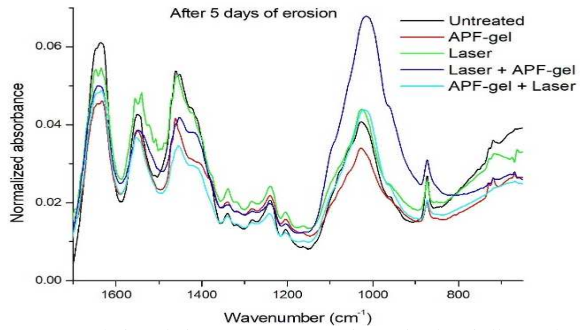

Fig. 2 Average infrared absorption spectra of root dentin of all experimental groups after 5 days of *in vitro* erosive challenge, in the region between 1700 – 650 cm^{-1}.

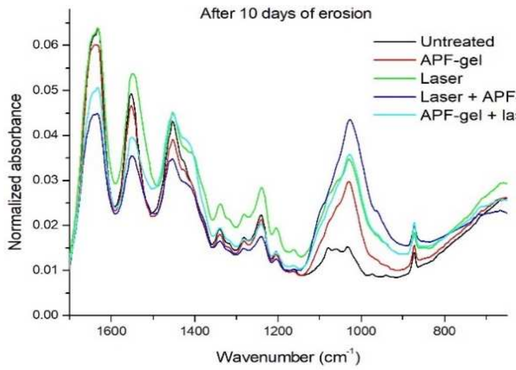

Fig. 3 Average infrared absorption spectra of root dentin of all experimental groups after 10 days of *in vitro* erosive challenge, in the region between 1700 – 650 cm^{-1}.

After 10 days of erosive cycling (Fig. 3), the mineral loss intensifies as expected, being confirmed by the lower intensity of the phosphate and carbonate peaks, as well as greater exposure of dentin organic content in the untreated group. During this period, all the treatments provided less reduction in the intensity of the phosphate and carbonate peaks and, therefore, the preventive effect is confirmed. Laser irradiation alone promoted a preventive effect like that observed in the group treated with APF-gel+laser; thus, the thermal action promoted by irradiation resulted in a probable decrease in mineral loss in 10 days. However, greater intensity was observed in the phosphate and carbonate peaks in the group treated with laser+APF-gel, which suggests a synergistic effect. These findings once again confirm a likely higher formation of CaF_2-like material because of micro-ablations [2], which resulted in a greater preventive effect.

Fig. 4 shows the average of the FTIR spectra of the untreated group, for the different experimental times and in Fig. 5 the results of the analysis of the area under the main absorption bands, normalized by the phosphate band, are shown. Note that the loss of phosphate has a positive relation with the erosive cycling time; still, this fact is reinforced by the greater exposure of dentin organic content, reported here by the bands of amides 1, 2 and 3 and carbonate. These findings once again emphasize the effectiveness of erosive cycling used in this study, for simulating erosion lesion and loss of surface mineral, as well as the potential of the FTIR technique in detecting these small changes.

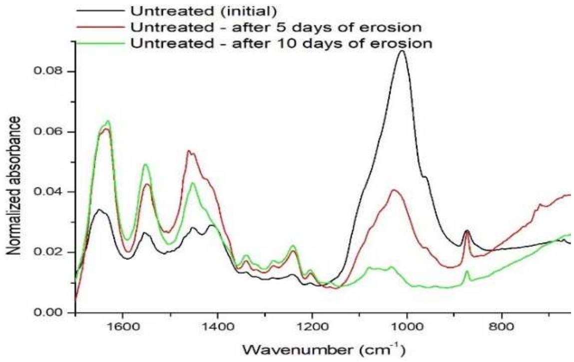

Fig. 4 Average infrared absorption spectra of untreated root dentin compared to dentin after 5 and 10 days of *in vitro* erosive challenge, in the region between 1700 – 650 cm^{-1}.

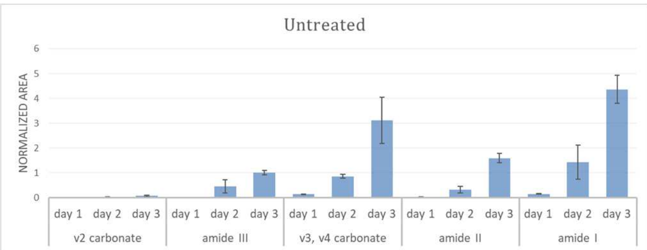

Fig. 5 Untreated group mean values of phosphate-normalized areas from infrared spectra. Error bars show standard errors. All comparisons of the evaluated areas, at all experimental times, showed a statistically significant difference at the level of 5%.

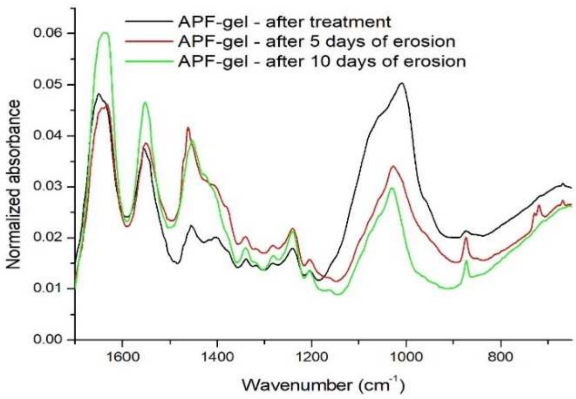

Fig. 6 Average infrared absorption spectra of root dentin treated with APF-gel after treatment, after 5 and 10 days of *in vitro* erosive challenge, in the region between 1700 – 650 cm^{-1}.

In the group treated with APF-gel, it is noted a smaller phosphate loss (Fig. 6) when compared to the untreated group. Although the exposure of organic material has also shown a positive relation with the time of erosion challenge (Fig. 7), this is not observed with carbonate. This fact is consistent with the greater preventive effect of APF-gel.

While there was a great loss of phosphate in the first 5 days of erosive cycling (Fig. 8), we can suggest that the laser-treated group has a long-term preventive effect (after 5 days of erosion, Fig. 9). This fact is noticed by the absence of statistical significance in the comparison between the areas under the bands of amides 1, 2 and 3 and carbonate between days 5 and 10 of erosive cycling.

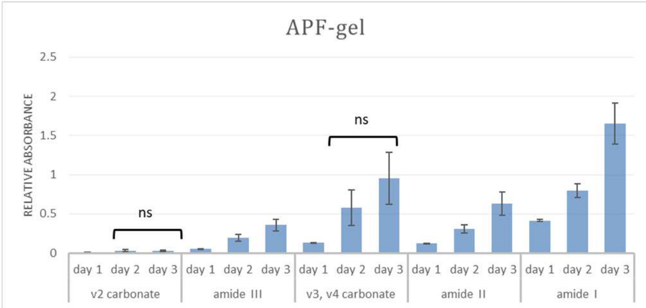

Fig. 7 APF-gel group mean values of phosphate-normalized areas from infrared spectra. Error bars show standard errors. Most comparisons of the evaluated areas, at all experimental times, showed a statistically significant difference at the level of 5%; ns – not significant.

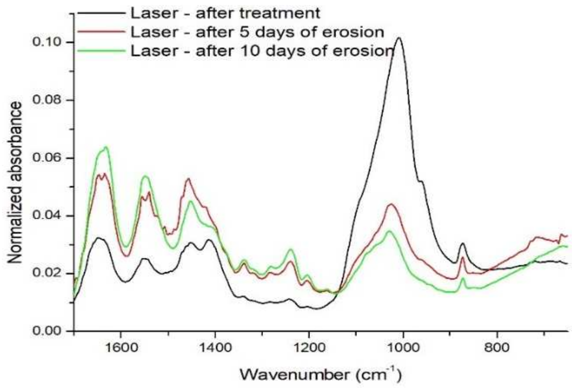

Fig. 8 Average infrared absorption spectra of laser treated root dentin after treatment, after 5 and 10 days of *in vitro* erosive challenge, in the region between 1700 – 650 cm^{-1}.

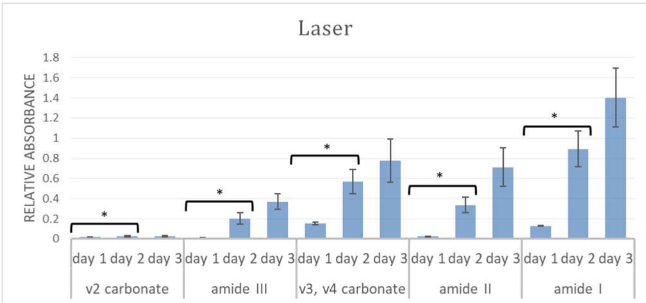

Fig. 9 APF-gel group mean values of phosphate-normalized areas from infrared spectra. Error bars show standard errors. Stars (*) denote the statistically significant differences at 5% level.

In sequence, from the analysis of Fig. 10 and Fig. 11, it is noticed that the group treated with laser+APF-gel was the one that showed a reduction in the loss of phosphate during the erosive cycling, even though the higher exposure of organic content. By semi-quantitative analysis, a significant difference is observed only in relation to the content of amide 3 over time, that is, the rate of mineral loss seems to have been lower in this experimental group.

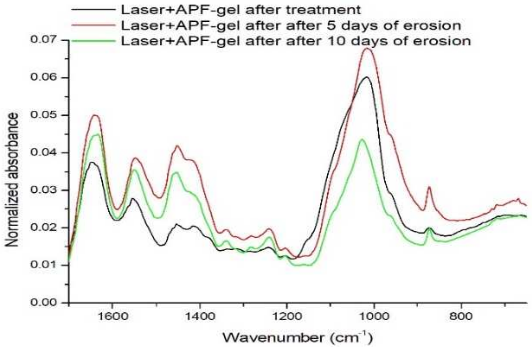

Fig. 10 Average infrared absorption spectra of laser+APF-gel treated root dentin after treatment, after 5 and 10 days of *in vitro* erosive challenge, in the region between 1700 – 650 cm^{-1}.

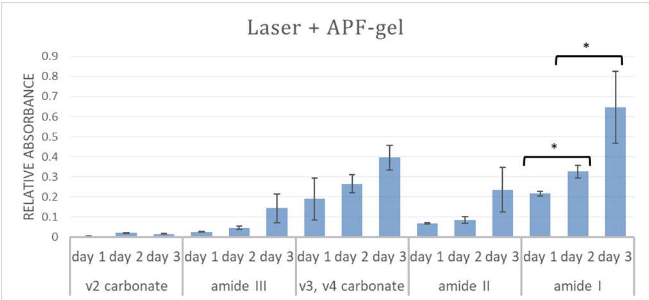

Fig. 11 Laser + APF-gel group mean values of phosphate-normalized areas from infrared spectra. Error bars show standard errors. Stars (*) denote the statistically significant differences at 5% level.

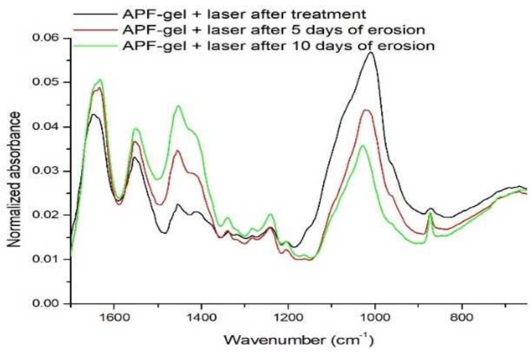

Fig. 12 Average infrared absorption spectra of APF-gel+laser treated root dentin after treatment, after 5 and 10 days of *in vitro* erosive challenge, in the region between 1700 – 650 cm^{-1}.

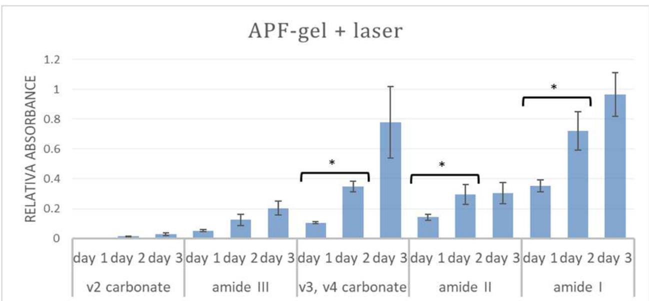

Fig. 13 APF-gel+laser group mean values of phosphate-normalized areas from infrared spectra. Error bars show standard errors. Stars (*) denote the statistically significant differences at 5% level.

Although we have seen a preventive effect from the treatment with APF-gel before laser irradiation (Fig. 12), the data suggest that this effect was delayed, a fact evidenced by the significant increase in the proportion of carbonate and amides 1 and 2 only in the first 5 days of cycling (Fig. 13). This finding indicates greater loss of phosphate in the first days, with a reduction in the rate of progression of lesions between 5 to 10 days. Therefore, even if there was some absorption of fluoride, this did not result in a synergistic effect, as demonstrated in previous studies. Therefore, future investigations on the formation and retention of loosely and firmly bound fluoride due to the laser irradiation are necessary to confirm such hypotheses.

IV. CONCLUSIONS

Based on the methodology used and the results obtained in the present study, it was concluded that the effects promoted by the association of laser irradiation with APF-gel application are more promising for preventing dental erosion than the isolated application of these treatments. For a more effective effect, laser irradiation must be performed before applying the APF-gel.

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