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ORIGINAL ARTICLE

Effect of the carbon dioxide 10,600-nm laser and topical fluoride gel application on enamel microstructure and microhardness after acid challenge: an in vitro study

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Abstract

The aim of this in-vitro study was to evaluate positive effects of the carbon dioxide laser (CO_2, 10,600 nm) with acidulated phosphate fluoride (APF) gel on enamel acid resistance. Twenty extracted human third molars (40 surfaces) were randomly assigned into four groups: group C, untreated control; group L, CO_2 laser alone group; group F, APF 1.23% fluoride gel; and group FL, APF 1.23% gel and laser. Samples from group L were irradiated with a CO_2 laser for 30s. The parameter settings used were average power, 0.73 W; time on, 100 μs; time off, 40 ms; tip-to-tissue distance, 20 mm; tip diameter 700 μm; and energy density with movements, 5 J/cm^2. Samples from group F were treated with the APF gel for 4 min, and the gel was washed off with distilled water. The enamel samples from group FL were treated with APF gel for 4 min and then irradiated with the CO_2 laser for 30s without removing the gel. Each enamel sample was placed in 50 ml soft drink ($pH = 2.75$) for 10 min then rinsed with deionized water and stored in artificial saliva at 37 °C for 1 h. Samples were assessed for Vickers hardness number (VHN) before and after treatments and subjected to SEM analysis. Data were analyzed using a one-way analysis of variance (ANOVA) and Tukey's test ($\alpha < 0.05$). After the acid challenge, the untreated C group was demineralized to a great extent and the enamel surface was with the lowest mean score of microhardness. The observed VHN in the control (C group) had a mean value of 176.13, the scores in the CO_2 laser group (L group) were with mean value of 238.40, the F group with a mean value of 218.45, and the fluoride-treated and laser-irradiated FL group—with a mean of 268.28 VHN. Paired t test performed to compare groups C, L, F, and FL has shown that group FL has greater resistance to decrease in microhardness of dental enamel ($P \leq 0.05$) on exposure to acidic protocol. After the acid challenge, the fluoride-treated and laser-irradiated samples (group FL) showed the least diminution in enamel surface microhardness. The sub-ablative carbon dioxide laser irradiation in combination with fluoride treatment is more effective in protecting enamel surface and resisting demineralization than CO_2 laser irradiation or fluoride alone.

Keywords Enamel · Demineralization · Carbon dioxide laser · Fluoride · Prevention · Microhardness

Introduction

Enamel demineralization is a significant clinical problem. Demineralization can lead to loss of tooth structure [1, 2].

Dental erosion is a pathological condition of increasing prevalence, characterized by demineralization and loss of hard tooth structure. It may be further defined as loss of dental hard tissue as a result of non-bacterial chemical attack, usually by acidic-containing substances. The acidic attack leads to a demineralization and softening of the tooth surface [1]. It was shown that patients suffering from erosions had an enamel wear over 6 months in a range from 17.6 to 108.2 μm [2]. The prevalence of dental erosion in children, adolescents, and adults has increased in recent years [3, 4].

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Consumption of soft drinks shows a continuing upward trend. Exogenic acids, originating from acidic food or beverages, might act as erosive substrates [4]. The increasing consumption of acidic soft drinks is an increasingly important factor involved in the etiology of dental erosions [5, 6].

The increased prevalence of dental erosion [7, 8] has encouraged different investigations into controlling these lesions. Some innovative and currently used methods and techniques have shown to be successful in stable movement toward enamel protection [9, 10]. These methods include fluoride therapy for inhibition of demineralization. A lot of studies were made to provide increased acid resistance or remineralize the dental structure with the use of amine fluoride (AmF) [11–13], titanium tetrafluoride (TiF_4) [14–18], and sodium fluoride (NaF) [11].

Topical fluoride application is a widely applied method for prevention that can enhance the subsurface remineralization. Topical fluoride application deposits calcium fluoride (CaF_2) on the surface crystals. It is a reservoir releasing fluoride in the demineralization process [11, 17].

Most popular and used fluorides for hard tissue prevention are sodium fluoride, stannous fluoride and acidulated phosphate fluoride (APF) [19]. Comparing these systems, APF has proved to be better than the others, since it produces more fluorapatite during its chemical reaction with hydroxyapatite [19].

Laser-mediated caries prevention to alter the composition, or solubility of dental hard tissues, depends on laser photonic energy being strongly absorbed and converted efficiently to heat, but without damage to underlying or surrounding tissues. The wavelengths must be chosen to correspond to specific chromophore components in dental hard tissues, such as hydroxyapatite, and water. Most suitable choices for that reason are carbon dioxide (CO_2) and erbium family of lasers. The chemical changes induced by laser action depend on the temperature rise during irradiation [20].

Sub-ablative irradiation of enamel by a CO_2 laser usually involves a melting and recrystallization process. This process leads to apparent morphological and crystallographic changes in the enamel [21]. The carbon dioxide laser is reported to inhibit enamel demineralization and the melting and fusion of carbonated hydroxyapatite crystals. The subsequent sealing of the enamel surface achieves this inhibition with low laser fluence [21].

Topical fluoride application before or after laser irradiation leads to an increase in fluoride uptake and a decrease in the dissolution rate in acidic solution [22].

The aim of this study was to investigate the possible effect of enamel surfaces irradiation with CO_2 laser and topical fluoride gel application on prevention of demineralization after acid challenge.

It was achieved by

- calculating the changes in surface microhardness (SMH) as directly correlating to mineral loss.
- evaluating microstructural changes in enamel surface after treatment by SEM analysis.

Material and methods

Preparation of the enamel samples

Twenty extracted human third molars, free from cracks, erosion, caries, or any structural defect, were chosen from anonymous source from the department of oral surgery at the Medical University of Plovdiv, Bulgaria. These teeth were destined to be destroyed and were removed from patients who had consented independently for third molar surgery. The teeth were stored in 0.1% thymol solution before the experiment. The teeth were sectioned mesio-distally in two halves.

Experimental design Forty enamel surfaces were included and were randomly assigned into 4 groups according to the method of treatment:

- Group C—untreated control
- Group F—treated with APF 1.23% gel
- Group L—CO_2 laser alone group
- Group FL—treated with APF 1.23% gel and laser

The enamel surfaces from each tooth were divided into the four groups ($n = 10$). This approach provided a sample from each tooth in each group. This is the equivalent of a within-subject control design.

The tooth slabs were prepared in the following way by a crystallographer from the Bulgarian Academy of Science, Sofia:

(a) The tooth was cut into two parts by cutting machine “Minosekar-2” using a diamond blade.
(b) The two parts of the object were dried and placed in round shaped polyvinyl chloride cylinders of dimensions 2.5 cm diameter and 1.0 cm thickness. The samples were embedded in polyester resin using the cylinders with the enamel surfaces facing upwards.
(c) After 12 h, the obtained samples were removed from the mold and processed sequentially with the following abrasivity 230, 400, 800, and 1000.
(d) After the processing, the surfaces were polished on the same woolen cloth with chrome trioxide, potassium dichromate, and aluminum oxide for 40 min.

Fluoride treatment and laser irradiation

No treatment was provided in the control group (C).

The samples from group F were treated with the APF gel (Elmex APF 1.23% fluoride gel, Switzerland) for 4 min, according to the manufacturer's instructions, and then the gel was washed off with distilled water.

The samples from group L were irradiated with a CO_2 laser (Ultra Dream Pulse , DS_40U, Daeshin Enterprise, Seoul, South Korea), emission wavelength 10,600 nm, for 30s. The parameter settings used were

Time on—100 μs; time off—40 ms;
Average power—0.73 W; peak power—292.73 W;
Speed of movement—2 mm/s;
Energy density with movements—5 J/cm^2;
Tip-to-tissue distance—20 mm; tip diameter 700 μm;
Irradiation time—30 s.

The measured values were confirmed using power meter.

The enamel samples from group FL were treated with APF gel for 4 min and then irradiated with the CO_2 laser for 30s without removing the gel. The gel was then washed off.

Demineralization Each enamel sample was placed in 50 ml soft drink (pH = 2.75) for 10 min [23]. After the erosive challenge, specimens were rinsed for 10 s with deionized water and stored in artificial saliva at 37 °C for 1 h, then rinsed with deionized water and dried with adsorbed paper.

Surface microhardness evaluation Surface microhardness was assessed at the baseline, after treatment, and after acid challenge.

Vickers hardness was determined using a microhardness tester (Tukon 1102, Wilson Hardness, Germany). The load applied was 50 g, with an indentation time of 10 s (Vickers pyramid: diamond right pyramid with a square base and an angle of $a = 136°$ between the opposite faces at the vertex and ×600 magnification of microscope).

The indentations were made for each specimen at three different locations (≥ 100 μm from each other), and the average of three measurements was calculated and obtained as one reading. Indentation result can be seen at projector screen in the form of shadow shaping rhomb; the diagonal length is measured with micrometer. Three indentations were made for each specimen and were independently averaged and reported in Vickers hardness number (VHN).

To reassure the blinding of the study, two examiners, working at the Department of Pediatric Dentistry, Plovdiv, Bulgaria, without knowing the procedures made to the samples, performed the microhardness evaluation.

Statistical analysis

The enamel surface microhardness data were statistically analyzed by descriptive statistics using mean values and standard deviation. The normality distribution of the data was checked by Kolmogorov-Smirnov test. After evaluating the assumptions of normality, data were analyzed using one-way ANOVA. The intra-group comparison was done by Tukey's post hoc test ($\alpha < 0.05$). The level of significance was established at 95%. All P values were two tailed.

SEM analysis

After the acid challenge, 10 samples from each subgroup (L, C, F, and FL) were prepared according to the previously described methods to be examined under scanning electron microscopy (SEM). The samples were then fixed (2.5% glutaraldehyde, 12 h, 4 °C), dehydrated (25–100% ethanol in increasing concentrations), dried, and spatter coated with gold and examined under scanning electron microscopy at ×100, ×1000, and ×2000 magnifications. The SEM preparation, the examination, and the analysis was done by two chemists working at the Department of Phisico-Chemistry, Bulgarian Academy of Science, Sofia, Bulgaria.

Results

Microhardness

The mean values for baseline enamel hardness in the control (C group), laser (L group), fluoride (F group), and fluoride and laser (FL group) groups are presented at Table 1. The established mean VHN in the four groups did not differ significantly ($p > 0.05$).

All the mean measurements of the specimens used for this in vitro study after treatment are presented in Fig. 1. The lowest measured microhardness values were observed at the control (group C). The other three groups showed significantly higher mean microhardness levels (Fig. 1).

There is different level in the microhardness (VHN) from the control to the L and FL groups. The mean VHN in the group Fluor and CO_2 laser (FL group) was significantly higher than that in the control group (C group).

Table 1 The descriptive statistics comparing microhardness mean values and SD (standard deviation) among the study groups before treatment

Groups	No	Mean (kgf/mm^2)	SD	Minimum	Maximum
Control	10	276.95	24.15	244.50	318.50
L	10	281.54	21.03	245.40	304.20
F	10	263.78	22.96	233.60	298.60
FL	10	280.57	16.45	255.60	307.60

Significance at $p \leq 0.05$ kgf/mm^2 —kilogram force per square millimeter

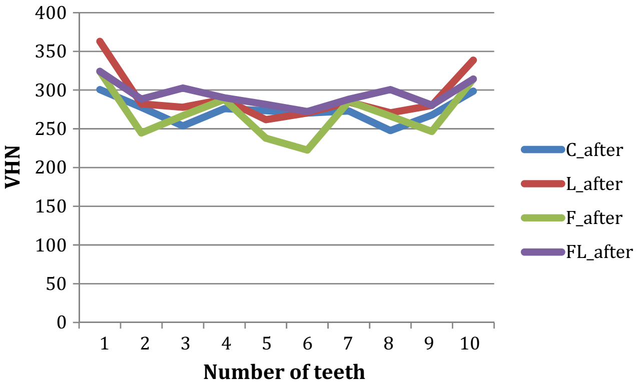

Fig. 1 The mean microhardness values after sample treatment

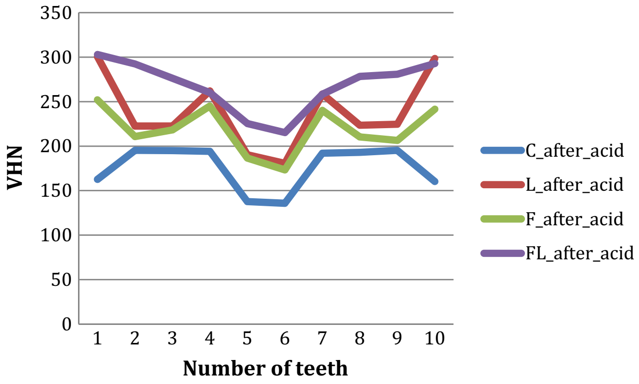

Fig. 2 The mean microhardness values after demineralization

A comparison of microhardness between the groups revealed differences that observed structural changes. The exact *t* test was calculated to measure the difference between all the groups (Table 2). Although the difference did not reach statistical significance, the mean VHN in the CO_2 laser group (L group) was lower than this in the Fluor and CO_2 laser group (FL group) ($p > 0.05$). Significant difference in the mean microhardness was observed between the CO_2 laser (L group) and the APF fluoride gel group (F group) ($p < 0.05$), as well as between the APF fluoride gel group (F group) and the Fluor and CO_2 laser (FL group) ($p < 0.05$). Compared to the control group, the differences were significant with the CO_2 laser group (L group) and the Fluor and CO_2 laser (FL group) ($p < 0.05$).

After the acid challenge was performed, the microhardness of the samples showed significant difference. The untreated group (C group) after soft drink challenge had significantly decreased microhardness. It ranged between 136 and 195 VHN, with a mean value of 176.13. This was the group with the lowest measured mean microhardness value. The scores in the CO_2 laser group (L group) were between 180 and 300 VHN with a mean of 238.40. The fluoride group (F group) showed a range of 173 and 252 VHN with a mean of 218.45. The combined application of CO_2 laser and fluoride (group FL) showed a range of 215–302 VHN with a mean of 268.28 (Fig. 2).

The mean measured microhardness values for all the specimens from all the groups, after acid challenge with demineralized solution are presented in Fig. 2. The untreated group (C group) had the lowest mean microhardness values. The laser and fluoride group (FL group) showed the highest mean microhardness levels. Both CO_2 laser (L group) and fluoride (F group) groups were with very close measurements observed. (Fig. 2).

The results showed that mean microhardness of enamel subsurface performed on permanent teeth was statistically different ($p \leq 0.05$). After the acid challenge, the untreated group was demineralized to a great extent and the enamel surface was with the lowest mean score of microhardness.

The differences between the groups were established. Additionally, post hoc paired *t* tests were carried out to compare the three examined moments—before treatment, after treatment, and after acid challenge for each of the treatment groups (Table 3).

The mean VHN was found to be statistically significantly higher in the FL group compared to that of the laser group ($p < 0.01$). The fluoride group (F group) was statistically significant lower than the laser group (L group) and fluoride and CO_2 laser group (FL group). Additionally, and more importantly, all the groups showed levels of mean microhardness significantly higher than the untreated control group (C group) after the acid challenge (Fig. 3).

After the acid challenge, the untreated control group decreased its microhardness by 44.98%. The carbon dioxide laser group presented a mean difference of 62.27 compared to the control group, which is 35.35% relatively higher. The

Table 2 Intra-group comparison of the treated groups

	Compared groups	Lower	Upper	*t*	Sig. (two tailed)
Pair 1	L_after—F_after	9.48874	34.65126	3.968	0.003*
Pair 2	L_after—FL_after	− 17.59453	12.46453	− 0.386	0.708
Pair 3	F_after—FL_after	− 39.39751	− 9.87249	− 3.775	0.004*
Pair 4	C_after—L_after	− 3.283.47	− 278.953	− 2.682	0.025*
Pair 5	C_after—F_after	− 1.497.416	2.349.016	0.501	0.629
Pair 6	C_after—LF_after	− 3.252.977	− 822.423	− 3.793	0.004*

Paired sample test. Paired differences, 95% confidence interval of the difference

*Indicates statistical differences between pairs

Table 3 Intra-group comparison of the treated groups after the acid challenge

	Compared groups	Lower	Upper	*t*	Sig. (two tailed)
Pair 1	L_after_acidF_after_acid	6.91792	32.98208	3.463	0.007*
Pair 2	L_after_acidLF_after_acid	− 50.49974	− 9.26026	− 3.278	0.010*
Pair 3	F_after_acidLF_after_acid	− 65.66654	− 33.99346	− 7.118	0.000*
Pair4	C_after acid—L_after_acid	− 9.287.186	− 3.166.814	− 4.603	0.001*
Pair 5	C_after_acid—F_after_acid	− 6.166.599	− 2.297.401	− 4.949	0.001*
Pair 6	C_after_acid—LF_after_acid	− 11.017.738	− 7.412.262	− 11.563	0.000*

Paired samples test. Paired differences, 95% confidence interval of the difference

*Indicates statistical differences between pairs

fluoride group (F group) showed difference of 42.32 which is 24.03% higher in comparison, and the laser and fluoride group showed a significant mean difference of 92.15, which is 52.32% higher in comparison (Fig. 4).

Scanning electron microscopy

All the SEM figures were done after the acid challenge.

In Fig. 5 are presented SEM micrographs representative of samples from the control group. The micrographs show exposed prisms due to enamel acid dilution. The acid demineralization resolved the untreated surface.

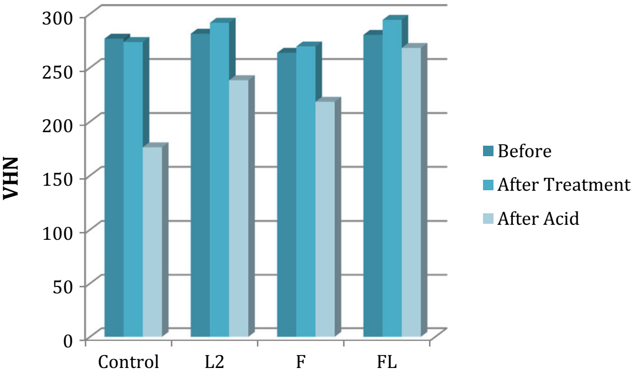

Fig. 3 Distribution of the microhardness values among all the groups

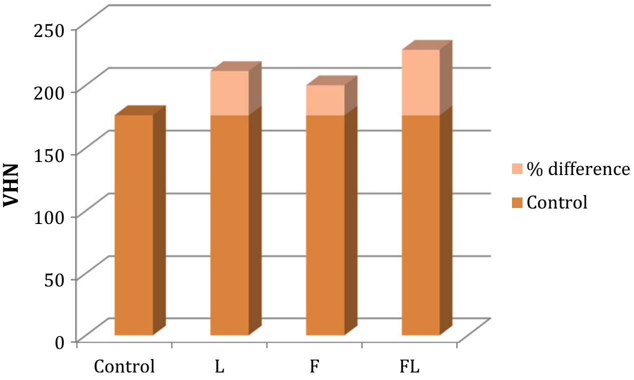

Fig. 4 Comparison and percentage difference of microhardness between the control group and the treated groups after the acid challenge

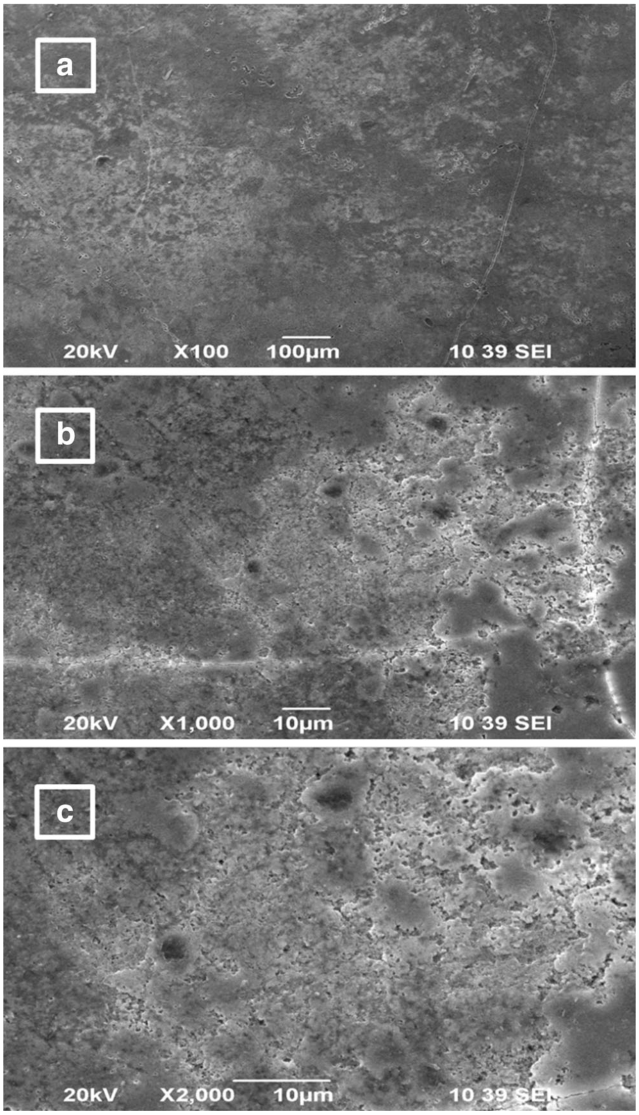

Fig. 5 Scanning electron micrographs of the enamel surface of the control sample. Magnification ×100 (**a**), ×1000 (**b**), and ×2000 (**c**)

Scanning electron micrographs of the enamel surface treated with CO_2 laser (L group) are shown on Fig. 6. The micrograph of CO_2 laser showed a relatively homogeneous and confluent surface coatings that masked the under lying enamel surface. Laser-induced microfractures on enamel surfaces after laser irradiation can be seen.

Scanning electron micrographs of the enamel surface treated with APF 1.23% are shown in Fig. 7. Surface morphology showed globular fluoride deposits on the enamel surface.

Figure 8 presents SEM micrographs of enamel surface after fluoride application and CO_2 laser irradiation. Almost invisible prisms full with fluoride and some enlighten showing presence of small cracks and lamella.

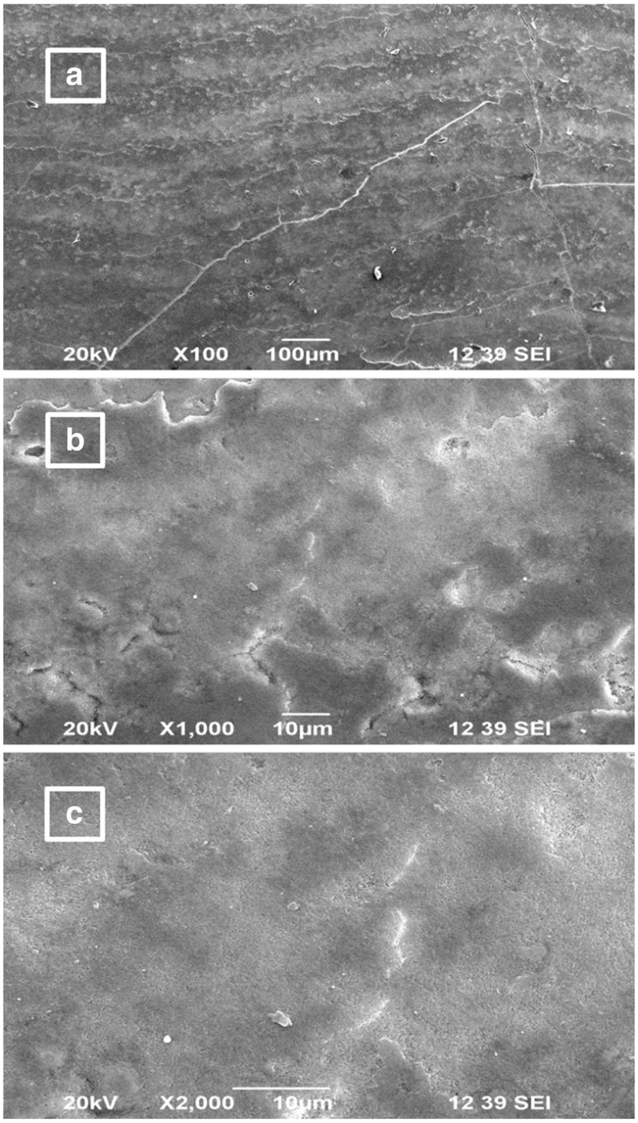

Fig. 6 Scanning electron micrographs of the enamel after CO_2 laser treatment sample. Magnification ×100 (**a**), ×1000 (**b**), and ×2000 (**c**)

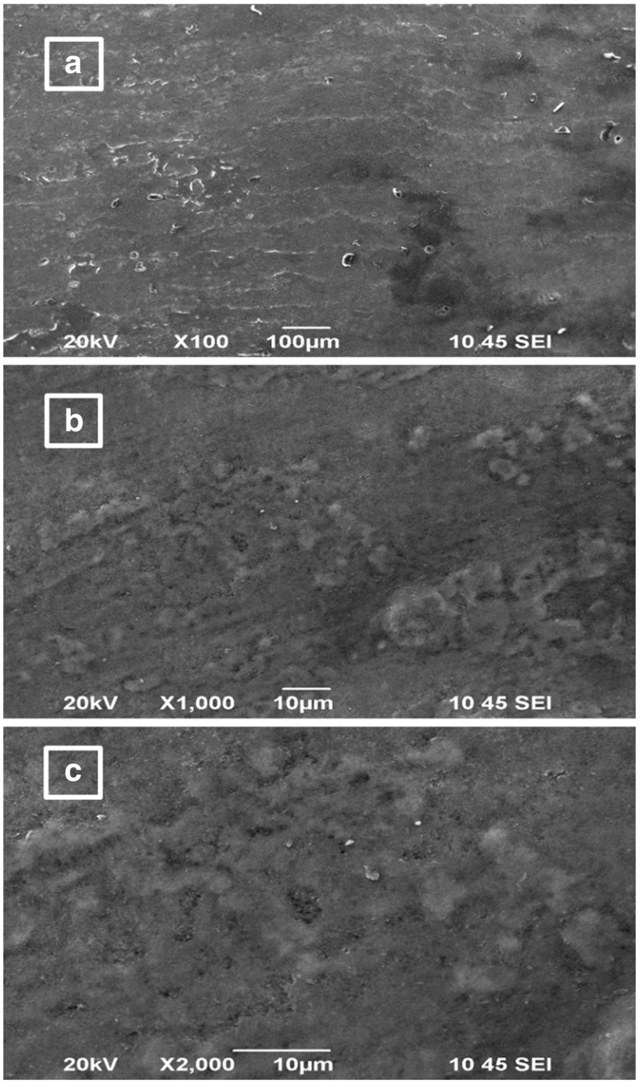

Fig. 7 Scanning electron micrographs of the enamel surface treated with APF 1.23%. Magnification ×100 (**a**), ×1000 (**b**), and ×2000 (**c**)

Discussion

The decreased solubility after CO_2 laser irradiation is mainly due to the thermal decomposition of the more soluble carbonated hydroxyapatite into the less soluble hydroxyapatite with changes in the crystallinity [24–26]. Thermal analysis studies of Holcomb and Young [27] and subsequent studies by Kuroda and Fowler [28] showed that there is substantial loss of carbonate and water at temperatures between 100 and 400 °C. This loss is sufficient to change the crystallinity of the intrinsic mineral and to form a purer phase that is the more acid resistant form of hydroxyapatite. These findings were reflected by the present results obtained with the CO_2 laser group (L group) and CO_2 laser and fluoride group (FL group). After the acid challenge, the untreated group was demineralized to a great extent and the enamel surface was with the lowest mean score of microhardness. The laser-

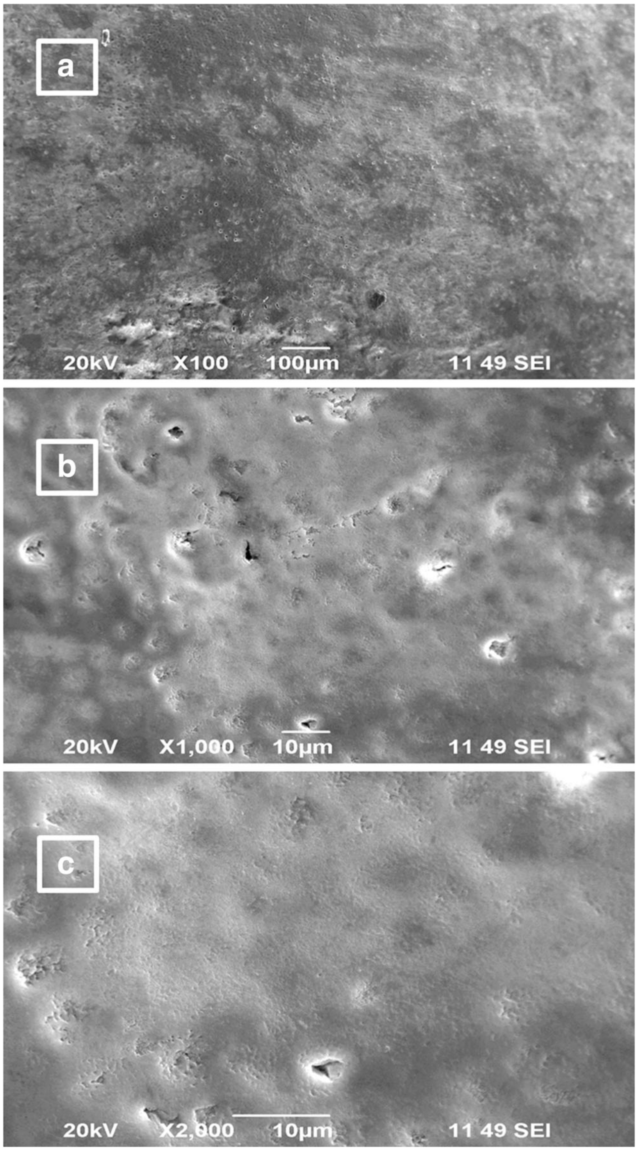

Fig. 8 Scanning electron micrographs of the sample surfaces from the CO_2 laser and fluoride group (FL group). Magnification ×100 (**a**), ×1000 (**b**), and ×2000 (**c**)

irradiated and fluoride-treated samples (groups FL) showed the least diminution in enamel surface microhardness followed by the L group.

The results of the current study were also in accordance with many previous studies. Tagomori and Morioka [29] suggested that the CO_2 laser-modified enamel had increased the uptake of acidulated phosphate fluoride. The authors state that the APF gel application before laser irradiation is more effective, because greater fluoride uptake was achieved. In our study, we established greater acid resistance in the group of specimens treated first with the APF gel and then irradiated with CO_2 laser. Correa-Afonso et al. [30] and Paulos et al. [31] suggested that the combination of CO_2 irradiation with fluoride in solution was more effective than CO_2 laser alone. Our study showed that the CO_2 laser alone is also significantly effective but the highest acid resistance is achieved by combined application of fluoride gel and CO_2 laser irradiation. Jeng et al. [32] also investigated the efficiency of this combination. They applied fluoride compounds before irradiation and found increased mechanical properties of the calcium fluoride-like deposits. The wear resistance of the calcium fluoride-like deposits improved about 40% following irradiation for 10 s. The same results are obtained by Vieira et al. [33]. The authors showed that it was possible to transform hydroxyapatite crystals to fluorapatite crystals instantaneously in the presence of fluoride using a CO_2 laser irradiation.

In our study, the CO_2 laser treatment alone significantly increased the enamel resistance. The group treated only with fluoride gel did not show significant difference from the control group. The combination CO_2 laser irradiation with fluoride was found to have advantage over laser and fluoride application alone. These findings were as well in line with some previous other works, which showed that the laser energy in combination with topical fluoride treatment could increase the resistance of tooth structure to mineral loss [17, 34].

Another important factor is the specific laser parameters that can influence on controlling enamel demineralization and may lead to different results over fluoride uptake [35].

According to the studies conducted by Fried et al. [36, 37], for the same pulse duration of 100 μs, the highest percentages of inhibition of mineral loss were observed with energy density around 12 J/cm^2. In our study, the highest microhardness values were observed with a fluence of only 5 J/cm^2. So with the same pulse duration, the amount of energy required to modify the hard dental tissue leading to an increase in acid resistance of enamel was decreased. And this was in accordance with many thorough studies [38–40] where they used a short pulse width and low energy and they obtained an increase in the enamel acid resistance.

In our study, SEM micrograph of enamel surface after fluoride application and CO_2 laser irradiation showed homogenous structure, almost invisible prisms full with fluoride. CO_2laser irradiation fused the surface, creating a smooth recrystallized aspect. Wu et al. also observed under SEM fusion between hexagonal-shaped crystals of the enamel surface after the 10,600 nm CO_2 laser treatment [41].

The effect of extrinsic and intrinsic acids on dental hard tissues can be investigated by the microhardness test. This technique applied in our investigation can evaluate early stages of enamel and dentin dissolution, which are associated with weakening and softening of the surface [23, 42]. The combined application of CO_2 laser and fluoride (group FL) showed the highest microhardness score 268.28 VHN. After the acid challenge, the enamel microhardness of the untreated group decreased by 44.98%. In the CO_2 laser group, the microhardness increased by 35.35%, in the fluoride group by 24.03%, and the laser and fluoride group (FL group) showed a significant increase of 52.32%. Some studies in the literature [43] showed very limited effect in the increase of the microhardness (around 30%) when laser irradiation was combined with fluoride. However, this was accompanied with excessive surface damage. The use of a high-energy density and longer pulse widths during laser irradiation was probably the reason behind this. Our in vitro study used a CO_2 laser (Ultra Dream Pulse) with a short pulse duration 100 μs and interval time of 40 ms, so higher thermal relaxation time. The irradiation was done at a distance of 2 cm in the focal point of the laser beam using low energy density of 5 J/cm^2. No thermal surface damages occurred.

The experimental results of our study showed promising opportunities with some clinical limitations. The CO_2 laser treatment was performed only once followed by a high percentage reduction in enamel mineral loss revealed by the enamel microhardness values. This could be an advantage for the treatment of patients because it would not involve dependence on frequent use of mouth rinses. However, future studies should be conducted in order to evaluate the long-term predictability of this innovative CO_2 laser demineralization and caries preventive therapy. Other studies may be conducted to possibly evaluate the quantity of mineral loss.

Conclusion

Under the in vitro conditions in which several variables acted directly on the samples, it could be concluded that the treatment with CO_2 laser (10,600 nm) associated with the acidulated phosphate fluoride (APF) was able to cause a significant prevention of enamel demineralization at all analyzed groups both in relation to the control and to to fluoride group.

The optimum CO_2 laser parameters obtained in the present investigation (5 J/cm^2, 0.73 W) were able to decrease enamel demineralization by 53% without causing surface and subsurface thermal damage observable by scanning electron microscopy.

Microhardness values found in the current study showed a significant difference between lased and unlased groups as well as between sample groups with and without fluoride application. The laser-irradiated and fluoride-treated samples (FL group) showed the least diminution in enamel surface microhardness.

In short, carbon dioxide laser irradiation at sub-ablative low power settings in combination with fluoride treatment is more effective in protecting enamel surface and resisting demineralization than CO_2 laser irradiation or fluoride application alone.

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Consent was not required owing to the anonymous nature of the sourcing of teeth extracted for oral surgery purposes at Medical University, Plovdiv, Bulgaria.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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