



A modified Er,Cr:YSGG laser protocol associated with fluoride gel for controlling dentin erosion

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Abstract

Propose Effective strategies to control the development of dental erosion are still needed. This study evaluated the effect of associating a modified Er,Cr:YSGG laser protocol with topical fluoride application on dentin erosion.

Methods Sound and eroded dentin specimens ($n = 10$ /substrate) were allocated into groups: control (no treatment); APF gel (1.23% F⁻, for 1 min, one application, removed with cotton roll); Er,Cr:YSGG laser P1 [0.25W, 20Hz, $\cong 6.5$ J/cm², 2 mm away from the surface, two irradiation of 10 s each, with sweeping movements, under 25% air, without water, with a sapphire tip measuring 750 μ m in diameter and with of 6 mm (S75)]; Er,Cr:YSGG laser P2 (same settings with P1 except 1 mm away from the surface and $\cong 8.3$ J/cm²); APF gel before Er,Cr:YSGG laser P1; APF gel before Er,Cr:YSGG laser P2. Specimens underwent a 5-day erosion-remineralization cycling. Erosion depth (surface loss — SL) was determined. Environmental scanning electron microscopy images ($n = 2$) were obtained. Data were statistically analyzed ($\alpha = 0.05$).

Results Sound substrate: APF gel presented lowest SL, differing significantly from control and other groups. Laser P1 and P2 had highest SL. Eroded substrate: laser P1 showed highest SL, differing significantly from all other groups. For the control, APF gel, and laser P1, the eroded substrates had significantly higher SL than the sound. For laser P2, SL from sound specimens was higher than the eroded. Melted areas were observed in the laser-treated groups.

Conclusions Modified Er,Cr:YSGG laser parameter was unable to control progression of dentin erosion, not even when it was combined with fluoride.

Keywords Dental erosion · Dentin · Er,Cr:YSGG laser · Fluoride · Optical profilometry

Introduction

Dental erosion, a subject of increasing interest among clinicians and researchers worldwide, is related to the dissolution of minerals that occurs at and near the tooth surface, caused by recurrent episodes of exposure to sources of non-bacterial

acid [1]. The development of erosive tooth wear involves the interaction of several factors, such as the patient's eating and oral hygiene habits, presence of gastro esophageal reflux disease or recurrent vomiting, characteristics of saliva, and use of certain medications [2]. In advanced stages, loss of dental structure may compromise the tooth integrity, resulting in undesirable consequences, such as dentin hypersensitivity [3].

Differently from the caries process, in which demineralization occurs mostly at the subsurface, in dental erosion, the outer superficial portion of the tooth is dissolved, layer by layer. Initially, a loss of surface hardness occurs, resulting in a thin layer of softened tissue [4]. With continuous exposure to acid, or due to the incidence of mechanical impacts, this layer is eventually lost, leaving a demineralized surface at the base of the lesion [5]. In dentin, demineralization

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progresses, leaving a surface layer of insoluble collagen matrix that has been found to be resistant to mechanical impacts [6].

For the prevention of erosion, the use of fluoride toothpastes, rinses, gels, and varnishes have been extensively studied [7–9]. Considering the thinness of the remaining softened layer, which leaves little room for mineral deposition, the most likely mode of action of these products is to provide surface protection [10]. Depending on their composition, pH, and concentration, fluoridated products are capable of inducing the formation of precipitates on tooth surfaces, which would act as a first barrier against the erosive acids. These deposits would also serve as an ionic reservoir, releasing fluoride and calcium into the environment at the time of the erosive challenge, favoring mineral deposition [10]. However, as the erosive challenges are aggressive, fluoride provides only short term protection; therefore, frequent applications are usually necessary, suggesting a preference for over-the-counter fluoride products [10].

Whereas products intended for professional application have higher fluoride concentrations, and although they can increase the chances of fluoride toxicity [11], their use does not depend on patient cooperation [8]. Studies have shown evidence that these products were relatively effective against erosion [12, 13], which makes it increasingly desirable to increase their protective effect. This could be done by associating them with other therapies, such as high-power lasers [9]. When combined with fluoride, high-power laser irradiation may potentially increase the amounts of fluoride deposited on and incorporated into the dental substrate [14]. In the context of caries, high power lasers have been shown to be capable of increasing the acid resistance of dental surfaces, leading to a reduction in the caries process [15]. However, where dental erosion is concerned, further investigation is needed to determine the protective effect of the high power lasers, especially when used on the dentin substrate [16].

A previous investigation observed that the association of Er,Cr:YSGG laser irradiation (with the following parameters: 0.50 W, 20 Hz, 5.7 J/cm², 1136 W/cm², tip S75, beam diameter of 750 µm, pulse width of 60 µm, with 30% air without water, 1 mm away from the surface) with fluoride application was able to control the progression of enamel erosion; however, when the two treatments were used alone, no effect was detected. Fluoride was applied before using the laser, because the authors hypothesized that the high temperature induced by the laser could promote an increased retention of fluoride on the enamel surface [17]; however, for dentin, no significant protective effect could be observed. When visualizing the images in the micrographs, as opposed to the observations on enamel, it seemed that the laser caused ablation of the eroded dentin substrate, even at a lower energy density than that used on enamel (0.25 W, 20 Hz, 2.8 J/cm², tip S75, beam diameter of 750 µm, 1 mm

away from the surface) [16], which was the lowest possible value obtainable with the equipment used, considering that irradiation was performed in focused mode.

In view of the promising results observed for the combination between Er,Cr:YSGG laser and fluoride for the purpose of preventing enamel erosion, finding a suitable parameter for dentin is warranted. One option to avoid undesirable dentin loss resulting from irradiation would be to apply the laser in unfocused mode. This would reduce the energy absorbed by the tissue and prevent overheating. Thus, the aim of this *in vitro* study was to evaluate the effect of a modified Er,Cr:YSGG laser protocol, associated with fluoride application or not, on the prevention and control of dentin erosion. The null hypotheses were (1) the different laser protocols would not be able to prevent or control the progression of dentin erosion and (2) the different laser protocols would not be able to increase the protective effect of fluoride against dentin erosion.

Materials and methods

Specimen preparation

A total of 120 bovine root dentin slabs (4 mm × 4 mm × 2 mm) were sectioned using an automatic sectioning machine (Isomet, Buehler, Lake Bluff, IL, USA). The pulp surfaces of the specimens were flattened in a polishing machine (Tegramin 30, Struers Inc., Ballerup, Denmark), fitted with a #800-grit abrasive discs (Struers Inc.), under constant water cooling. Subsequently, the buccal surfaces were ground flat and polished using a sequence of abrasive discs with range of decreasing granulations, #800, #1200, and #4000 (Struers Inc.), and polishing cloth sprayed with diamond suspension (1 µm, Struers Inc.) for 3 min. After using each abrasive disc and at the end of the polishing procedures, the specimens were sonicated with distilled water for 3 min. All the specimens were analyzed with an optical profilometer to select those with curvature below 0.3 µm and specimens without fractures or any other visual imperfections. In half of the selected specimens (60 specimens), with the purpose of producing an initial erosion lesion *in vitro*, unplasticized polyvinyl chloride (UPVC) tapes were placed on their polished surfaces (Fig. 1), leaving a central window of 4 mm × 1 mm exposed. Then the specimens were immersed in 1% citric acid solution (pH~2.4), at room temperature for 10 min. After immersion, the specimens were rinsed with deionized water. The specimens were analyzed with an optical profilometer to select those with surface loss values ranging from 3 to 5 µm. The selected specimens were randomly assigned to the 12 experimental groups (*n* = 10/substrate).

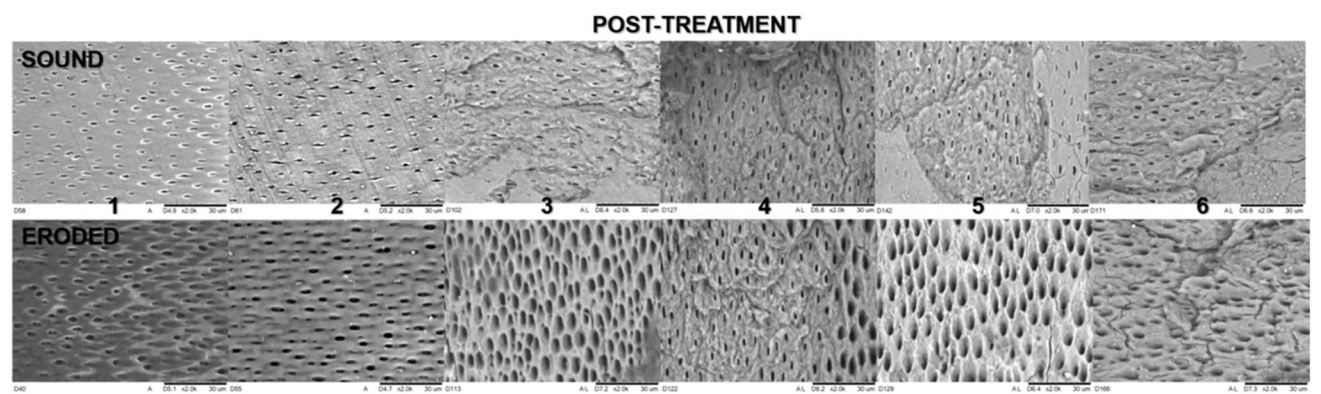


Fig. 1. Representative micrographs obtained for all the groups post-treatments at $\times 2000$ magnification. **a** Sound dentin and **b** eroded dentin. 1, control; 2, APF gel; 3, Er,Cr:YSGG laser P1; 4, Er,Cr:YSGG laser P2; 5, APF gel + Er,Cr:YSGG laser P1; and 6, APF gel + Er,Cr:YSGG laser P2

Surface treatments

This study was based on a completely randomized experimental design with two experimental factors, as shown in Table 1.

Tapes were once more placed on the polished surfaces of all the specimens. APF gel (acidulate phosphate fluoride, 1.23% F, pH: 3.6–3.9, Nova DFL, Rio de Janeiro, RJ, Brazil) was applied once with the aid of swabs for 1 min and then removed with cotton rolls. Regarding laser irradiation, the equipment used was an Er,Cr:YSGG laser, (model Waterlase iPlus, Biolase, San Clemente), wavelength of 2.78 μm , pulse width of 60 μs (H/short pulse mode) and repetition rate of 20 Hz and a power rate ranging from 0 to 10 W. The energy was delivered through an optical fiber with a beam diameter of 430 μm , with a sapphire tip measuring 750 μm in diameter and with of 6 mm (S75). For the modified parameter P1, the following protocol was used: power of 0.25 W, repetition rate of 20 Hz, energy density of $\cong 6.5 \text{ J/cm}^2$, 2 mm away from the surface, in unfocused mode, under 25% air, without water. The protocol for parameter P2 was as follows: power of 0.25 W, repetition rate of 20 Hz, energy density of

$\cong 8.3 \text{ J/cm}^2$, 1 mm away from the surface (focused mode), under 25% air, without water. For both parameters (P1 and P2), two irradiations were performed, vertically and horizontally, each one for 10 s. So, a 20-s irradiation time in total was performed. The equipment power was checked with a power meter before each irradiation. Ten-second irradiations were performed, making three horizontal sweeping movements, covering the entire surface of the lesion formed or the surface that was about to be submitted to cycling. In the groups in which APF gel was associated with Er,Cr:YSGG laser, the gel was applied immediately before laser irradiation. After treatments application, specimens were once again analyzed by optical profilometry.

Erosive challenge

The effect of the treatments on reducing erosion was tested in an erosion-remineralization model for 5 days, using dentin specimens ($n = 10/\text{substrate}$) obtained from bovine incisor roots. Twelve well cell culture plates were used to allow simultaneous immersion of all the specimens in the solutions. After treatments had been applied, all specimens were attached to

Table 1. Experimental factors

Surface treatment (6 levels)	Type of dentin substrate (2 levels)
1. Negative control (no treatment)	1. Sound
2. APF gel (acidulated phosphate fluoride, 1.23% F, pH 3.6–3.9) applied once for 1 min, and then removed	2. Eroded
3. Irradiation with Er,Cr:YSGG laser [parameter P1 (modified): 0.25W, 20Hz, $\cong 6.5 \text{ J/cm}^2$, tip S75, beam diameter of 750 μm , 2 mm away from the surface, unfocused mode, under 25% air, without water, using two 10 s irradiations, under sweeping movements]	
4. Er,Cr:YSGG laser irradiation (parameter P2: 0.25W, 20Hz, $\cong 8.3 \text{ J/cm}^2$, tip S75, beam diameter of 750 μm , 1 mm away from the surface, focused mode, under 25% air, without water using two 10 s irradiations, under sweeping movements)	
5. APF Gel + Er,Cr:YSGG laser parameter 1	
6. APF Gel + Er,Cr:YSGG laser parameter 2	

the lids of the plates, using sticky wax. With the purpose of simulating a patient at high risk for dental erosion, the specimens were immersed in 0.3% citric acid (pH~2.6), for 5 min, followed by a 60-min immersion in artificial saliva. This procedure was repeated four times a day for 5 days. The artificial saliva was renewed each day before the cycle began and the acid was renewed after each episode of exposure [18].

Profilometric analysis

For post-treatment and post-cycling, the tapes were removed from the specimens and their surfaces were analyzed. The data was obtained by using the instrument sensor (S11/03, resolution of 0.012 μm) to scan an area measuring 2 mm long (x-axis) and 1 mm wide (y-axis), located at the center of the specimen. The equipment was set to travel 200 steps along the x-axis, with each step measuring 0.01 mm. In the y-axis, there were 20 steps measuring 0.05 mm each. The depth of the treated area was calculated using a dedicated software (Proscan Application software v. 2.0.17) that subtracted the mean height of the test area from the mean height of the two reference surfaces. The specimens were scanned in a moistened condition to prevent collagen shrinkage.

Environmental scanning electron microscopy (ESEM)

To qualitatively evaluate the post-treatment surface morphology of the specimens, two randomly selected specimens from all the groups were further analyzed by ESEM. Representative micrographs were taken at 2000 $\times$ magnification in the center of each specimen, using the Analy observation conditions (15 Kv). No specimen preparation was required.

Statistics

Normality and homoscedasticity of surface loss (SL) data were checked with the Shapiro-Wilk and Brown-Forsythe tests, respectively. Since data did not follow a normal distribution, they were converted to Log, and then evaluated by two-way ANOVA and Tukey tests, considering a significance level of 5%. The software SigmaPlot 13.0 (Systat Software Inc., Chicago, IL, USA) was used for all calculations.

Results

Profilometry

The mean (*SD*) of the baseline specimen profile values was 0.08 μm (0.07). The mean (*SD*) of dentin specimen SL values after initial lesion formation was 4.61 μm (1.34).

The means (*SD*) of dentin SL for the groups in the sound and eroded specimens are presented in Table 2. There was significant difference among the levels of the factor treatments ($p < 0.001$), and in the interaction between treatment and substrate ($p < 0.001$). There were no significant differences between the levels of the factor substrate ($p = 0.185$).

For the factor treatments, in the sound substrate, Group F showed the lowest SL value, differing significantly from the control ($p = 0.002$) and from the other groups ($p < 0.001$). Laser P1 and laser P2 showed the highest SL values, without significant difference between them ($p = 0.994$). Laser P2 also did not differ significantly from F + laser P2 ($p = 0.099$). F + laser P1 and F + laser P2 did not differ significantly from the control group ($p = 0.611$ and $p = 0.172$, respectively). In the eroded substrate, group laser P1 showed the highest SL value, differing significantly from all the other groups ($p < 0.05$). There were no significant differences among the other groups ($p > 0.05$).

For the factor substrate, in groups control, F and laser P1, the eroded substrates had significantly higher SL values than the sound types ($p = 0.023$; $p < 0.001$; and $p = 0.045$, respectively). For group laser P2, the SL value of the sound was higher than that of eroded substrate ($p < 0.001$). For groups F + laser P1 and F + laser P2, there were no significant differences between the eroded and sound substrates ($p = 0.67$ and $p = 0.525$, respectively).

ESEM evaluation

In the ESEM image post-treatment (Fig. 2), the specimens of the control group showed a regular surface, with many opened dentin tubules, which had increased diameters in the eroded specimens, when compared with those of the sound types. In group APF gel, the morphology was similar to that of the control, but the presence of some particles on dentin could be observed, which were suggestive of calcium fluoride-like deposits. In the laser-treated groups of sound specimens, both parameters used showed areas of melting and re-solidification, in which no dentin tubules could be

Table 2. Means (*SD*) of dentin SL (in μm) for the groups in the sound and eroded specimens. In columns, different capital letters denote significant difference among groups, within substrate ($p < 0.05$). In rows, different lower-case letters denote significant difference between substrates, within groups ($p < 0.05$).

	Sound		Eroded	
Control	10.67 (2.47)	Cb	14.76 (2.40)	Ba
F	6.73 (3.55)	Db	10.52 (2.55)	Ba
Laser P1	24.79 (8.33)	Ab	32.01 (6.64)	Aa
Laser P2	22.03 (4.55)	ABa	13.46 (5.64)	Bb
F + laser P1	13.28 (2.18)	Ca	13.28 (5.13)	Ba
F + laser P2	15.11 (3.97)	BCa	13.72 (3.05)	Ba

seen. For the eroded substrate, melted areas were also visible, but the dentin tubules were enlarged and had an etched appearance. In the APF + laser-treated groups, for both parameters the same surface characteristics of laser-treated groups were observed for sound and eroded dentin. For all groups treated with laser (laser-treated and APF + laser groups), ablation was not observed, just a minor modification of the surface and dehydration of the dentinal tubules due to the temperature.

Discussion

The majority of studies testing high power lasers for use against dentin erosion were conducted with sound dentin specimens [19, 20]. However, clinically, high power laser irradiation would most probably be used on established erosion lesions for the purpose of controlling their progression. Furthermore, a previous study has shown that the laser protocols usually applied on sound dentin might have a different effect on eroded dentin [12]. Considering that eroded dentin has more water and less mineral content than sound dentin, the absorption of laser by these substrates might not be the same [21]. Thus, it is necessary to determine specific protocols for this modified type of substrate. In this present study, irrespective of the type of substrate, none of the laser protocols tested showed an ability to protect the dentin from erosion, not even when associated with fluoride; therefore, both of our null hypotheses were accepted.

In a previous study, promising results were observed with the association between fluoride and the Er,Cr:YSGG laser (0.5 W, 5.7 J/cm²) for preventing the progression of enamel erosion [17]. However, for dentin, no suitable parameter has yet been found [16]. The distinct results found for both substrates could be related to their different morphological aspects. On enamel, it was shown that Er,Cr:YSGG laser irradiation at 8.5 J/cm² was able to increase the acid resistance of the substrate against cariogenic acids, but lower energy densities have also produced a more resistant substrate [22]. Since enamel is 85% carbonated hydroxyapatite, with 12% water and 3% protein and lipid by volume [23], laser irradiation, which is absorbed by water and hydroxyapatite, is expected to promote an increase in temperature that is high enough to modify the chemical structure of enamel and reduce its solubility [22]. When used with irradiation protocols similar to those of the present investigation, Er,Cr:YSGG laser irradiation has been shown to be capable of reducing the carbonate and organic content of enamel, in addition to promoting water loss [24]. The formation of tricalcium phosphate and tetracalcium phosphate have also been reported [25], and these changes may have a substantial effect on reducing the solubility of the substrate. Furthermore, melted areas have been observed with

the Er,Cr:YSGG laser irradiation on enamel with the use of sub-ablative parameters, which could potentially reduce the diffusion pathways of the acids [26]. On dentin, increased acid resistance has been observed when Er,Cr:YSGG laser irradiation at 4.64 J/cm² and 8.92 J/cm² (without water cooling) was associated with 2% NaF [27]. The findings suggested that the absence of water cooling increased the temperature at the dentin surface, resulting in effects similar to those found for enamel. In addition, the dentinal tubules were sealed through a mechanism of melting and re-solidification, thereby reducing the permeability of the tissue, hindering the penetration of the acids into the deeper layers of dentin [27].

In a previous investigation, it was observed that Er,Cr:YSGG laser parameters that were suitable for enamel were extremely aggressive for dentin [16]; thus ablation instead of surface modification was detected. Taking this result into consideration in the present study, the parameter was changed and the lower settings achieved by the equipment were tested (0.25W, 20Hz, 2.8J/cm²), with the aim of minimizing the laser ablation side effects on dentin tissue. This change included a reduction in the energy delivered to the tissue, by unfocusing the tip of the laser (irradiation was performed 2 mm away from the surface) to prevent overheating of the tissue. Nonetheless, considering that the laser-treated groups showed no significant reduction in surface loss when compared with the control, for both sound and eroded dentin, it seems that other treatment options should be considered.

For erbium lasers, the irradiation distance is known to be capable of affecting its preventive potential against demineralization [28]. The majority of studies using Er,Cr:YSSG laser for preventing erosion have used a focused mode. By increasing the distance of irradiation from the tissue in parameter 1 (modified), irradiation was performed 2 mm away from the dentin surface, which could have enhanced the perimeter affected by the laser. At same time, however, the energy was less concentrated. Moreover, it should be pointed out that no water irrigation was used — a procedure which is thought to increase the laser interaction with the dental hard tissues, thereby, its ablative effect. Nevertheless, air was used to avoid overheating the tissue [29].

Surface loss from the sound specimens was higher than that from the eroded types in group laser P2, although the eroded specimens had an initial erosion lesion approximately 2–5 µm deep. These finding needs to be better explored in future investigations because, due to the higher water content, eroded dentin was expected to show higher surface loss values. It well known that the ablation of dental hard tissues with Er,Cr:YSSG laser occurs through the absorption of laser energy by water molecules within the dental tissues. This would increase the vibration of these molecules, resulting in higher temperature and higher internal pressure [30].

Perhaps the eroded dentin was already so demineralized when the laser irradiation with this high energy parameter was performed, so that the internal pressure within this tissue was lower than that of sound dentin, thereby reducing the amount of tissue removed.

One study observed that the firmly bound fluoride integrated into the crystalline structure might increase the crystal stability and its acid resistance [27]. In addition, the tightly bound fluoride can serve as a fluoride reservoir [14, 27]. For dentin, the association of a fluoride varnish (5% NaF) with Er,Cr:YSGG laser (parameter: 0.5 W, 5 Hz, air 55%, focused mode) reduced dentin demineralization that had occurred after 5 min of immersion in citric acid, with 6 h interval, twice a day, for 14 days before laser treatment began, leaving the surface more acid resistant [31]. However, in the present study, this synergism was not observed. Although some reduction in surface loss from both types of substrates treated with APF + Er,Cr:YSGG laser P1 was found, when compared with Er,Cr:YSGG laser P1; the result of APF + Er,Cr:YSGG laser P1 was not significantly better than that of the control. A similar fact was observed for APF + Er,Cr:YSGG laser P2. This lack of effect could be due to the high aggressiveness of the erosive challenges performed in this present study, in which the acid immersion was performed at more frequent intervals (5 min immersion, with 1 h interval, 4 times a day), in addition to the lower frequency of fluoride and laser application (only once, pre-cycling). The erosive cycling used in the present study was an attempt to simulate a situation of high risk for erosion, similar to the situation used in our previous investigation on enamel [17], allowing a better comparison of results.

It should, however, be noted that the groups treated with the association of fluoride with the Er,Cr:YSGG laser irradiation had a tendency to reduce the progression of surface loss from the eroded substrates, as their values were not significantly different from those of the sound specimens, which started the cycling without any initial erosion lesion. This indicated that this association was somewhat positive in controlling the process, however, not up to a point that it could differ significantly from the control. A possible explanation, based on the idea that the eroded dentin would be able to retain more fluoride than sound dentin would retain, might be that it was an effect that was potentiated by the laser [31].

In the fluoride-treated groups, significant protection against erosion was observed only for the sound dentin; however, on the eroded substrate, there was a (non-significant) trend towards protection. This could be attributed to the low frequency of gel application, as it was applied only once before cycling. Monovalent fluoride compounds act on erosion mainly by protecting the surface by means of fluoride deposition such as CaF_2 -like precipitates that would act as

a first barrier against erosive acids. These deposits can also serve as a reservoir, releasing fluoride and calcium into the medium at the time of the erosive challenge [10]. However, since several erosive challenges were performed, it could be suggested that these deposits were lost [32], therefore, offering little or no protection.

The findings of the present study showed that the protocols of Er,Cr:YSGG laser irradiation tested, alone or combined with fluoride, could not prevent the progression of dentin erosion. Considering the controversial results relative to the use of high-power lasers for preventing erosion, further investigation is suggested, with special focus on the most appropriate laser parameters and the combination with different types of fluoride compounds.

In conclusion, considering the limitations of this laboratory study, the modified Er,Cr:YSGG laser parameter tested was unable to control the progression of dentin erosion, not even when it was combined with fluoride.

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Denise Maria Zezell: laser irradiation and manuscript review

Ana Cecília Corrêa Aranha: manuscript final review

Taís Scaramucci: conception and design of the study, data analysis, and manuscript final review

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Declarations

Conflict of interest The authors declare no competing interests

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