Lasers Med Sci (2018) 33:41–49
DOI 10.1007/s10103-017-2326-3

ORIGINAL ARTICLE

Sub-ablative Er,Cr:YSGG laser irradiation under all-ceramic restorations: effects on demineralization and shear bond strength

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Received: 16 March 2017 / Accepted: 11 September 2017 / Published online: 17 September 2017

Abstract This study evaluated the caries resistant effects of sub-ablative Er,Cr:YSGG laser irradiation alone and combined with fluoride in comparison with fluoride application alone on enamel prepared for veneer restorations. And also, evaluated these treatments' effects on the shear bond strength of all-ceramic veneer restorations. One hundred and thirty-five human maxillary central teeth were assigned to groups of 1a–control, 1b–laser treated, 1c–fluoride treated, 1d–laser + fluoride treated for shear bond testing and to groups of 2a–positive control(non-demineralised), 2b–laser treated, 2c–fluoride treated, 2d–laser + fluoride treated, 2e–negative control (demineralised) for microhardness testing ($n = 15$, $N = 135$). Demineralisation solutions of microhardness measurements were used for the ICP-OES elemental analysis. The parameters for laser irradiation were as follows: power output, 0.25 W; total energy density, 62.5 J/cm^2 and energy density per pulse, 4.48 J/cm^2 with an irradiation time of 20 s and with no water cooling. Five percent NaF varnish was used as fluoride preparate. ANOVA and Tukey HSD tests were performed ($\alpha = 5\%$). Surface treatments showed no significant effects on shear bond strength values ($p = 0.579$). However, significant differences were found in microhardness measurements and in elemental analysis of Ca and P amounts ($p < 0.01$). Surface-treated groups showed significantly high VNH values and significantly low ICP-OES values when compared with non-treated (−control) group while there were no significance among surface-treated groups regarding VHN and ICP-OES values. Sub-ablative Er,Cr:YSGG treatment alone or combined with fluoride is as an effective method as at least fluoride alone for preventing the prepared enamel to demineralization with no negative effect on shear bond strength.

Keywords Laser · Sub-ablative · Caries · Laminate veneer

Introduction

Although the preventive measures, such as water fluoridation and the use of fluoride containing cariostatic agents have led to distinct decline in the prevalence of dental caries, this disease is still have a high manifestation [1–3]. By topical fluoride applications, deposition of surface CaF_2 crystals occur which are highly soluble [4–6]. Thus, it is stated that several fluoride applications are necessary to maintain the anti-caries effect by topical treatments [7]. In some cases, perhaps faster acting applications may be more useful by alone or with fluoride. And to identify patients with an elevated risk of caries and give them appropriate and individual preventive support may be the right move.

The use of lasers in this field of dentistry has been a subject of discussion ever since lasers were introduced into dental medicine [8]. The possibility of increasing the acid resistance of enamel after laser irradiation was first demonstrated by Sognnaes and Stern in the 1970s using a CO_2 laser [9]. When laser energy absorbed by the specific components of dental enamel, it is directly converted into heat which is the main factor leading to the structural and chemical changes in enamel [10]. The theories suggested to explain the reduced acid solubility of heated dental enamel are the decrease of the water permeability of dental enamel after heating [11] and when compared with unheated enamel more hydroxide and pyrophosphate but less carbonate content of lased enamel [12, 13]. Nowadays it is well known that, since they are highly

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absorbed by water and hydroxyapatite, the wavelengths emitted by the erbium (Er:YAG and Er,Cr:YSGG) and CO_2 lasers have higher interaction with dental hard tissues allowing either to cut these tissues or make them acid-resistant depending on the laser energy transmitted [8–15].

The Er,Cr:YSGG (erbium,chromium: yttrium-scandium-gallium-garnet) laser works at a wavelength of 2.78 μm which is suitable for cavity preparation also investigated for caries-prevention purposes. Nevertheless, it is so important that to ensure the caries-prevention effect, lased surfaces must not be ablated only morphological or chemical changes must be provided. For this purpose, several studies have been performed with low energy densities so-called sub-ablative laser irradiation [8–17].

For a few dacades, since porcelain laminate veneers (PLVs) require less invasive tooth preparation with high esthetic appeal and patient satisfaction there is an increasing demand for treating unaesthetic anterior teeth by using PLVs. These restorations are also biocompatible, resistant to staining and wear [19–21]. Improvements in adhesive systems and in new-generation porcelain technology have supported the use of these materials. Currently, different ceramic systems are available in the market, and also there are different methods to fabricate all-ceramic restorations [22, 23]. In recent years, there has been a significant increase in chair side dental computer-aided design/computer-aided manufacturing (CAD/CAM) materials like lithium disilicate glass ceramics, leucite-reinforced glass ceramics, feldspathic glass ceramics, some kind of zirconia polycrystals, polymer-infiltrated ceramics and nanohybrid-composites with inorganic ceramic fillers [24]. Among them, glass ceramics have highly natural looking outcomes, excellent optical properties, mechanical and chemical stability and biocompatibilty.

Although studies have reported the long-term clinical success rates, but also, there are still failures even if in low rates. Researchers stated that the most frequent failure was fracture/chipping, but in these studies, caries formation was also detected in changing rates between 1 to 3.5% [19–21, 25–32].

So the aim of this in vitro study was to evaluate the acid-resistant effect of sub-ablative laser treatment (alone and combined with fluoride) on dental enamel by using inductively coupled plasma-optical emission spectrometry (ICP-OES) and Vicker's microhardness tests to analyze mineral solubility and to evaluate the effects of this laser treatment on the shear bond strength of all glass ceramic restorations to enamel. There were two null hypothesis:

1. Sub-ablative laser treatment has the potential decreasing effect on demineralisation of dental enamel caused by acidic conditions.
2. This laser treatment has the negative effect on shear bond strength of glass ceramic laminate restorations to enamel.

Materials and methods

Sample preparation

The protocol of this study plan was approved by the Ethics Committee of Kirikkale University (Approval number 18/13, 29.06.2015). One hundred and thirty-five noncarious human maxillary central teeth extracted within the last 6 months were used in this study. After removal of dental plaque, calculus, and periodontal fibers, the teeth were stored in 0.1% thymol-containing distilled water under refrigeration at 4 °C until they were required for the study. Before the experiment the teeth were observed in a stereomicroscope for cracks, caries, defects and the presence of calculus. After cleansing, the selected teeth crowns were separated from the roots at the cement–enamel junction using a section machine (Minitom, Struers Inc., Westlake, OH, USA) with a diamond disk (Isomet; 10.2 cm × 0.3 mm, arbour size 1/2 in., series 15HC diamond; Buehler Ltd., Lake Bluff, IL, USA) at low speed. Then the teeth were rinsed with distilled water for 15 s and dried with oil-free compressed air. Samples were then assigned randomly to one of the four groups for shear bond testing and to one of the five groups for microhardness testing (n = 15, N = 135). Demineralisation solutions of specimens of microhardness measurements were used for the ICP-OES analysis.

Shear bond groups	*Microhardness and ICP-OES groups*
Group 1A—control group	Group 2A—positive (+) control group (non-demineralised)
Group 1B—laser-treated group	Group 2B—laser-treated group
Group 1C—fluoride-treated group	Group 2C—fluoride-treated group
Group 1D—laser + fluoride-treated group	Group 2D—laser + fluoride-treated group
	Group 2E—negative (−) Control group (demineralised)

Enamel preparation

At first, by using a depth preparation bur (Diatech, Coltene/Whaledent, AG, Switzerland) depth-orientation grooves (0.5 mm in depth) were placed on the facial surfaces of the teeth. The preparation surfaces were covered with a water insoluble pen. Then, the surfaces were grinded with silicon carbide abrasive papers of grit sizes of 100, 400, and 600 (Leco^R VP 100, Leco Instrumente GmbH, Germany) without exceeding the depth-orientation grooves. The prepared enamel surface areas were approximately 5 mm in diameter. Until the colour was removed from the middle third of the covered facial surfaces preparations were continued. In total, 135 teeth were prepared, among them 60 specimen used for shear bond testing and 75 for microhardness measurements. A 4 × 4 mm area in each of the 135 samples of tooth enamel was identified, with the bottom edge located 2.0 mm above of the cemento-enamel junction. The parts outside this defined area were coated with two layers of acid-resistant varnish sealer.

Enamel surface treatments

Laser treatment In groups 1B and 2B, samples were irradiated immediately after removal from the distilled water in order to prevent drying out of the dental hard substance and any associated corruption of the results. Defined 4 × 4 mm areas were irradiated with an Er,Cr:YSGG laser device (Waterlase Millennium™, Biolase Technologies Inc., San Clemente, CA, USA). This equipment emits photons at a wavelength of 2.78 μm. The power output was 0.25 W. The repetition rate was fixed at 20 Hz and pulse duration was fixed on 140 μs. The energy density per pulse was 4.46 J/cm^2 which calculated as follows: power, 0.25 W; frequency, 20 Hz; pulse energy, 12.5 mJ; focal spot area, 0.28×10^{-2} cm^2 and the energy density, $12.5 \text{ mJ} \times 10^{-3} / 0.28 \times 10^{-2} = 4.46 \text{ J/cm}^2$.

MGG6 sapphire tip was used. The beam diameter at the focal area for the handpiece was 600 μm. These parameters (Table 1) were selected after a careful review of the literature [8–18]. The tip was positioned 1.0 mm from the enamel surface [focused mode]. To ensure consistent spot size with the hand irradiation, an endodontic file was fixed to top of handpiece to keep the distance of 1 mm during the irradiation. The handpiece was positioned 3–5° angle to the enamel surface to protect the tip from reflected rays, and the samples were irradiated by hand in focused mode, screening the test surface with a uniform sweeping motion, moving the handpiece horizontally and vertically for 40 s (20 s vertically and 20 s horizontally) [14, 17].

Fluoride treatment In groups 1C and 2C, 5% sodium fluoride containing Flor-Opal Varnish (Ultradent Products Inc., South Jordan, UT, USA) was applied immediately after removal from the distilled water and left untouched for 30 min.

Fluoride + laser treatment In groups 1D and 2D, the specimens were firstly treated with fluoride with the same way and then laser irradiated according to the procedure used in groups 1B and 2B. In groups 1A, 2A and 2E, no treatment was done. All specimens kept in distilled water after surface treatments.

Shear bond strength test

Tooth specimen preparation

Teeth were placed in a flat plane with their whole facial surfaces completely horizontally touched to modelling wax. Then a custom-made Teflon mold put on the wax and self-curing acrylic resin was placed into the mold from the top. After curing, resin blocks were removed from mold and the wax on the facial surfaces of the specimens washed with hot water by gently brushing until all the wax removed. This process was separately done for all surface-treatment groups.

Ceramic specimen preparation

VITABLOCS Mark II (Vita Zahnfabrik, Bad Säckingen, Germany) feldspathic glass ceramic CAD/CAM blocks were used for this study. Sections (n = 60) with a thickness of 2.5 mm were prepared from the blocks by using a slow-speed water-cooling diamond blade (Ernst Leitz GmbH, Wetzlar, Germany). All of the ceramic sections were then ground with silicon carbide abrasive paper of grits 400, 600 and 1200 (Leco1 VP 100, Leco Instrumente GmbH, Germany) from the both sides. A 4 mm in diameter Teflon molds placed on the sections and ceramic sections marked with a fine-tipped water insoluble pen through the mold. Then ceramic sections were carefully milled by hand, using water cooled micro-motor with diamond-coated fissure bur. The edges of discs were grounded with Sof-Lex polishing discs from coarse to fine (3M ESPE, St Paul, MN, USA) and discs were subsequently inspected with a magnifying glass for any existing damage.

Bonding procedure

The ceramic specimens were randomly divided (n = 15) into four groups according to the surface treatment groups of the teeth. Ten percent HF gel (Angelus) was applied to the ceramics for 60 s and rinsed with deionized water for 2 min and dried with oil-free air. On the other hand, enamel surfaces were etched with 37% orthophosphoric acid (AXJA-ETCH, Scientific Pharmaceutic Inc. USA) for 15 s according to the manufacturer's instructions and rinsed with deionized water for 2 min and dried with oil-free air. Then Scotchbond Universal adhesive (3M ESPE, St Paul, MN, USA) was

Table 1 Surface treatment methods

Er,Cr:YSGG Laser	Power output [W], 0.25	Total energy density [J/cm2], 62.5	Energy density per pulse [J/cm2], 4.46	Irradiation time [s], 20	Water cooling [ml/min], 0
Flor-opal varnish	Manufacturer, Ultradent	Fluoride source and con-centration, 5% NaF	Carrier, hydrogenated rosin [< 60%]	Other active ingredient, xylitol	Usage, apply a thin smooth layer to dry tooth using a painting motion

applied both to the enamel and ceramic surfaces for 20 s and gently air dried for 5 s. Resin cement RelyX Ultimate (3M ESPE, St Paul, MN, USA) was placed to the ceramic through the mixing tip and ceramic placed to enamel surface with a gently press. The excess cement was removed, and the specimens were then light cured on each side (perpendicular, from right and left) for 20 s, a total exposure of 60 s with a LED light-curing unit (Elipar S10, 3M Espe, St. Paul, MN) with an irradiance of 1200 mW/cm^2 according to the manufacturer's instructions. The same operator was prepared all the specimens in same conditions of room temperature and humidity. All operations (etching, priming and ceramic bonding) were carried out according to the manufacturer's instructions. After storing in 37 °C distilled water for 24 h, specimens were thermocycled in distilled water for 5000 cycles in a 5–55 °C water bath with a dwell time of 30 s and a transfer time of 5 s.

Shear bond strength test

Specimens were mounted on the jig of a universal testing machine (MCE 2000ST, Quicktest Prüfpartner GmbH, Langenfeld, Germany). Bond strength was determined at a crosshead speed of 0.5 mm/min until fracture occurred. Shear bond strength was calculated by dividing the maximum load at failure (N) with the bonding area (mm^2) and recorded in megapascals (MPa).

Vickers microhardness measurements

Seventy-five teeth were used for this part of the study. At first, for groups 2B to 2E, demineralisation treatment was carried out. The other 15 teeth of group 2A left untouched to use as positive control (non-demineralised and non-surface treated).

Demineralisation procedure

The applied demineralisation protocol was based on the method that by White has been used in various studies [35–37]. Artificial lesions were formed by immersing the specimens for 48 h individually in plastic tubes (Falcon TubesTM, BD, Franklin Lakes, USA) which were containing 0.1 M lactic acid buffer solution[0.75 mM $CaC1_2 \cdot 2H_2O_2$, 0.45 mM $K(H_2P0_4)$] with a pH value of 4.75. Demineralisation was performed at 37 °C at a ratio of 10 ml of solution per specimen. At the end of the 48 h, specimens were removed from the solutions and ultrasonically cleaned with distilled water and gently dried. Then, all specimens including group 2A were prepared for microhardness measurements by embedding in self curing acrylic resin as with the same way described before in shear bond strength test section. On the other hand, immersed solutions were used for ICP-OES elemental analysis of Ca and P amounts.

Microhardness test

Microhardness measurements were made with a Vickers diamond microhardness tester (Buehler Micromet 5101, obj. lens, 50×) in Vickers hardness units/numbers (VHN). Three indentations were made using a 300 g load perpendicularly orientated to the indentation surface for 15 s, and the average values were recorded.

ICP-OES analysis

The amounts of calcium and phosphorous released into the demineralization solutions were measured with an inductively coupled plasma-optical emission spectrometer (ICP-OES; Spectro Flame Blue EOP TI, Spectro Analytical Instruments GmbH and Co. KG, Kleve, Germany) in the conditions of the following: plasma power, 1430 W; pump speed, 30 rpm; coolant flow, 13 L/min; auxilory flow, 0.80 and nebulizer flow, 0.75. Before the determination of the elements, calibration was performed with calcium and phosphorous standard solutions (Merck KGaA, Darmstadt, Germany). From each solution, 3 ml samples were taken in triplicate, and three measurements of calcium and three of phosphorous were performed to ensure the precision of the measurements. Both calcium and phosphorous contents were measured in parts per million.

Statistical analysis

Assumptions of normal distribution were checked with Shapiro-Wilk tests for all the variables tested. Descriptive and inferential statistical analyses were performed using the SPSS 16.0 for Windows statistical program at a signicance level of $p < 0.05$. Differences between the groups for all tests (shear bond, microhardness, ICP-OES) were statistically analyzed by one-way analysis of variance (ANOVA) and Tukey HSD tests.

Results

Shapiro-Wilk tests showed that all data obtained in this study had normal distribution. Mean values and standard deviations of all the tests and the tested groups are shown in Table 2. There were slightly differences in shear bond strength values among groups, but ANOVA revealed that the surface treatments had no significant effects on shear bond strength values ($p = 0.579$ at Table 3). However, significant differences were found in microhardness measurements ($p < 0.01$, VHN at Table 4) and in elemental analysis ($p < 0.01$, ICP-OES at Table 5). In VHN and ICP-OES tests, parallel results were obtained. Such that surface-treated groups showed significantly different high VNH values and significantly different low ICP-OES values when compared with non-treated "- control"

Table 2 Mean values and standard deviations of all the tests and the tested groups. Different letters show statistical significance

	Tested groups				
	Values				
Tests	2A (+ control)	2B (laser)	2C (fluoride)	2D (laser + fluoride)	2E (− control)
VHN	390.1 ± 37.7^{a}	131.4 ± 21,9^{b}	126.9 ± 15.4^{b}	129.9 ± 25.7^{b}	26.5 ± 7.8^{c}
Ca-PPM	4.7 ± 0^{d}	10,8 ± 1.6^{e}	12.1 ± 2.2^{e}	10.9 ± 2.1^{e}	46.5 ± 0.7^{f}
P-PPM	0.25 ± 0^{g}	3.4 ± 0.7^{h}	4.1 ± 0.9^{h}	3.3 ± 0.8^{h}	18 ± 0.4^{i}
	1A (control)	1B (laser)	1C (fluoride)	1D (laser + fluoride)	
MPa	23.8 ± 8.2^{j}	25.4 ± 4.3^{j}	22.3 ± 4.7^{j}	23.3 ± 6.7^{j}	

group. Although there was a slight difference on behalf of the laser-treated group, there was no significance among surface-treated groups regarding VHN and ICP-OES values.

Discussion

It is well established in literature that sub-ablative laser treatment have the potential of increasing the caries resistance of tooth enamel. And there are various studies stated the caries-preventive effects of different laser types like Nd:YAG, Argon, CO_2 and Erbium family lasers [14–18, 32–35]. Among them, Er,Cr: YSGG laser can be widely used for many dental purposes, ranging from hard tissue operations to soft tissue operations. In this study, this laser type was investigated to contribute to its usage for another field, because of the high surface absorption due to hydroxyapatite. Although many researchers have investigated the caries resistant effect of this laser, it was the first that in this study, this effect evaluated on the enamel which prepared for all-ceramic laminate restorations and additionally, the ICP-OES analysis was used to support the microhardness measurement results in determining the mineral loss. According to the results obtained from this study, the first hypothesis "sub-ablative laser treatment have the decreasing effect on demineralization of dental enamel" is accepted, but the second "this laser treatment have the negative effect on shear-bond strength of all glass ceramic laminate restorations to enamel" is rejected.

In high-risk areas such as around orthodontic brackets or around composite restorations, it has been demonstrated through several studies that laser use alone or combined with fluoride treatment can control caries [32, 36, 37]. However, it is a mystery that laser treatment has such an effect under or around all ceramic restorations. Numerous clinical studies with evaluation periods varying from 3 to 25 years have reported the high success rates of ceramic laminate restorations but these studies still refer to the formation of caries between rates of 1 to 3.5% [19–21, 25–29]. No doubt, possible treatment methods that can be made to raise this rate to 100% cannot be ignored. But at the same time, these methods should not adversely affect the bonding performance of the restorations to tooth hard substances. Fortunately, according to the results of this study ANOVA (Table 3) showed that there were no significant differences of shear bond strength values among the groups. Moreover, considering the demineralisation prevention there were significant positive effects of treatment methods compared to control group.

It is well known that to prevent ablation is has the primary importance in laser-assisted caries prevention [13–18, 32–38]. To increase the enamel's acid resistance by using laser

Table 3 One-way analysis of variance (ANOVA)

		Sum of squares	df	Mean square	*F*	Sig.
VHN	Between groups	1,103,650.853	4	275,912.713	481.080	.000
	Within groups	40,146.933	70	573.528		
	Total	1,143,797.787	74			
Ca (ppm)	Between groups	15,589.158	4	3897.289	646.525	.000
	Within groups	421.964	70	6.028		
	Total	16,011.122	74			
P (ppm)	Between groups	2919.909	4	729.977	817.630	.000
	Within groups	62.496	70	.893		
	Total	2982.405	74			
Shear (MPa)	Between groups	76.442	3	25.481	.662	.579
	Within groups	2154.900	56	38.480		
	Total	2231.342	59			

Table 4 Multiple comparisons with Tukey HSD test (microhardness values)

Dependent Variable	I group	J group	Mean difference (I–J)	Std. error	Sig.
VHN	+Cont.	Las.	258.66667*	8.74473	*.000*
		Las. + fluor.	260.20000*	8.74473	*.000*
		Fluor	263.13333*	8.74473	*.000*
		−Cont.	363.60000*	8.74473	*.000*
	Las.	+Cont.	− 258.66667*	8.74473	*.000*
		Las. + fluor.	1.53333	8.74473	1000
		Fluor.	4.46667	8.74473	.986
		−Cont.	104.93333*	8.74473	*.000*
	Las. + fluor.	+Cont.	− 260.20000*	8.74473	*.000*
		Las.	− 1.53333	8.74473	1.000
		Fluor	2.93333	8.74473	.997
		−Cont.	103.40000*	8.74473	*.000*
	Fluor.	+Cont.	− 263.13333*	8.74473	*.000*
		Las.	− 4.46667	8.74473	.986
		Las. + fluor.	− 2.93333	8.74473	.997
		−Cont.	100.46667*	8.74473	*.000*
	−Cont.	+Cont.	− 363.60000*	8.74473	*.000*
		Las.	− 104.93333*	8.74473	*.000*
		Las. + fluor.	− 103.40000*	8.74473	*.000*
		Fluor.	− 100.46667*	8.74473	*.000*

irradiation at sub-ablative parameters, photothermal effect is more dominant rather than photomechanical effect [21]. In several studies, it is stated that to achieve this effect, temperature raise ranging from 100 °C to 600 °C is necessary [12, 35, 38]. And regarding the use of Er,Cr:YSGG laser, the required energy density to ensure enamel acid resistance effect changes between 4 to 13 J/cm^2 [8, 14, 20, 32, 35]. So following a detailed review of the literature sub-ablative parameters that given in Table 1 were selected to use in the present study.

For preventing enamel demineralisation, the combined use of fluoride and laser irradiation evaluated in several studies and it is stated that due to increased fluoride retention capacity, lased enamel may show more acid resistance [39]. It is also stated that the heating effect of laser irradiation can cause melting in enamel structure and in the presence of fluoride the transformation of hydroxyapatite to fluorapatite may induced in this melted layers [40]. In this study, considering this synergistic effect, laser treatment done both alone and in combination with fluoride application and corresponded with each other, with fluoride application only and with control groups. When the results were evaluated in terms of caries preventing effects, although there was a slight difference in favor of the lased and fluoride-assisted lased groups there were no statistically significance in treatment groups. But when compared to the non-treated-control group there were significantly different preventing effects of treatment groups of lased, lased with fluoride combination and only fluoride applied groups. That is to say that laser treatment is found to be as effective as fluoride treatment alone or with laser combination. These results are consistent with some other previous studies [8, 28, 32, 41]. But at the contrary in some other studies, a synergistic effect between laser and fluoride that increases the acid resistance of sound enamel have been shown [18, 33, 35, 42]. This differences probably occured due to the differences among the studies. Namely, 1—in the present study only demineralisation process was applied but in Liu et al.'s [42] and de Freitas et al.'s [18] studies demineralisation-remineralisation cycles applied with different time intervals. 2—in Zezell et al.'s [33] and Anaraki et al.'s [35] studies different types of CO_2 and Nd:YAG lasers were used. This laser types differently interacts with tooth structures that may exhibit different melting effects. 3—the other point was the differences in the fluoride preparate types and the concentrations used in the studies.

To evaluate the treated enamel specimens' responses to the acidic condition, mineral loss was evaluated. For this purpose, Vickers microhardness test and ICP-OES elemental analysis were used. Vickers microhardness test is one of the widely preferred methods in the field of dental research. In the present study, ANOVA showed significant differences in VHN among groups (p = 0.00). When the results are evaluated considering Tukey HSD test, treatment groups of 2B, 2C and 2D showed significant higher VHN values corresponded to (−)control group of 2E; meanwhile, they showed significant lower VHN values

Table 5 Multiple comparisons with Tukey HSD test (Ca and P amounts in ICP-OES)

Dependent variable	I group	J group	Mean difference (I–J)	Std. error	Sig.
Ca	Control	Laser	− 6.53933*	.89652	*.000*
		Laser_flour	− 6.70667*	.89652	*.000*
		Flour	− 7.82867*	.89652	*.000*
		Non_treated	− 40.64667*	.89652	*.000*
	Laser	Control	6.53933*	.89652	*.000*
		Laser_flour	− .16733	.89652	1.000
		Flour	− 1.28933	.89652	.605
		Non_treated	− 34.10733*	.89652	*.000*
	Laser_flour	Control	6.70667*	.89652	*.000*
		Laser	.16733	.89652	1.000
		Flour	− 1.12,200	.89652	.721
		Non_treated	− 33.94000*	.89652	*.000*
	Flour	Control	7.82867*	.89652	*.000*
		Laser	1.28933	.89652	.605
		Laser_flour	1.12200	.89652	.721
		Non_treated	− 32.81800*	.89652	*.000*
	Non_treated	Control	40.64667*	.89652	*.000*
		Laser	34.10733*	.89652	*.000*
		Laser_flour	33.94000*	.89652	*.000*
		Flour	32.81800*	.89652	*.000*
P	Control	Laser	− 3.14733*	.34502	*.000*
		Laser_flour	− 3.10267*	.34502	*.000*
		Flour	− 3.87067*	.34502	*.000*
		Non_treated	− 17.76800*	.34502	*.000*
	Laser	Control	3.14733*	.34502	*.000*
		Laser_flour	.04467	.34502	1.000
		Flour	− .72333	.34502	.233
		Non_treated	− 14.62067*	.34502	*.000*
	Laser_flour	Control	3.10267*	.34502	*.000*
		Laser	− .04467	.34502	1.000
		Flour	− .76800	.34502	.182
		Non_treated	− 14.66533*	.34502	*.000*
	Flour	Control	3.87067*	.34502	*.000*
		Laser	.72333	.34502	.233
		Laser_flour	.76800	.34502	.182
		Non_treated	− 13.89733*	.34502	*.000*
	Non_treated	Control	17.76800*	.34502	*.000*
		Laser	14.62067*	.34502	*.000*
		Laser_flour	14.66533*	.34502	*.000*
		Flour	13.89733*	.34502	*.000*

corresponded to (+)control group of 2A. But there were no significance among the treatment groups of 2B, 2C and 2D. Since ICP-OES allows to determine the mineral content, it was used to support the VHN measurement values. For this purpose, methods such as SEM and energy dispersive spectrometry, ICP-AES (inductively coupled plasma–atomic emission spectrometry) or atomic absorption spectrometry can be used, but in SEM and energy dispersive spectrometry, measurement were not repeated exactly at the same point and also, it is necessary to coat the specimens for this method which may cause the specimens not to be used for further studies. In atomic absorption spectrometry and ICP-AES, it is necessary to dehydrate, burned and dissolved that again cause the specimens not to be used for further studies. ICP-OES does not require this situations which permits to use the specimens in further studies and in addition, this method can detect elements in parts per billion (micrograms per liter). Because of these advantages, ICP-OES was preferred. Similar to the results of VHN, ANOVA showed significant differences in ICP-OES among groups regarding Ca and P levels (p = 0.00). In both elements, when the results are evaluated considering Tukey HSD test, treatment groups of 2B, 2C and 2D were showed significant lower Ca and P amounts in demineralisation solutions corresponded to the untreated (−)control group of 2E; meanwhile, they were showed significant higher Ca and P amounts in demineralisation solutions corresponded to the control solution. But again there were no significance among the treatment groups of 2B, 2C and 2D in terms of Ca and P amounts in solutions.

So according to this result, it can be argued that these treatment methods have the capability of preventing enamel demineralisation, and there were no difference in efficiency between them.

Adhesion is one of the most important parameters affecting the success of restorations. And shear bond testing is the one of the most accepted and used methods for assessing the adhesion performance of dental materials to dental structures [43]. It has been stated in the literature that the required shear bond strength of adhesives to enamel to compensate the polymerization shrinkage stresses should be at least 20 MPa [43, 44]. In the present study, all preparation margins were in enamel and all groups showed the adequate shear bond strength values contrary to estimates. Moreover, although there was a slight difference in favor of the laser group, this difference was not significant. According to these results, the application of enamel preventive methods to demineralisation did not negatively affect the adhesive bonding to prepared enamel. Further studies are needed to support these results and also to evaluate these effects of laser treatment in prepared dentin structure.

Conclusions

Within the limitations of this study, the following conclusions can be addressed:

1. The sub-ablative Er,Cr:YSGG treatment alone or combined with fluoride is as an effective method as at least fluoride alone for preventing the prepared enamel to demineralisation. Thus, it can be an alternative choice of enamel prevention.

2. While sub-ablative Er,Cr:YSGG treatment shows preventive effects, it also does not negatively affect the shear bond strength to enamel.

Acknowledgements The author would like to thank to Prof. Dr. Aslihan USUMEZ DDS; PhD for the supports about the calculation of the laser energy density.

The author would like to thank the Department of Restorative Dentistry, Faculty of Dentistry, Kirikkale University, Turkey, for allowing the use of the Er,Cr:YSGG laser device.

Compliance with ethical standards

Conflict of interest The author declare no potential conflicts of interest with respect to the authorship and/or publication of this article, and the author do not have any financial interests in the companies whose materials and devices are included in this article.

Ethical approval and informed consent The protocol of this study plan was approved by the Ethics Committee of Faculty of Medicine, Kirikkale University (Approval Number: 18/13, 29.06.2015). Teeth donators signed an informed consent form and the experiments followed the principles of the Helsinki Declaration.

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