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Effect of Er,Cr:YSGG laser associated with fluoride on the control of enamel erosion progression

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ABSTRACT

Objective: To evaluate the effect of Er,Cr:YSGG laser associated or not with acidulated phosphate fluoride (APF) on the control of enamel erosion progression. Design: Enamel slabs (4 mm × 4 mm × 2 mm) from bovine incisors were flattened, polished, and received a tape on their test surfaces, leaving a 4 mm × 1 mm area exposed. Specimens were eroded (10 min in 1% citric acid solution) and randomly assigned into 8 experimental groups (n = 10): Control (no treatment); F (APF gel, 1.23% F, pH 3.6–3.9); Er,Cr:YSGG laser irradiation (P1: 0.25 W, 20 Hz, 2.8 J/cm^2, 56 W/cm^2); Er,Cr:YSGG laser irradiation (P2: 0.50 W, 20 Hz, 5.7 J/cm^2, 1136 W/cm^2); Er,Cr:YSGG laser irradiation (P3: 0.75 W, 20 Hz, 8.5 J/cm^2, 1704 W/cm^2); F + Laser P1; F + Laser P2; F + Laser P3. Specimens were then subjected to erosive cycling (5 min immersion in 0.3% citric acid solution, followed by immersion in artificial saliva for 60 min; 4 × /day for 5 days). At the end of cycling, surface loss (SL, in μm) was determined with optical profilometry. Selected specimens were further evaluated by environmental scanning electron microscopy (n = 3). Data were analyzed using Kruskal-Wallis and Tukey tests ($\alpha = 0.05$). Results: Group F + Laser P2 had the lowest SL value, differing significantly from the control; however, with no significant difference from the other groups. All groups, except F + Laser P2, showed no significant difference in SL when compared with the control. An irregular and rough surface, suggestive of a melting action of laser, was observed on enamel in Laser P2 and F + Laser P2 groups. Conclusions: Association of the Er,Cr:YSGG laser in parameter 2 with fluoride was the only treatment capable of controlling the progression of enamel erosion.

1. Introduction

Dental erosion is characterized by loss of dental hard tissues caused by acids of intrinsic or extrinsic origin, without bacterial involvement (Eccles, 1979; Lussi & Jaeggi, 2006). When the erosive acids reach tooth enamel, its mineral content begins to dissolve, resulting in a rough and irregular surface, with decreased hardness. As the process continues, bulk surface loss occurs (Ganss, Lussi, & Schlueter, 2014). Dental defects resulting from erosion are difficult to be detected in its early stages, because they only cause subtle changes on the tooth surfaces (Carvalho, Scaramucci, Aimée, Mestrinho, & Hara, 2018). However, in most advanced cases, concavities and the loss of the original tooth anatomy, shortening of maxillary incisors, and the yellowish appearance of the teeth can easily be noticed, sometimes even by the patient (Schlueter, Jaeggi, & Lussi, 2012).

Once diagnosed, the most appropriate treatment strategy needs to be decided, so that the progression of this process can be controlled. Frequently, it is not easy to eliminate the causative factors related to erosion (habits, diet, and daily medication) (Amaechi & Higham, 2005); therefore, additional measures should be implemented. Topical application of fluoridated products has been suggested, but different levels of protection have been shown, depending on the fluoride compound (Wiegand, Bichsel, Magalhães, Becker, & Attin, 2009), its concentration (White, Jones, & Barbour, 2012), sfrequency of application (Huysmans, Young, & Ganss, 2014), among other factors.

Another measure would be the use of high power lasers. Studies using Nd:YAG, CO$_2$, Argon and Er:YAG lasers - well known for promoting an increase in acid resistance of dental substrates, have shown controversial results regarding the control or prevention of dental erosion (dos Reis Derceli et al., 2015; João-Souza, Scaramucci, Hara, &

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Aranha, 2015; João-Souza, Bezerra, Borges, Aranha, & Scaramucci, 2015; Magalhães et al., 2008; Pereira, Joao-Souza, Bezerra, Aranha, & Scaramucci, 2017; Ramalho et al., 2013; Rios et al., 2009), hindering their clinical use for this purpose. The possible synergistic effect of high power lasers and fluoride have also been addressed, with similar distinct outcomes (Altinok et al., 2011; dos Reis Derceli et al., 2015; João-Souza, Scaramucci et al., 2015; João-Souza, Bezerra et al., 2015; Rios et al., 2009; Souza-Gabriel et al., 2015; Steiner-Oliveira, Nobre-dos-Santos, Zero, Eckert, & Hara, 2010).

Another high-power laser that could potentially have some effect against erosion is the Er,Cr:YSGG (2.78 μm) laser, which has affinity for the water and hydroxyl (OH) ions present in large amounts on the tooth surfaces. Through temperature increase, chemical changes, such as those occurring in the mineral content of enamel on tooth surfaces, would result in increased acid resistance (Bachmann, Rosa, da Ana, & Zezell, 2009). Another advantage would be the increase in CaF_2-like material deposition on enamel when this laser is associated with fluoride (Ana, Tabchoury, Cury, & Zezell, 2012). This may be of relevance for control of the erosion process; however, so far, little is known about the effects of Er,Cr:YSGG lasers, combined or not with fluoride, on enamel erosion. De Oliveira et al. found that the irradiation of enamel surfaces with Er,Cr:YSGG laser, at a pulse frequency of 30 Hz, a power of 0.50 W, and energy density of 6.6 J/cm^2 was the best parameter to prevent enamel erosion (de Oliveira et al., 2017). Dionysopoulos et al. observed that Er,Cr:YSGG laser irradiation with 0.25 W, at a pulse frequency of 20 Hz, and an energy density of 31.25 J/cm^2 could significantly reduce erosion when compared to the control However, in these studies, no association with fluoride was tested.

In view of the foregoing, the aim of this in vitro study was to evaluate the ability of several Er,Cr:YSGG laser protocols, associated with acidulated phosphate fluoride or not, to prevent tooth enamel demineralization after erosive challenges. The null hypotheses were: 1) The different laser protocols would not be able to control the progression of enamel erosion; 2) The different laser protocols would not be able to increase the protective effect of fluoride against enamel erosion.

2. Materials and methods

2.1. Study design

This study followed a single factor design, with surface treatment at 8 levels: 1. negative control (no surface treatment); 2. F (acidulate phosphate fluoride gel, 1.23% F, pH: 3.6–3.9); 3. Er,Cr:YSGG laser irradiation (parameter 1: 0.25 W, 20 Hz, 2.8 J/cm^2); 4. Er,Cr:YSGG laser irradiation (parameter 2: 0.50 W, 20 Hz, 5.7 J/cm^2); 5. Er,Cr:YSGG laser irradiation (parameter 3: 0.75 W, 20 Hz, 8.5 J/cm^2); 6. F + Laser P1; 7. F + Laser P2; 8. F + Laser P3. Before application of the treatments, the specimens were first eroded to create an initial erosion lesion. Then the treatments were tested with an erosion-remineralization cycling model of 5 days, using enamel specimens (n = 10) obtained from the crowns of bovine incisors. The response variable was enamel surface loss (SL, in μm), measured post-treatment and on conclusion of the cycling, by using an optical profilometer. As an additional test, the surfaces of 3 specimens from Groups Control, F, Laser P2, F + Laser P2 were qualitatively analyzed by environmental scanning electron microscopy (ESEM) post-treatment and post-cycling.

2.2. Specimen preparation

One-hundred and fifty enamel slabs, obtained from the crowns of bovine incisors, were cut (4 mm width × 4 mm length × 2 mm thickness) using a low-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA). The slabs were embedded in acrylic resin (Varidur, Buehler), and the resulting blocks were ground flat and polished, with Al_2O_3 abrasive disks, under water cooling, according to the following sequence: 400-, 1200-, 2400- and 4000-grit (Buehler). At the end of each polishing procedure, the specimens underwent an ultrasonic bath with deionized water for 3 min. After preparation, the baseline curvature was evaluated with an optical profilometer (Proscan 2000, Scantron, Venture Way, Tauton, UK), as previously described (11). Specimens with initial curvature higher than 0.3 μm, or exhibiting cracks or any other surface defect were discarded.

2.3. Initial erosion

An adhesive tape was placed on the polished surface of the selected specimens, leaving a 4 mm × 1 mm window exposed to the subsequent tests. To induce an initial erosion lesion in vitro, each specimen was immersed for 10 min in 1% citric acid solution (Anhydrous citric acid, Sigma Aldrich, pH ˜ 2,3), at room temperature (˜24 °C). After immersion, the specimens were rinsed with distilled water. Then, they were analyzed by using an optical profilometer, with the aim of selecting eighty specimens with initial lesion depth values between 2–5 μm (description of this measurement was given in the item 'Surface loss evaluation'). The specimens were kept under a condition of 100% relative humidity until the experiment began.

2.4. Surface treatments

The specimens were randomly allocated into the 8 experimental groups (Table 1). The application of the APF gel (acidulate phosphate fluoride, 1.23% F, pH: 3.6–3.9, Maquira, Maquira Industry of Dental Products S.A, Maringá, PR, Brazil) was performed with a flexible cotton swab, for 1 min. Afterwards, the gel was removed with cotton rolls [15]. The Er,Cr:YSGG laser (Biolase Inc., San Clemente, CA, USA) operates at a wavelength of 2.78 μm, with a pulse width of 140 μs, fixed repetition rate of 20 Hz, and an average power that can vary from 0 to 6 W. The energy is delivered through an optical fiber with a sapphire tip of 750 μm in diameter and 6 mm in length (MS75). This laser was applied at a distance of 1 mm from the surface, focused mode. For parameter 1, the following protocol was used (P1): average output power of 0.25 W, repetition rate of 20 Hz, energy density of 2.8 J/cm^2; power density of 56 W/cm2; for parameter 2 (P2): average output power of 0.50 W, repetition rate of 20 Hz and an energy density of 5.7 J/cm^2 and power density of 113 W/cm^2; for parameter 3 (P3): average output power of 075 W, repetition rate of 20 Hz, energy density of 8.5 J/cm^2 and power density of 17,004 W/cm^2. Ten-second irradiations were performed, making three horizontal sweeping movements, under 30% air cooling without water, covering the entire surface of the initial lesion. In groups 6, 7 and 8, the fluoride gel was applied immediately before laser irradiation. In the post-treatment period, another profilometric measurement was made to verify whether the different laser protocols had caused any tissue ablation.

2.5. Erosive challenge

The specimens were submitted to an erosion-remineralization cycle

Table 1
Experimental groups.

Codes	Groups
C	Negative control (no surface treatment)
F	APF gel
Laser P1	Irradiation with Er,Cr:YSGG laser (parameter 1: 0.25 W, 20 Hz, 2.8 J/cm^2)
Laser P2	Irradiation with Er,Cr:YSGG laser (parameter 2: 0.50 W, 20 Hz, 5.7 J/cm^2)
Laser P3	Irradiation with Er,Cr:YSGG laser (parameter 3: 0.75 W, 20 Hz, 8.5 J/cm^2)
F + Laser P1	APF gel + Irradiation with Er,Cr:YSGG laser Parameter 1
F + Laser P2	APF gel + Irradiation with Er,Cr:YSGG laser Parameter 2
F + Laser P3	APF gel + Irradiation with Er,Cr:YSGG laser Parameter 3

that consisted of 5 min of immersion in 0.3% citric acid solution (pH ˜ 2,6), followed by 60 min of exposure to the artificial saliva. This procedure was repeated 4 times a day, for 5 days. During the overnight period, the specimens were stored in a humid environment at 4 °C.

2.6. Surface loss evaluation

Prior to each profilometric analysis, the tapes were removed from the specimens. The optical profilometer (Proscan 2000, Scantron, Venture Way, Tauton, UK) was programed to scan an area of 2 mm long (on the x-axis) and 1 mm wide (on the y-axis) at the center of the specimens, covering the treated area and the two reference areas. On the x-axis, the step size was set to 0.01 mm and the number of steps was 200. On the y-axis, these values were 0.05 mm and 20, respectively. The depth of the treated area was calculated based on the difference between the mean height of the test area and the mean height of the two reference areas, by using a specific software program (Proscan Application software v. 2.0.17).

2.7. Environmental scanning electron microscopy (ESEM) evaluation

Three randomly selected specimens from control, F, Laser P2, and F + Laser P2 groups were further analyzed by ESEM to qualitatively verify their surface morphology post-treatment and post-cycling. Representative micrographs were taken at 2000x magnification in the center of each specimen, using Analy observation conditions, with 15Kv. No specimen preparation was required. In the qualitative assessment, the surface characteristics of micrographs were described.

2.8. Statistical analysis

The normality and homoscedasticity of the data were checked with the Shapiro-Wilk and Brown-Forsythe tests, respectively. Since data did not follow a normal distribution, they were analyzed by Kruskal-Wallis and Tukey tests, considering a significance level of 5%. SigmaPlot 13 software (Systat Software Inc., Chicago Illinois, USA) was used for the calculations.

3. Results

The mean (SD) curvature value for all the specimens was 0.14 μm (0.08). Post initial lesion, the mean (SD) surface loss value of the specimens was 2.79 (0.41). The medians (interquartile intervals) of surface loss for each group post-treatment and post-cycling are presented in Table 2. Post-treatment, there were no significant difference among groups ($p > 0.05$). Post cycling, the control showed the highest surface loss values, without significant difference when compared with all the groups ($p > 0.05$), except F + Laser P2 ($p = 0.029$). Group F + Laser P2 showed the lowest surface loss value, without significant difference when compared with F + Laser P3, F, Laser P1, F + Laser P1, and F + Laser P2 ($p > 0.05$).

Table 2
The medians (interquartile intervals) of surface loss (in μm) for each group post-treatment and post-cycling. In columns, different letters denote significant difference among groups ($P < 0.05$).

Groups	Medians (IQI) post treatment	Medians (IQI) post cycling
C	2.80 (2.64 – 3.24) a	4.32 (4.01–4.61) a
Laser P3	2.41 (1.74 – 3.36) a	3.13 (2.40–4.10) a
Laser P2	2.98 (2.55 – 3.42) a	4.13 (3.85–4.31) ab
F + Laser P1	2.46 (2.00 – 3.33) a	3.76 (3.48–4.67) ab
Laser P1	2.81 (2.54 – 3.06) a	3.80 (3.31–4.10) ab
F	2.74 (2.43 – 3.00) a	3.85 (3.40–3.90) ab
F + Laser P3	2.56 (1.64 – 3.25) a	3.24 (2.44–4.58) ab
F + Laser P2	2.56 (2.56 – 3.34) a	2.98 (2.65–3.80) b

Fig. 1 shows the representative micrographs of control, F, Laser P2, and F + Laser P2 groups, immediately after treatment (A) and post-cycling (B). Post-treatment, the micrographs of groups F + Laser P2 and Laser P2 showed the enamel with an irregular and rough surface, because of melting resulting from the laser irradiation. Apparently, the enamel crystals re-solidified after melting, creating larger crystallites. More regular and smoother surfaces could be observed in the control and fluoride groups. Post-cycling, the acid seemed to have removed the melted layer of F + Laser P2 group, and its surface appeared smoother and more regular. In Laser P2, the surface also appeared to be less irregular, but it was still rougher than that of F + Laser P2.

4. Discussion

In the present study, the use of Er,Cr;YSGG laser as a single treatment, in three different parameters, was not able to control the progression of enamel erosion, thus our first null hypothesis was accepted. Er,Cr;YSGG laser is a high power laser with a wavelength that coincides with the maximum peak of the hydroxyl ion (OH) in the hydroxyapatite structure (Fried et al., 1996). It was hypothesized that the heating caused by Er,Cr:YSGG laser irradiation, at similar energy densities as tested in the present investigation, would be able to cause chemical and crystallographic changes in enamel, such as loss of carbonate, reduction in the organic content and water (Ana et al., 2012; Fried et al., 1996), formation of tricalcium phosphate in α and β phases, and the formation of tetracalcium phosphate (Bachmann et al., 2009; Zezell, Ana, & Albero, 2009). All together, these changes would result in an increase in the overall acid resistance of enamel, as shown in previous studies testing this laser in the context of caries (de Freitas, Rapozo-Hilo, Eduardo, & Featherstone, 2010). The authors suggested that this effect would be dependent on the energy density applied (Apel, Meister, Schmitt, Gräber, & Gutknecht, 2002), which should, however, be below the ablative threshold (Ramalho et al., 2015).

In a previous investigation, different Er,Cr:YSGG laser irradiation protocols were tested as regards their ability to prevent enamel erosion (sound specimens were irradiated). The authors observed that the parameter of 0.5 W of power, 30 Hz frequency, and energy density of 6.6 J/cm^2 used to the irradiate the specimens was the only one that could maintain the surface hardness values of enamel after an erosive cycling that consisted of immersion in 0.01 M HCl solution for 2 min, 4 times (de Oliveira et al., 2017). Although this energy density of 6.6 J/cm^2 was within the values tested in the present study (2.8 J/cm^2, 5.7 J/cm^2 and 8.5 J/cm^2), the erosive challenge performed in the previous investigation cited (de Oliveira et al., 2017) was less aggressive, intended to simulate the initial stages of erosion, whereas in the present investigation, a more aggressive erosive model was used, in which bulk surface loss could be noted. This could be the reason for the discrepant results between the two studies. Perhaps, the laser-modified layer was removed by the more aggressive challenge during the first days of cycling, offering no protection at the end of the experiment. The images obtained in the ESEM analysis corroborated this hypothesis, because the surfaces of irradiated enamel seemed less rough and irregular post-cycling when compared with post-treatment values. Despite these characteristics, no tissue removal could be noted after irradiation with all the laser protocols, as shown in the post-treatment profilometric analysis.

Although Protocol Laser P2 without fluoride did not show any ability to control the progression of enamel erosion when tested alone, the combination of Er,Cr;YSGG laser irradiation (in protocol - P2) with fluoride was able to provide a protective effect, significantly reducing surface loss when compared with the control group. Based on this result, the second null hypothesis of this study was rejected. Laser irradiation associated with fluoride application has been widely studied, and it has been shown that this combination can lead to further decrease in enamel demineralization, and an increase in fluoride retention on the tooth surface (Geraldo-Martins, Lepri, Faraoni-Romano, &

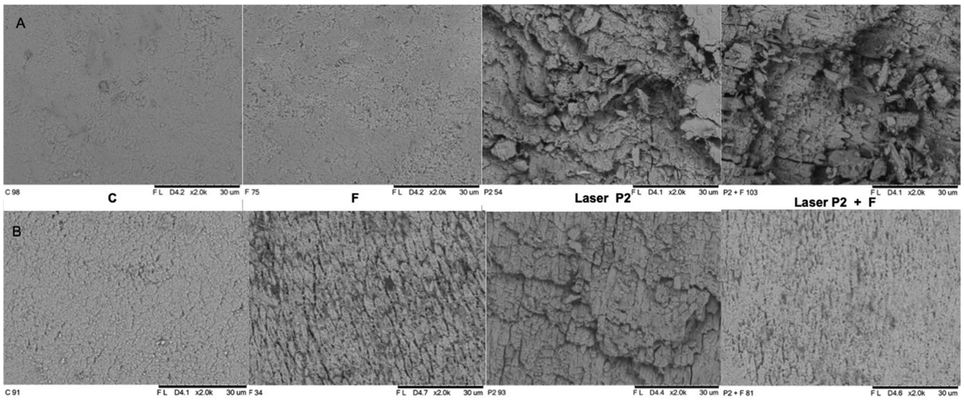

Fig. 1. Representative micrographs of control (C), APF (F), Laser P2 and F + Laser P2 groups, taken at 2000 × . (A: post-treatment; B: post-cycling).

Palma-Dibb, 2014). Indeed, Ana et al. (2012) observed a significant increase in CaF_2-like material formation on the enamel surface when it was irradiated with Er,Cr;YSGG laser before fluoride application (Ana et al., 2012). This may be the reason why this combination was able to control the progression of the erosive process in the present study. A study has suggested that monovalent fluoridated compounds act on erosion through the deposition of CaF_2-like material, which would temporarily protect the surface against demineralization. Upon its dissolution, the release of F and Ca would also contribute to remineralization of the eroded substrate (Magalhães, Wiegand, Rios, Buzalaf, & Lussi, 2011). Nevertheless, it should be mentioned that, differently from the study of Ana et al. (2012), in the present study, the choice was to apply the APF gel prior to the laser irradiation, because we hypothesized that the heating caused by irradiation would help to retain more fluoride on the substrate. This hypothesis, however, needs to be further elucidated, as no sign of globular structures suggestive of calcium fluoride could be seen in the ESEM images taken of the fluoride-containing groups post-treatment.

In this study, the group treated with APF gel did not show any ability to prevent the progression of enamel erosion. This may be related to the lower frequency of gel application, which was applied only once before cycling. Although highly concentrated and acidic fluoridated formulations, such as APF gel can predispose to the formation of more CaF_2-like deposits on the tooth substrates (Saxegaard & Rölla, 1988), a study has suggested that its protection may short-lived in highly erosive environments, which would require a frequent application of the agent (Huysmans et al., 2014). In agreement with this idea, in studies were the erosive challenge was milder, such as the model performed by Ramos-Oliveira, Ramos, Esteves-Oliveira, and Freitas (2014), significant protection against erosion was found with APF gel application. In this study, a single 3 min immersion of the specimens in 1% citric acid, pH of 4, was performed after the gel application.

The erosion-remineralization cycling model used in the present study was based on a previous investigation (Pereira et al., 2017) that attempted to simulate individuals with high risk for erosion, due to the high frequency of consuming acidic beverages. An initial erosion lesion was created to simulate a patient who was diagnosed with erosive tooth wear; and the laser and fluoride were measures proposed to control the progression of the process in already affected surfaces (Bezerra et al., 2018).

Although the findings of the present study showed that Er,Cr:YSGG laser-irradiated enamel combined with fluoride could be a promising alternative to prevent enamel erosion, further studies are required for better understanding of the behavior of irradiated enamel against erosion, by using more clinically relevant models. Another aspect that should be considered is whether the rougher and irregular surface resultant of the laser irradiation would facilitate bacteria accumulation. This also deserves to be further explored. While there are some open questions regarding the use of high power lasers for preventing erosion, the protocol tested in this study may be a promising alternative that warrants further investigation, with special focus on its mode of action.

Competing interests

The authors declare that they have no conflict of interest.

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