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ORIGINAL ARTICLE

Fluoride uptake and acid resistance of enamel irradiated with Er:YAG laser

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Abstract This study evaluated the resistance to demineralization and fluoride incorporation of enamel irradiated with Er:YAG. A total of 110 bovine teeth were selected and divided into eight groups: unlased, 37% phosphoric acid, and samples irradiated with the Er:YAG laser at several fluences (31.84 J/cm^2, 25.47 J/cm^2, 19.10 J/cm^2, 2.08 J/cm^2, 1.8 J/cm^2, and 0.9 J/cm^2). The application of acidulated phosphate fluoride was performed after treatments. All samples were immersed in 2 ml of 2.0 M acetic–acetate acid solution at pH 4.5 for 8 h, and fluoride, calcium, and phosphorus ions dissolved were analyzed by atomic absorption spectrometry and spectrophotometry. The phosphoric acid and 31.84 J/cm^2 groups presented the lowest dissolution of calcium and phosphorus ions. Higher fluoride incorporation was observed on 1.8 J/cm^2 and 0.9 J/cm^2 groups. Based on these results, Er:YAG laser was able to decrease acid dissolution and increase fluoride uptake and can be a promissory alternative for preventive dentistry.

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Introduction

The prevalence of dental caries has declined due to the worldwide preventive measures adopted, such as water fluoridation and the waste of products containing fluoride. Despite of that, dental caries is reported to be the most common chronic childhood disease, and its manifestation is still high in some groups. The main reasons for this high manifestation in some individuals are the growing sugar consumption and inadequate exposure to fluorides [1–4].

The caries process is characterized by mineral loss of enamel and dentin, which is caused by the acid by-products of bacterial metabolism [5, 6]. In this context, fluoride is a widely applied method for caries prevention that can enhance the subsurface remineralization of carious enamel, resulting in arrestment or reversal of caries lesions and inhibit the demineralization during the bacterially generated acid challenge [6, 7]. Topical fluoride application results in a deposition of surface crystals of calcium fluoride (CaF_2) that act as a reservoir releasing fluoride in the demineralization process. However, there is a small amount of fluoride that is retained in the enamel as fluoridated apatite or fluorapatite. The calcium fluoride formed presents higher solubility (12–15 mg/l) when compared with fluorapatite [8], which is almost insoluble and can provide a long-lasting protection against caries process [9]. Because of that, several applications of topical fluoride is necessary to maintain the anti-caries effect [10].

Considering its strong interaction with dental hard tissues, lasers are also used for caries prevention [11–18]. Since the first study carried out by Stern and Sognnaes [19] in the early 1970’s, it was demonstrated that laser irradiation could increase the acid resistance of enamel with promissory results. Lasers that have a higher absorption by dental enamel, such as CO_2 (9.6 μm and 10.6 μm) [17], Er:YAG (2.94 μm) [14, 20] and Er,Cr:YSGG (2.79 μm) [11, 21] were employed with success; however, the low-absorbed lasers such as Nd:YAG (1.064 μm) [15] and Argon [13] have also demonstrated to promote a similar effect in the acid resistance of enamel.

Despite its main application for cutting dental hard tissues, the use of erbium lasers such as Er:YAG and Er,Cr:YSGG have been also used for preventing enamel demineralization [11, 12, 14, 20, 21]. Er:YAG laser was the first laser approved by the Food and Drug Administration in 1997 for application in dental hard tissue [22]. This laser is emitted at 2.94 μm, and it has strong absorption by water and low absorption by hydroxyapatite, making possible the cutting of enamel by the ablation process that is achieved by the absorption of laser energy by the water droplets contents in enamel, which results in water micro-expansion and in the ejection of hard tissue [23].

Although the effect of laser irradiation on acid resistance of enamel is recognized, the benefit from laser irradiation on fluoride uptake is not clearly established. Thus, the aim of the present study was to investigate the increase of acid resistance of enamel and fluoride incorporation when irradiated with Er:YAG laser at fluences below and above the ablation threshold, combined with topical application of fluoride.

Materials and methods

For this study, there were selected 110 sound bovine teeth that were cleaned with pumice and stored in 0.1% thymol solution under refrigeration for no more than a week. Teeth with cracks or irregularities of the enamel structure were excluded.

Laser irradiation was performed using an Er:YAG laser (Opus 20, Opus Dent, Israel), 2.94 μm wavelength, pulsed with a duration of 400 μs. The energy is delivered through a sapphire fiber optic that has 1-mm beam diameter when in contact from irradiated surface.

Scanning electron microscopy analysis

For this analysis, 30 teeth were chosen. From each tooth, one enamel specimen with 4×4 mm was cut from the buccal surfaces with a slow speed diamond blade (Isomet, Buehler, IL, USA). Samples were cleaned on ultrasonic bath and, after that, randomly divided into six groups (n=5) according to the irradiation conditions described in Table 1.

All area of enamel surface was irradiated uniformly by hand over a period of 30 s. Laser irradiation was performed without refrigeration in all specimens.

After laser irradiation, the samples were cleaned on ultrasonic bath and were fixed with 2% glutaraldehyde solution for 2 h. The samples were immediately perfused with a 0.1 M-phosphate buffered solution at room temperature, rinsed with distilled water and then dehydrated in a graded series of alcohol solutions (50, 70, 80, 90, 95, and 100%) for 10 min at each concentration. These samples were sputtered with 15-μm thick of gold and submitted to analysis of scanning electron microscopy (Phillips XI-300, Eindhover, Holland).

Atomic absorption spectrometry and spectrophotometry analysis

For these analyses, 80 teeth were used. The crowns were separated from the roots by a low speed diamond saw (Isomet), and these crowns were individually embedded in acrylic resin. An area of 4×4 mm was isolated from the buccal surface of each sample using an acid-resistant varnish. Samples were kept in humid environment until the time of the experiment.

After cleaning with pumice and deionized water, the samples were randomly divided into eight groups (n=10) according to that described in Table 2.

In group 2, the samples were etched with 37% phosphoric acid (Scotchbond^{MR}–3 M, Brazil) during 15 s, and then washed with deionized water during 15 s and dried with absorbent paper. In lased samples, Er:YAG laser was applied in all area of enamel surface uniformly by hand over a period of 30 s. Laser irradiation was performed without air-water mist in all specimens.

After these procedures, all samples of this work had the application of an acidulated phosphate fluoride gel (Nupro–Dentsply, USA) containing 1.23% F^- and 0.1 M H_3PO_4 at pH 3.6. The fluoride application was performed during 4 min using a cotton swab [24], and then, samples were washed with deionized water for 1 min and dried with absorbent paper.

Each sample was demineralized by immersion in 2.0 ml acetate buffer solution (2 M, pH 4.5) for 8 h [25] in a water

Table 1 Irradiation conditions on groups for scanning electron microscopy analysis

SEM group	Energy per pulse (mJ/pulse)	Repetition rate (Hz)	Fluence (J/cm^2)	Distance from surface
1	250	7	31.84	Contact mode
2	200	7	25.47	Contact mode
3	150	7	19.10	Contact mode
4	250	7	2.08	3.0 cm
5	200	7	1.8	2.6 cm
6	100	7	0.9	2.6 cm

Table 2 Discrimination of groups for atomic absorption spectrometric and spectrophotometric analyses

Group	Treatment	Laser parameters			
		Energy per pulse (mJ/pulse)	Repetition rate (Hz)	Fluence	Distance from surface
1	Untreated (control)	–	–	–	–
2	Etching with 37% phosphoric acid	–	–	–	–
3	Er:YAG irradiation	250	7	31.84 J/cm^2	Contact mode
4	Er:YAG irradiation	200	7	25.47 J/cm^2	Contact mode
5	Er:YAG irradiation	150	7	19.10 J/cm^2	Contact mode
6	Er:YAG irradiation	250	7	2.08 J/cm^2	3.0 cm
7	Er:YAG irradiation	200	7	1.8 J/cm^2	2.6 cm
8	Er:YAG irradiation	100	7	0.9 J/cm^2	2.6 cm

bath with a controlled temperature of 37°C. After this, the specimens were removed from the demineralization solution and the calcium, phosphorus, and fluoride contents of the solutions were determined by an atomic absorption spectrometer (Analyst 300–Perkin Elmer, Germany) and a spectrophotometer (DR 2000-HACH, USA).

Results

Scanning electron microscopy analysis

The results of this analysis are shown in Figs. 1, 2, and 3. In Fig. 1, at laser fluence of 31.84 J/cm^2, ablation areas can be seen with fissures and conical craters with sharp enamel projections. In Fig. 2a and b, at laser fluences of 25.47 J/cm^2 and 19.10 J/cm^2, ablation areas with the exposition of the enamel rods were observed. In both Figs. 1 and 2, there is no evidence of thermal damages, cracks, or melting.

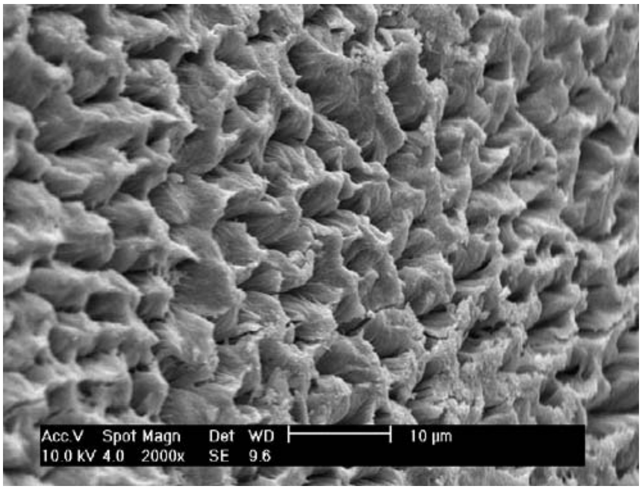

Fig. 1 Scanning electron microscopy of lased enamel with 31.84 J/cm^2 when an irregular surface with ablation areas and sharp enamel projections was produced. Original magnification=2,000×

In Fig. 3a–c, enamel surfaces were irradiated with the sub-ablative fluences of 2.08 J/cm^2, 1.8 J/cm^2, and 0.9 J/cm^2, respectively. After irradiation, flatten surfaces can be noted, similar to unlased enamel. There is no evidence of ablation or thermal damage in enamel, and there were no exposition of the enamel rods.

Atomic absorption spectrometry and spectrophotometry

The calcium, phosphorus, and fluoride contents of the demineralization solution of the individual samples was averaged arithmetically and the standard deviation calculated for statistical analysis [analysis of variance (ANOVA) and Duncan's test]. Table 3 shows the means and standard deviations for calcium, phosphorus, and fluoride contents in each group of this study.

The solutions of the control group (G1) presented the highest amount of calcium, phosphorus, and fluoride related to the other groups. In relation to the calcium content, G1 (115.7+18.7 mg/l) presented values statistically higher than groups G2 (67.5+27.4 mg/l) and G3 (64.6+14.9 mg/l). These groups, however, presented the lowest media compared to the other groups. The groups G4 (98.5+26.2 mg/l) to G8 (106.4+30.9 mg/l) presented amounts of calcium similar to the control group (Fig. 4).

For the phosphorus contents, the group G1 (1,273.6+239.1 mg/l) presented values similar to the groups G4 (1,061.1+253.5 mg/l) and G5 (1,069.0+219.3 mg/l). A marked reduction in phosphorus solubility was observed in groups G2 (635.5+283.0 mg/l), G3 (600.5+181.8 mg/l), G6 (736.9+212.6 mg/l), and G7 (696.5+248.1 mg/l), which had the less phosphorus contents compared to the groups G1, G4, G5, and G8 (953.6+370.8 mg/l). These results can be seen in Fig. 5.

An increase in fluoride incorporation tended to be seen in samples of groups G7 and G8 when lowest laser fluences were used (Fig. 6). These groups had means of 31.1+13.6 and 38.7+17.8 mg/l, respectively, and the group G7 presented a mean significantly lower ($p<0.0001$) when

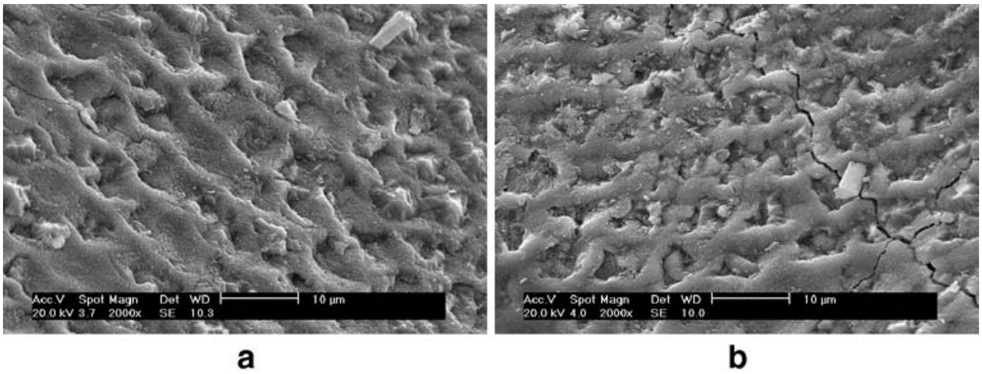

Fig. 2 SEM photographs of lased samples with 25.47 J/cm^2 (**a**) and 19.10 J/cm^2 (**b**) when ablation areas with the exposition of enamel rods can be seen. Original magnification=2,000×

compared to the other groups. However, the groups G4 (85.4+22.4 mg/l) and G5 (88.7+19.8 mg/l) had the higher fluoride liberation, and this liberation was statistically similar to the control group (96.5+21.9 mg/l). The G2 group, when phosphoric acid was applied before fluoride application, showed a mean of fluoride incorporation (54.4+16.7 mg/l) statistically similar to the G3 (59.0+17.8 mg/l) and G8 (38.7+17.8 mg/l) groups when the highest and the lowest fluences were used, respectively.

Discussion

Although there are several researches that confirm the preventive effect of lasers, there is a need to determine whether Er:YAG laser at sub-ablative conditions can reduce the enamel solubility to acids and increase the fluoride incorporation on enamel.

The erbium laser became an important tool in dentistry, making possible the laser application in several procedures with the same equipment. For the use of this wavelength in caries prevention, the sub-ablative conditions are important to promote chemical changes without morphological damages in enamel surfaces [21, 26]. Because of its higher absorption in water contents of enamel, Er:YAG laser causes micro-explosions that result in ablation process [11, 23]. This process promotes an irregular surface that could propitiate a higher plaque accumulation.

The ablation threshold of Er:YAG laser was determined by several authors, however, without agreement between them. The fluence of 7.2 J/cm^2 with a repetition rate of 5 Hz and 18.6 J/cm^2 with a repetition rate of 2 Hz was found by Li et al. [27] as the ablation threshold; Fried et al. [11] reported 7 J/cm^2 of threshold, and Apel et al. [28] reported a threshold between 9 and 11 J/cm^2. The present work tested fluences of 0.9 J/cm^2, 1.8 J/cm^2, and 2.08 J/cm^2 to assure fluences that are below the ablation threshold, avoiding mechanical damages on the enamel.

To choose an appropriate irradiation condition for a clinical application, energies that do not promote thermal damages in pulp and periodontal tissues must be considered. It was demonstrated by Zach and Cohen [29] that increments of 5.6°C are tolerable by dental pulp; however, above this threshold, the temperature rises are potentially threatening and can result in pulpitis and pulpal necrosis. Concerning Er:YAG laser, temperature rises of 3°C in pulp chamber when Er:YAG laser was irradiated with energies of 500 and 850 mJ/pulse, and 10 Hz of repetition rate [30] were reported. Therefore, the energies applied in the present

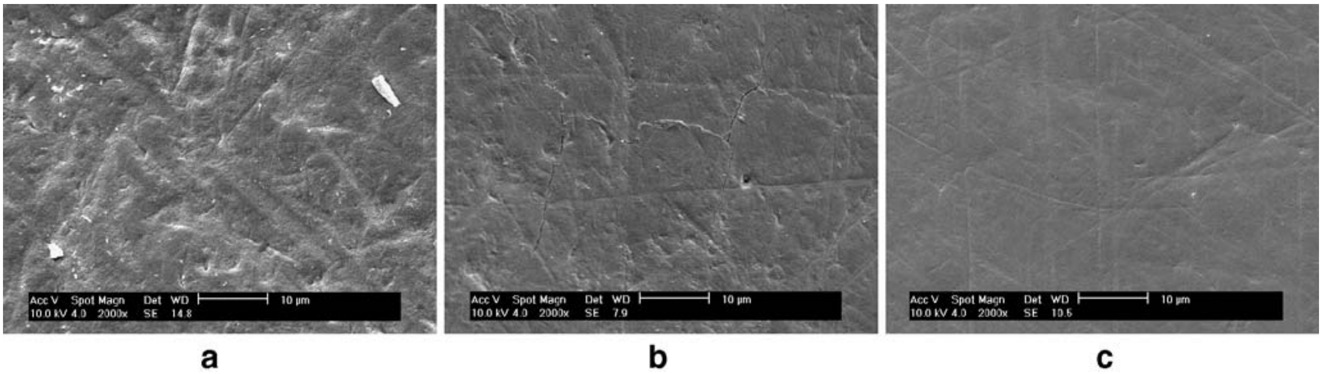

Fig. 3 SEM photographs of lased samples with 2.08 J/cm^2 (**a**), 1.8 J/cm^2 (**b**) and 0.9 J/cm^2 (**c**). Flatten surfaces and any visible morphological changes on enamel surface can be seen. Original magnification=2,000×

Table 3 Means and standard deviation of calcium, phosphorus, and fluoride contents in demineralization solution (mg/l)

Group	F		Ca		P	
	Mean	SD	Mean	SD	Mean	SD
G1	96.5 a	21.9	115.7 a,c	18.7	1,273.6 a	239.1
G2	54.4 b,c	16.7	67.5 b	27.4	635.5 b	283.0
G3	59.0 b	17.8	64.6 b	14.9	600.5 b	181.8
G4	85.4 a	22.4	98.5 a	26.2	1,061.1 a,d	253.5
G5	88.7 a	19.8	123.9 c	28.1	1,069.0 a,d	219.3
G6	63.3 b	21.1	95.4 a	16.2	736.9 b,d	212.6
G7	31.1 d	13.6	93.6 a	19.6	696.5 b,d	248.1
G8	38.7 c,d	17.8	106.4 a,c	30.9	953.6 d	370.8

Means followed by distinct letters are statistically different by ANOVA and Duncan's test ($p<0.05$).

study (100 up to 250 mJ/pulse) were considered safe concerning the pulp vitality.

A water layer is shown to be an important initiator for erbium lasers, increasing the interaction of laser irradiation with dental hard tissues and, consequently, increasing the ablative process [23, 31, 32]. However, it was reported that Er:YAG laser without coolant had more effectiveness in caries prevention when compared with Er:YAG laser with water mist [20, 33]. Due to this fact and to reach sufficient temperature at the surface to promote crystallographic changes, all irradiation conditions used in the present work were used without water mist [12].

The results of this study showed no changes in the morphology of the enamel when irradiated with fluences of 0.9 J/cm^2, 1.8 J/cm^2, and 2.08 J/cm^2, which confirm that these fluences are below the ablation threshold. All irradiated surfaces presented a smooth pattern, without ablation or cracks. However, the fluences of 19.10 J/cm^2, 25.47 J/cm^2, and 31.84 J/cm^2 promoted the removal and exposition of enamel rods, with a slight ablation of the surface, which agrees with some previous findings that reported an etched pattern [25, 32]. Even irradiated without water mist, Er:YAG laser irradiation did not promote cracks, carbonization, or melting of enamel surface. It has been reported that the acid solubility of irradiated enamel can be a function of the energy density [21]. In the present study, the use of fluences above the ablation threshold is due to confirm this hypothesis.

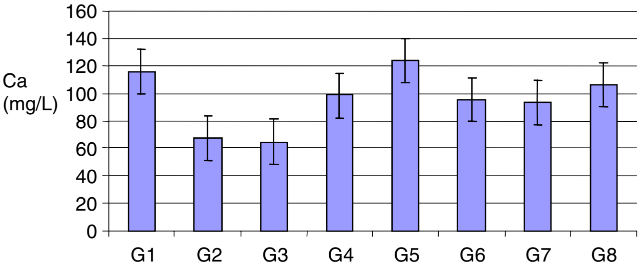

Fig. 4 Means of calcium contents in the demineralization solution and respective 95% confidence interval

For preventing dental caries, laser irradiation should promote chemical changes in dental enamel that decrease its solubility. There are some theories that explain the mechanism of the increase of enamel's acid resistance induced by laser irradiation: (1) the decrease of enamel permeability due to melting and recrystallization of enamel surface [34]; (2) the decrease in enamel's solubility due to the formation of less soluble substances, such as tetracalcium diphosphate monoxide [35]; (3) the decrease in enamel's solubility due to changes in its ultra-structure, as the reduction of water and carbonate contents, the increase in the hydroxyl ion contents, formation of pyrophosphates, and the decomposition of proteins. The crystallographic changes on enamel are promoted by heating the surface with laser irradiation. It has been reported that temperature rises of 100 to 650°C are necessary to promote water evaporation, oxidation of organic components, conversion of acid phosphate to pyrophosphate, and the beginning of carbonate loss [17, 36]. The decomposition and oxidation of carbonate occurs in temperature ranges from 650 to 1,100°C [36]. At 1,100°C, all carbonate is eliminated, and there is the formation of new crystalline phases (α-TCP and β -TCP phases), which are less resistant to demineralization [37]. At the ablation threshold, Er:YAG laser can provide temperature rises up to 300°C [11], promoting the water evaporation and loss of carbonate. Therefore, reaching this temperature range, Er:YAG has a theoretical potential for inducing acid resistance in enamel.

To simulate the demineralization process that occurs *in vivo*, an acetate buffer solution was employed [21, 25]. However, the samples were immersed no more than 8 h [25] to reduce the demineralization process and to avoid the removal of the surface of enamel. Calcium and phosphorus are the components that prevail as the crystalline components of the enamel. In the demineralization process, calcium and phosphate groups of the hydroxyapatite

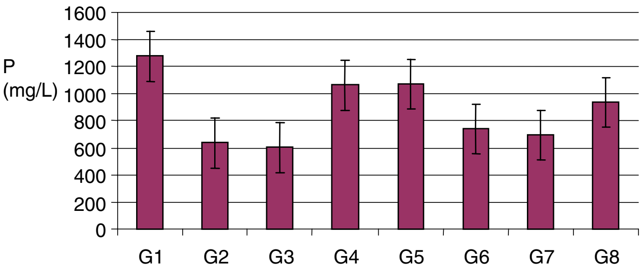

Fig. 5 Means of phosphorus contents in the demineralization solution and respective 95% confidence interval

dissolve, which characterizes the caries lesions [21]. In the present study, calcium and phosphate dissolution were measured to evaluate the resistance of enamel to acid after the treatments, and fluoride dissolution was measured to detect the fluoride incorporation on enamel.

All lased groups showed an increase in fluoride incorporation when compared to the control group, which agrees with the results obtained by Apel et al. [21] and Delbem et al. [14]. The higher fluoride incorporation was noted in samples irradiated with the lowest fluences (1.8 J/cm^2 and 0.9 J/cm^2). These results indicate that the low fluences generated some structural changes in the enamel surface that propitiated the retention of fluoride. Similar findings were reported when Argon laser was irradiated at low fluence on enamel when it was suggested that laser irradiation could increase the fluoride diffusion through the enamel structure or generated reservoirs for fluoride deposition into enamel [13]. In the present study, however, the enamel irradiation with fluences of 25.47 J/cm^2 and 19.10 J/cm^2 had fluoride liberation similar to the control group. The fluences above the ablation threshold did not increase the fluoride incorporation on enamel, which led us to suppose that the microspaces created by laser irradiation was not able to retain fluoride ions as suggested by previous studies [17, 38].

A decrease in calcium solubility was found in samples irradiated with the highest laser fluence (31.84 J/cm^2). The other fluences used in the present work showed similar calcium solubility than the control group, which agrees with the theory that acid resistance is greater when higher fluences are applied on the surface [21]. When analyzing the phosphate dissolution, the results were similar to the calcium contents.

Concerning the use of Er:YAG laser for caries prevention, a significant increase in acid resistance of enamel irradiated with Er:YAG at 0.39 J/pulse [20] and an inhibition of 40% of enamel dissolution when laser with Er:YAG at incident fluence of 12 J/cm^2 and 9 J/cm^2 were reported [11]. In another study, Er:YAG laser demonstrated to decrease the enamel demineralization even when applied at sub-ablative fluences [39]. However, when irradiated at sub-ablative fluences, Er:YAG laser showed calcium solubility similar to unlased group [21], and this finding is similar to those in the present work. In fact, when irradiated with fluences nearly the ablation threshold, it was expected to reach temperature ranges of 300–400°C that induce loss of carbonate and increase the acid solubility of enamel [11]. When enamel is irradiated at the sub-ablative fluences used in the present work, the peak temperature rise is expected to be below 100°C, and it seems to be not sufficient to induce crystallographic changes on enamel surface [11]. In this study, the acid-etched group also revealed a higher decrease in solubility of enamel, and this finding is confirmed by Shirazuka et al. [40].

In the present work, the group irradiated with 1.8 J/cm^2 presented the higher fluoride retention; however, it was the group that presented the greatest dissolution of calcium and phosphate. In fact, the calcium dissolved in the demineralizing solution could also be from the calcium fluoride crystals formed after topical application of acidulated phosphate fluoride and not only from the crystalline structure of enamel.

The results of the present study clearly show that the effect of Er:YAG laser irradiation associated with topical application of fluoride is located at enamel surface and depends exclusively on the fluence applied. Further studies are necessary to investigate the reduction on the progression of caries lesions in depth and to understand the involved mechanisms of induced acid solubility when dental enamel is irradiated with Er:YAG at sub-ablative fluences.

Conclusion

The combined Er:YAG laser irradiation and topical application of fluoride showed a beneficial effect on enamel's acid resistance and an increase of fluoride incorporation. However, these effects depend on the fluence applied over the surface [2, 3, 16].

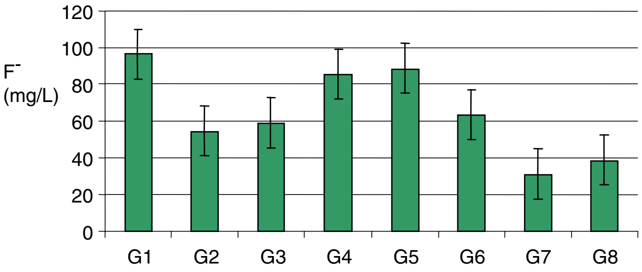

Fig. 6 Means of fluoride contents in the demineralization solution and respective 95% confidence interval

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