



# Evaluation of a clinical preventive treatment using Er,Cr:YSGG (2780 nm) laser on the susceptibility of enamel to erosive challenge

Dimitrios Dionysopoulos<sup>1</sup> · Kosmas Tolidis<sup>1</sup> · Dimitrios Strakas<sup>1</sup> · Thrasyvoulos Sfeikos<sup>1</sup>

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## Abstract

The purpose of this in vitro study was to evaluate the effect of a clinical preventive treatment using Er,Cr:YSGG laser irradiation on bovine enamel susceptibility after erosive challenge. Twelve sound bovine incisors were used and twenty-four enamel specimens were prepared in total. Two experimental groups ( $n = 12$ ) were assigned as follows: Group 1 was the control group and in Group 2, the enamel specimens were irradiated with an Er,Cr:YSGG (2780 nm) laser system for 20 s, with average output power of 0.25 W, pulse repetition rate at 20 Hz without water or air flow and the pulse duration was fixed at 140  $\mu$ sec. The tip diameter was 600  $\mu$ m, the tip to tissue distance was 1 mm, the speed of handpiece movement was 2 mm/s, the power density was 88.34 W/cm<sup>2</sup>, and the fluence was 31.25 J/cm<sup>2</sup>. The specimens were submitted to erosive challenge using a common soft drink. Surface microhardness changes, surface roughness changes, and surface loss were evaluated after erosive challenge. The data were statistically analyzed using one-way ANOVA and Tukey's *post-hoc* test at a level of significance  $\alpha = 0.05$ . Er,Cr:YSGG laser-treated enamel exhibited significantly less decrease in surface microhardness and significant less surface loss compared to control enamel after the erosive challenge ( $p < 0.05$ ). The experimental groups did not show significant differences in surface roughness increase after the erosive challenge ( $p > 0.05$ ). Er,Cr:YSGG laser treatment may be promising for the limitation of enamel erosive tooth wear induced by excessive consumption of soft drinks. Clinical studies are needed to clarify whether this protective effect is clinically significant.

**Keywords** Er,Cr:YSGG laser · Enamel erosion · Soft drinks · Surface loss · Surface microhardness · Surface roughness

## Introduction

Erosive tooth wear is defined as a chemical-mechanical process which leads to a cumulative loss of hard dental tissues not attributed to oral bacteria [1]. In previous years, it was proposed that enamel surface loss, occurred by a constant layer-by-layer dissolution, which was enhanced as the acidic activity, was repeated more often and lasted for longer time [2]. Recently, new concepts have been suggested for the mechanism of the erosive tooth wear, which deal with the fact that erosive demineralization on enamel

takes place not only at the enamel/acidic agent interface, but also within a partly demineralized thin layer beneath the enamel surface, during a process namely near-surface demineralization [3]. Moreover, the critical pH value of enamel erosive wear is calculated from the calcium and phosphate concentrations in the erosive solution itself, which means that it depends on the composition of this erosive solution [4].

The etiology of erosive tooth wear includes a combination of various chemical, biological, and behavioral factors which affect its pathogenesis and different interactions of these factors are attributed the different clinical expressions in patients [5]. Clinically, erosive tooth wear is often characterized by a loss of the natural morphology and contour of the tooth. In initial stages, a shiny, silky-glazed, dull, and excessively smooth enamel surface can be observed [2]. In advanced stages, the clinical symptoms are more intense and include cupping of the cusps of the posterior teeth and flattening of the occlusal surfaces, while

✉ Dimitrios Dionysopoulos  
ddiondent@gmail.com

<sup>1</sup> Department of Operative Dentistry, School of Dentistry, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

the smooth surfaces appear flattened with an intact rim along the gingival margin and concavities may also be detected [1].

The mechanism of dental erosion begins with an attack of hydrogen cations ( $H^+$ ) derived from the acidic agents on enamel surfaces [6]. More specifically, when a solution with pH below the critical pH value of enamel ( $<5.5$ ) comes in contact with tooth enamel, the hydrogen cations start to form chemical bonds with carbonate ( $CO_3^{-2}$ ) and phosphate ( $PO_4^{-3}$ ) anions of hydroxyapatite crystals leading to their dissolution of the surface and diffuse into the interprismatic areas of enamel and dissolve minerals in the subsurface region. As a consequence, a gradual softening and loss of enamel volume takes place [7].

In order to prevent erosive tooth wear, firstly, causal therapy is needed comprising the identification and elimination of the causal factors, and secondly, an individually tailored preventive program including measures such as diet modification, instructions of oral hygiene, fluoride use, and regular dental supervision has to be scheduled [8]. Currently, numerous clinical methods have been suggested to limit the progress of erosive tooth wear by increasing the resistance of tooth tissues to erosive agents. Most of those methods act by modifying the tooth surfaces in a way that hydroxyapatite crystals become less acid soluble [3] or by forming protective layers on the tooth surfaces [9]. Monovalent and polyvalent fluorides have been used for different vehicles such as mouthrinses, toothpastes, gels, or varnishes and the most effective formulations were the high-concentrated, acidic, and polyvalent fluorides [10].

The use of laser technology has already contributed to this purpose. In particular, the possibility of increasing enamel resistance to demineralization after laser irradiation has been investigated in previous studies using carbon dioxide ( $CO_2$ ) laser [8], erbium:chromium:yttrium-scandium-gallium-garnet (Er,Cr:YSGG) laser [11], erbium:yttrium-aluminum-garnet (Er:YAG) laser [12], and neodymium:yttrium-aluminum-garnet (Nd:YAG) laser [13]. The preventive laser treatments against enamel erosion are in-office clinical techniques and as a result, the main advantages are the shorter time of the therapy and their implementation exclusively by the dentist.

The aim of this in vitro study was to evaluate the effect of a clinical preventive treatment using Er,Cr:YSGG laser irradiation on enamel susceptibility after erosive challenge induced by a soft drink. For this reason, we evaluated enamel surface microhardness and surface roughness changes, as well as enamel surface loss. Moreover, scanning electron microscopy (SEM) observation and energy dispersive X-ray spectroscopy (EDS) analysis of the mineral composition of the enamel were also performed for better explanation of the outcomes of the study.

Three null hypotheses were formulated prior to the experiment. The first null hypothesis ( $H_{01}$ ) was that there were no significant differences in surface microhardness decrease between the experimental groups of the study after the erosive challenge. The second null hypothesis ( $H_{02}$ ) was that there were no significant differences in surface roughness increase between the experimental groups after the erosive challenge. Finally, the third null hypothesis ( $H_{03}$ ) was that there were no significant differences in surface loss between the experimental groups after the erosive challenge.

## Materials and methods

### Specimens' preparation

Twelve sound bovine incisors were carefully extracted and stored in a 0.5% chloramines T solution at 6 °C for up to 1 month. The crowns were separated from the roots, and each crown was sectioned into two halves using a water-cooled diamond disc (Isomet, Buehler, Lake Bluff, IL, USA). The dimensions of each enamel specimen were 4 mm long, 4 mm wide, and 1.5 mm height. The 24 tooth fragments were not allowed to be dehydrated and observed by means of optical microscope for any detection of surface structural deficiency. The specimens were distributed into two groups ( $n = 12$ ) in a way that each group included one fragment of each tooth. Subsequently, the specimens were embedded in epoxy resin (Epofix resin, Struers Tech A/S, Denmark) with the facial or lingual surface facing up and were ground and polished on a polishing machine (Jean Wirtz TG 250, Dusseldorf, Germany) using up to 1200 grit silicon carbide abrasive papers (Struers, Copenhagen, Denmark). After polishing, the specimens were immersed in an ultrasonic bath (Euronda Spa, Montecchio Precalcino, Vicenza, Italy) to remove any impurities and stored in a remineralizing solution for 24 h at 37 °C. The composition of the remineralizing solution was according to Dionysopoulos et al. [14]: 0.103 g/l of  $CaCl_2$ , 0.019 g/l  $MgCl_2 \cdot 6H_2O$ , 0.544 g/L  $KH_2PO_4$ , 2.24 g/l KCl, and buffer (TCP-KOH) were added to adjust the pH to 7. This solution was used for the storage of the specimens during the experimental period.

### Experimental groups

The two experimental groups ( $n = 12$ ) of the study were the following:

- 1) Group 1 specimens (control group) did not receive any treatment during the experimental period.
- 2) Group 2 specimens were irradiated with an Er,Cr:YSGG (2780 nm) solid-state laser system (Waterlase MD Turbo, BIOLASE, Irvine, CA, USA). The power output of the

device was 8 W, the beam divergence was  $\alpha_{\text{total}} = 14^\circ$ , and the energy distribution follows the Gaussian profile. The pulse repetition rate was 20 Hz without water or air flow and the pulse duration was fixed at 140  $\mu\text{s}$ . The beam diameter at the focal area for the handpiece was 600  $\mu\text{m}$ . A Z-type glass cylindrical tip (MZ6) with a 600  $\mu\text{m}$  diameter and 6 mm length, which was adapted to a gold handpiece of the laser system, was positioned 1 mm from the enamel surface (focused mode). To ensure consistent spot size with the hand irradiation, an endodontic file was fixed at the handpiece, and kept a distance of 1 mm from the surface during irradiation. The energy per pulse was 12.5 mJ and the peak power was 89.28 W. The handpiece was positioned perpendicularly to the enamel surface, and the samples were irradiated by hand once in each direction in a scanning mode, moving the handpiece slowly horizontally and vertically with a speed of 2 mm/s, in purpose to promote homogeneous irradiation and to cover the entire specimen area. All the specimens were irradiated by the same researcher. The irradiation time was 20 s (10 s vertically and 10 s horizontally) and the average output power was 0.25 W, which yielded to a power density of 88.34 W/cm<sup>2</sup> and fluence was 31.25 J/cm<sup>2</sup>. The laser parameters were adjusted according to Geraldo-Martins et al. [11].

## Erosive challenge

For the experiment, a common soft drink (Coca Cola, 3E Company, Greece) was used as erosive agent. The composition of the soft drink was as follows: water, sugar, carbon dioxide, caramel color E 150d, phosphoric acid, natural flavors, and caffeine. Each enamel specimen was rinsed with distilled water for 10 s and immediately immersed into 6 ml of fresh soft drink in a plastic container for four intervals of 2 min and then rinsed again with distilled water and stored in fresh remineralizing solution. The erosive challenges were applied at 0, 12, 24, 36, 48, and 60 h. This cycling treatment was according to Wang et al. [15]. The pH of the soft drink was measured using a digital pH-meter (Orion Star™ Series ISE Meter, Thermo Scientific, Beverly, USA) and was stable (pH = 2.47 ± 0.09) for at least 15 min at room temperature (23 ± 1 °C).

## Evaluation of surface microhardness

Surface microhardness of each enamel specimen was evaluated prior and after the erosive challenge. For laser-treated specimens (Group 2), surface microhardness was also evaluated after laser irradiation. The method used for the assessment of surface microhardness was the Vickers method with a hardness tester (HMV-2000, Shimadzu, Tokyo, Japan) at a load of

200 g and indentation time of 10 s. Five indentations were carried out on the top surface of each enamel specimen, one in the center and one in every quadrant (500  $\mu\text{m}$  apart). Data were independently averaged and reported in Vickers Hardness Numbers (VHN), which is defined by the ratio  $F/A$ , where  $F$  is the force applied to the diamond in kilogram-force and  $A$  is the surface area of the resulting indentation in square millimeter:  $VHN = F/A = 1.8544F/d^2$  where  $d$  is the diagonal of the indentation.

## Evaluation of surface roughness

Surface roughness of the enamel specimens was evaluated according to ISO 25178 (non-contact type), which is related to the analysis of 3D areal surface textures. The measurements were made prior and after the erosive challenge. Likewise as surface microhardness test, for laser-treated specimens (Group 2), surface roughness was also evaluated after laser irradiation. Surface roughness was evaluated by Vertical Scanning Interferometry (VSI) using an optical profilometer (Bruker, ContourGT, Berlin, Germany). Three images were obtained from each specimen (magnification × 20) in the four quadrants of the enamel surface which correspond to a surface of 0.317 × 0.238 mm<sup>2</sup>. Vision64™ software (Bruker, ContourGT, Berlin, Germany) was used to acquire the data and compute the mean surface roughness in Sq units of each image. The values of the 12 images of each specimen were averaged and the mean value was calculated. The root mean square height (Sq) parameter is defined as the root mean square value of the surface departures,  $z(x,y)$ , within the sampling area,  $A$ :  $Sq = \sqrt{1/A \iint_A z(x,y) dx dy}$ .

## Surface loss measurements

Before the erosive challenge, half of each enamel surface of the specimens was covered with one-sided silver adhesive tape (Wonder® Tape, P.V.C. Electrical Tape). Measurements were carried out using VSI method following the erosive challenge. After removal of the adhesive tape, four images were obtained in the center of each specimen's surface. The enamel surface loss was calculated after superimposing the baseline and post-erosion profiles. The depth of the eroded area for each specimen was calculated based on the subtraction of the two profiles. The measurements were performed by two different researchers who did not know which experimental group was tested.

## SEM and EDS analysis

Aiming to investigate the modification of the enamel surface after the laser treatment, three specimens of each experimental group were prepared and mounted on aluminum stubs, sputter coated with carbon to a thickness of approximately 200 Å in a

vacuum evaporator (at low vacuum), and examined under Scanning Electron Microscope (JEOL Ltd., JSM-840, Tokyo, Japan) at accelerated voltage of 19 kV. Photomicrographs were performed using  $\times 2000$  magnification on the surface area of the enamel specimens in order to detect any changes in surface morphology after the laser treatment. The alterations of mineral composition of the enamel specimens after the laser treatment were also evaluated using Energy Dispersive X-ray Spectroscopy (EDS).

### Statistical analysis

The sample size of each testing was calculated considering 80% power and a significance level of 0.05. The data were statistically analyzed using SPSS Statistics 20.0 software (IBM Corp, ILL, Chicago, USA). Data were preliminary tested for normality and homogeneity using Shapiro-Wilk test and Levene test, respectively. Surface hardness, surface roughness, and surface loss data of the enamel specimens were statistically analyzed using one-way ANOVA and Tukey's *post-hoc* test was used to detect statistical differences at a level of significance  $\alpha = 0.05$ .

## Results

### Surface microhardness

Means and standard deviations of surface microhardness (VHN) of the experimental groups of the study are presented in Table 1. Surface microhardness was significantly reduced in both experimental groups after the erosive challenge ( $p < 0.05$ ). Laser treatment group exhibited significantly less decrease in surface microhardness compared to the control group after the erosive challenge ( $p < 0.01$ ). This decrease was 7.5% less than that of control group. In addition, after the Er,Cr:YSGG laser irradiation, the enamel surface microhardness was not changed significantly ( $p > 0.05$ ).

### Surface roughness

Means and standard deviations of surface roughness (Sq,  $\mu\text{m}$ ) of the experimental groups of the study are shown in Table 2.

**Table 1** Means and standard deviations of surface microhardness (VHN) of the experimental groups of the study before and after the erosive challenge. The same uppercase superscripts in columns indicate

Groups	Before laser irradiation	Before erosive challenge	After erosive challenge	Mean $\Delta$ VHN	Mean %VHN decrease
Control	–	278.4 $\pm$ 19.2 <sup>Aa</sup>	156.6 $\pm$ 21.5 <sup>Ab</sup>	121.8 $\pm$ 24.2 <sup>A</sup>	43.8%
Er,Cr:YSGG laser treatment	282.5 $\pm$ 24.9 <sup>a</sup>	277.1 $\pm$ 26.2 <sup>Aa</sup>	175.8 $\pm$ 13.6 <sup>Bb</sup>	101.3 $\pm$ 22.3 <sup>B</sup>	36.3%

$\Delta$ VHN, reduction of surface microhardness in VHN after the erosive challenge; %VHN decrease, % decrease of surface microhardness after erosive challenge

Surface roughness was significantly increased in both experimental groups after the erosive challenge ( $p < 0.05$ ). However, the laser treatment group did not present significant different increase in surface roughness compared to the control group ( $p > 0.05$ ). Moreover, in the laser treatment group, enamel surface roughness significantly increased after laser irradiation ( $p < 0.05$ ) around 0.013 Sq.

### Surface loss

Means and standard deviations ( $\mu\text{m}$ ) of surface loss of the experimental groups of the study after the erosive challenge are presented in Table 3. Representative topographic surface maps ( $\times 20$  magnification) and surface analysis of the experimental groups of the study are shown in Fig. 1a–b. Surface loss was observed in both experimental groups after the erosive challenge. The laser treatment group presented significant less surface loss than control group ( $p < 0.01$ ). This decrease in surface loss was approximately 25.1%.

### SEM observations and EDS analysis

Representative photomicrographs of the enamel surface of the experimental groups of the study are illustrated in Fig. 2a–b. SEM images revealed alterations in enamel surface morphology after the laser treatment. The EDS spectrum of each representative photomicrograph of the enamel surfaces also appears below the SEM images. EDS analysis indicated that there was a significant decrease in Ca concentration of the enamel surface after the laser treatment compared to the control group ( $p < 0.05$ ) as shown in Table 4.

## Discussion

According to the results of the present study, H<sub>0</sub>1 was rejected. After the erosive challenge, the experimental groups presented reduced enamel surface microhardness. This reduction may be attributed to the loss of minerals of the enamel surface due to the activity of the acidic components of the soft drink. This is in agreement with previous studies [16, 17]. Er,Cr:YSGG laser treatment decreased the surface microhardness drop compared to the control group. For the prevention of enamel

no significant differences among treatments ( $p > 0.05$ ). The same lowercase superscripts in rows indicate no significant differences between before and after erosive challenge values ( $p > 0.05$ )

**Table 2** Means and standard deviations of surface roughness (Sq) of the experimental groups of the study before and after the erosive challenge. Same uppercase superscripts in columns indicate no significantdifferences among treatments ( $p > 0.05$ ). Same lowercase superscripts in rows indicate no significant differences between before and after erosive challenge values ( $p > 0.05$ )

Groups	Before laser irradiation	Before erosive challenge	After erosive challenge	Mean $\Delta$ Sq ( $\mu\text{m}$ )	Mean %Sq increase
Control	–	0.201 $\pm$ 0.011 <sup>Aa</sup>	0.219 $\pm$ 0.008 <sup>Ab</sup>	0.018 $\pm$ 0.010 <sup>A</sup>	8.2%
Er,Cr:YSGG laser treatment	0.191 $\pm$ 0.008 <sup>a</sup>	0.204 $\pm$ 0.008 <sup>Ab</sup>	0.218 $\pm$ 0.007 <sup>Ac</sup>	0.014 $\pm$ 0.007 <sup>A</sup>	6.4%

$\Delta$ Sq, increase of surface roughness in Sq after the erosive challenge; %Sq increase, % increase of surface roughness after erosive challenge

erosion, laser irradiation must be strongly absorbed and converted efficiently to heat without damaging the underlying or surrounding tissues, with the advantage of altering the composition and reducing the solubility of enamel [18]. Thus, laser wavelengths must be selected according to their higher absorption to constituents of enamel. For this reason, Er,Cr:YSGG laser (2780 nm) irradiation, which exhibits high absorption in water and hydroxyapatite, was utilized.

The use of Er,Cr:YSGG laser irradiation has been recently introduced as an in-office preventive measure for dental erosion [19]. Geraldo-Martins et al. [11] demonstrated that Er,Cr:YSGG laser irradiation with the same setting parameters of the current study reduced the drop of enamel microhardness at a level of 23.58% (at 20  $\mu\text{m}$  depth) compared to the control group after artificial caries challenge. Although the mechanism by which tooth demineralization occurs differs when considering caries or erosion lesions, in the present study, enamel surface microhardness of the Er,Cr:YSGG laser-treated specimens was 17.12% lower than control specimens after the erosive challenge, which is comparable with the results of the investigation mentioned above.

In the current study, we followed the protocol of Geraldo-Martins et al. [11] for Er,Cr:YSGG laser treatment. In their study, they evaluated two different power outputs for laser irradiation (0.25 and 0.5 W) with or without water spray. According to their results, Er,Cr:YSGG laser acted in the superficial layers of the enamel and energy densities of 62.5 and 125  $\text{J}/\text{cm}^2$  were capable of increasing the acid resistance of enamel, while water spray during laser irradiation did not make enamel surface less susceptible to acidic activity. As a result, we adjusted power output at 0.25 W without water spray during irradiation as recommended by the authors. Moreover, de Oliveira et al. [19], who focused on laser irradiation pulse frequency and power parameters, reported that

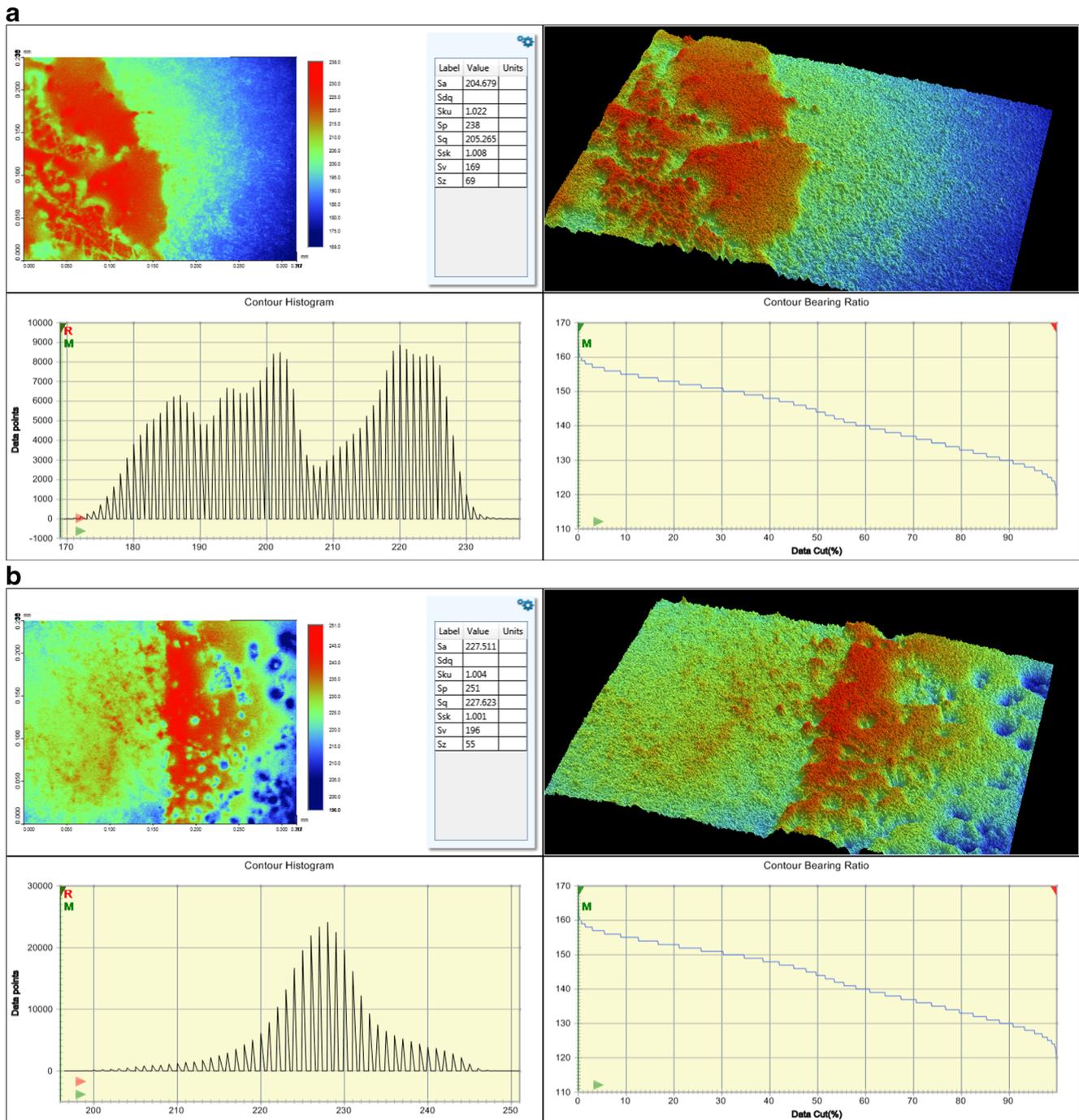
Er,Cr:YSGG treatment may alter enamel erosive resistance differently. In particular, they found that pulse frequency of 30 Hz and power of 0.50 W were considered the best parameters to prevent enamel acid erosion.

The interaction of a laser beam with enamel is affected by the irradiation parameters such as wavelength, emission mode (pulsed or continuous), pulse duration, average power, frequency, spot size, delivery method, laser beam features, and optical properties of enamel such as the refractive index, scattering coefficient, and absorption coefficient [18]. Er,Cr:YSGG laser wavelength (2780 nm) exhibits very high absorption in water and hydroxyapatite, two of the main constituents of the enamel [20] with absorption coefficient for enamel approximately to 50  $\text{mm}^{-1}$ . This means that the laser beam may penetrate in enamel around 21  $\mu\text{m}$  from the surface and as a result, Er,Cr:YSGG laser irradiation is absorbed mainly in the superficial layers of enamel, which is also beneficial for the protection of the dental pulp [21]. It is important for this treatment the laser beam not to ablate the treated enamel surface, but only to change it morphologically or/and chemically in order to promote a more resistant surface to erosive activity [22].

Two main mechanisms have been suggested for the explanation of the laser-induced prevention of enamel erosion. The increased resistance of enamel surface after Er,Cr:YSGG laser treatment may be attributed to photo-thermal effects that take place when the temperature rises from 100 to 650  $^{\circ}\text{C}$  and leads to chemical and morphological changes. The most important chemical alterations that occur in the enamel are the decrease in carbonate ( $-\text{CO}_3^{-2}$ ) anions and evaporation of water which reduce enamel solubility after acidic attack [22]. Simultaneously, the formation of pyrophosphate ions increases, leading to inhibition of the dissolution of enamel hydroxyapatite crystals [23]. The second suggested mechanism

**Table 3** Means and standard deviations of surface loss ( $\mu\text{m}$ ) of the experimental groups of the study after the erosive challenge. The decrease in surface loss of the laser treatment group compared to thecontrol group is also presented. Different uppercase superscripts in columns indicate significant difference between the experimental groups ( $p < 0.05$ )

Groups	Treatments	Mean surface loss ( $\mu\text{m}$ )	% decrease loss compared to control
1	Control	66.9 $\pm$ 8.7 <sup>A</sup>	–
2	Laser (Er,Cr:YSGG)	50.1 $\pm$ 7.1 <sup>B</sup>	25.1%



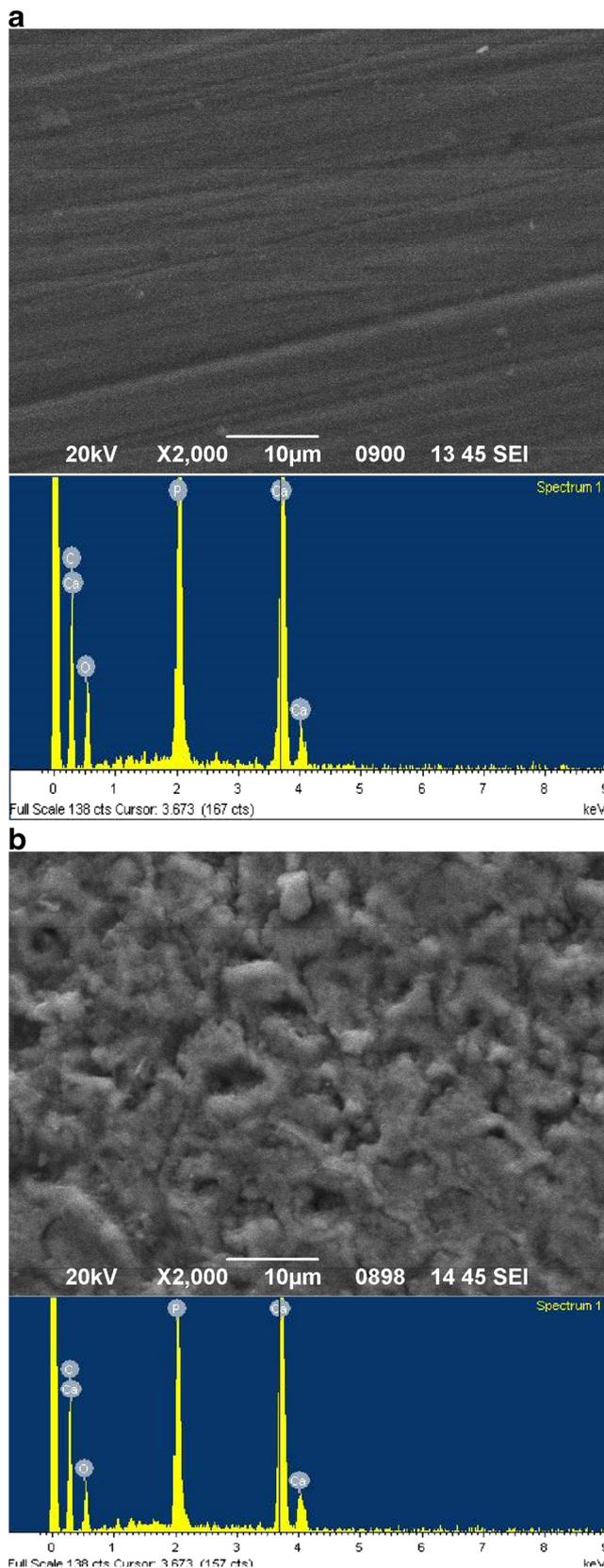
**Fig. 1 (a–b)** Representative topographic surface maps and surface analysis of enamel specimens of the experimental groups of the study ( $\times 20$  magnification) at the center of the specimens indicating the depth

of the erosive lesions. The *S* values are also presented as well as the contour histogram and the contour bearing ratio. Control group (**a**) and laser-treated group (**b**)

includes the partial denaturation of the organic matrix of the enamel during Er,Cr:YSGG laser irradiation which blocks the inter- and intra-prismatic voids resulting to decreased ion diffusion of the enamel and retardation of enamel demineralization. It has been found that this phenomenon may occur when enamel surface approaches temperatures near  $400\text{ }^{\circ}\text{C}$  [24] and that laser fluence per pulse around  $5\text{ J/cm}^2$  may increase the

temperature on enamel surface up to  $300\text{ }^{\circ}\text{C}$ , which is similar to that used in the present investigation ( $4.42\text{ J/cm}^2$ ).

As statistical analysis revealed  $H_02$ , stated that there were no significant differences in increase in surface roughness between the experimental groups after the erosive challenge, was accepted. The two experimental groups presented the same behavior regarding the increase in enamel surface



**Fig. 2** a–b Representative SEM images of the enamel surface of the experimental groups of the study (magnification  $\times 2000$ ) revealing morphological alterations. The EDS spectrum of each enamel surface appears below its SEM image. Control group (a) and laser-treated group (b)

roughness after the erosive challenge. It has been demonstrated that the morphological changes of enamel after erosive activity were attributed to the collapse of demineralized apatite crystals which lead to the increased surface roughness [25].

For the tested laser protocol, the energy density per pulse was  $4.42 \text{ J/cm}^2$ . Since the ablation threshold for enamel of Er,Cr:YSGG is in the range of  $10\text{--}14 \text{ J/cm}^2$  per pulse [26], there is no risk of damaging the enamel tissue. Nevertheless, the use of Er,Cr:YSGG laser pretreatment on enamel before the erosive challenge significantly increased surface roughness. This is in agreement with de Oliveira et al. [19], who demonstrated that experiment groups with power settings at 0.25, 0.5, 0.75, and 1 W exhibited minor alterations in surface roughness after Er,Cr:YSGG laser irradiation. This evidence may be explained by the exposition of the enamel prisms after laser irradiation and as shown in Fig. 2b of the present study. Apel et al. [27] reported that subablative Er:YSGG laser irradiation may form fine cracks on enamel surface over  $100 \mu\text{m}$  in depth which may act as starting points for erosive attack and facilitate deep demineralization. However, in the current investigation, this phenomenon was not observed. In spite of statistically significant increase in surface roughness after the erosive challenge, this increase may not be clinically important for enamel integrity.

The outcomes of the current study did not support  $H_03$ , stated that there were no significant differences in surface loss between the experimental groups after the erosive challenge. Therefore,  $H_03$  was rejected. The laser treatment decreased significantly the surface loss after the erosive challenge compared to the control group. This evidence coincides with previous reports regarding laser treatments against enamel erosion [8, 13, 19]. On the other side, Scatolin et al. [12] reported that there were no significant differences in surface loss after an Er:YAG laser treatment following erosive challenge.

Although the dental products containing stannous and fluoride exhibit currently the best evidence of anti-erosive effects

**Table 4** Means and standard deviations of wt% content of the main constituent elements of the enamel surface of the experimental groups of the study. Values in rows followed by different lowercase superscripts are statistically significant different ( $p < 0.05$ )

Elements	Control	Laser treatment
Ca	$38.67 \pm 2.86^a$	$34.12 \pm 3.01^b$
P	$20.03 \pm 2.65^a$	$21.21 \pm 2.73^a$
O	$41.30 \pm 2.35^a$	$42.03 \pm 3.25^a$

[1, 10], these preventive treatments are not controlled by the dentists and need a long period of application to be effective. In contrast, laser preventive treatments are in-office techniques which are applied by the dentist and last much shorter time. Notwithstanding Sn-containing toothpastes reduce enamel loss around 33–60% [28], tooth brushing enhances enamel loss during this therapy [29].

Surface loss after an erosive challenge is the most important method to evaluate the resistance of a tooth surface to erosion because it results to a permanent decrease of tooth volume [2]. In the present investigation, VSI method revealed a drop in surface loss at a level of 25.1% or 16.8  $\mu\text{m}$  enamel loss in comparison with the control group. This means that the tested laser treatment improves the resistance to enamel erosion but there is still a need for additional preventive measures in order to minimize the erosive action of soft drinks because there is still a loss of around 50.1  $\mu\text{m}$  of tooth surface.

SEM observations revealed changes in enamel surface after Er,Cr:YSGG laser treatment compared to control group of the study. Previous studies also reported morphological changes on enamel surface after erbium laser irradiation [12]. Scatolin et al. [12] reported that only small changes on enamel surface irradiated with Er:YAG laser were observed without melting or recrystallization areas. In the current study, after Er,Cr:YSGG laser irradiation, morphological alterations were observed revealing enamel prisms and melting areas which may partially explain the effectiveness of the method against erosion. These changes are mainly attributed to the absorption of the light energy from the superficial water and hydroxyapatite molecules of enamel and its conversion to heat [22]. EDS analysis also revealed significant changes in mineral composition, and more specifically a slight decrease in Ca concentration after laser irradiation, which partially explains the surface changes due to laser beam interaction with enamel surface.

## Conclusions

Within the limitations of this *in vitro* study, it could be concluded that the tested Er,Cr:YSGG laser treatment may be promising for the limitation of enamel erosive tooth wear induced by excessive consumption of soft drinks. Nevertheless, this laser treatment did not completely inhibit the development of enamel erosion which means that it may be recommended as a part of an individually tailored preventive program. Further clinical studies are needed to clarify whether this protective effect of the tested laser treatment is clinically significant and if it is possible to improve its effectiveness by changing the laser parameters.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Research involving human participants and/or animals** There are no human participants or animals in this study.

**Informed consent** For this type of study, formal consent is not required.

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