EFFECT OF ER:YAG LASER ON SODIUM FLUORIDE VARNISH UPTAKE BY PRIMARY TOOTH ENAMEL: AN *IN-VITRO* STUDY

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ABSTRACT: This *in vitro* study evaluated the effect of Er:YAG laser irradiation on the fluoride ion (F) uptake of primary enamel. It is believed that Er:YAG laser irradiation on the dental structure can lead to a more F uptake and a more acid-resistant surface. Ninety enamel samples obtained from 90 primary canines were ground and randomly divided into six groups (n=15): group 1 (G1): no treatment (negative control); G2: F varnish #1; G3: F varnish #2; G4: Er:YAG laser (2 Hz, 80-90 mJ, 60 s/cm^2, handpiece RO2, beam diameter at the focal area 300 µm); G5: F varnish #1 + Er:YAG laser, and G6: F varnish #2 + Er:YAG laser. After the surface treatment, the samples were submitted to an acid challenge consisting of daily immersion for 5 days in demineralizing and remineralizing solutions for 3 and 21 hr per day, respectively. The F concentrations were then calculated with a potentiometer. Chi-squared and variance analysis with post hoc Tukey tests were used for the statistical analyses ($p<0.05$). There were statistical differences between G1 and G4 when compared with G2, G3, and G6 ($p<0.05$). G2, G3, G5, and G6 exhibited a higher F uptake compared with G4 and the non-irradiated samples (G1). The findings revealed that Er:YAG lasers can be a suitable tool for enhancing primary enamel resistance to acids.

Key words: Er:YAG laser; Fluoride uptake; Primary teeth.

INTRODUCTION

In recent years, high-power lasers have been introduced as an adjunctive technique to prevent caries since they increase the resistance of tooth enamel to acids. The effect of lasers depends on the interaction between the laser beam and some components of the enamel. The enamel is composed of carbonated calcium hydroxide (85%), water (12%), and proteins and lipids (3%). Use of a wavelength of laser that is absorbed by both water and hydroxyapatite results in thermal, chemical, or morphologic changes in the enamel.[1] High-intensity lasers have been used in pediatric dentistry as an acceptable and pain-free therapeutic tool, with many studies reporting their use for the preparation of cavities, the removal of caries, and the prevention of caries in children.[2, 3] Irradiation by laser beams on tooth hard tissues results in morphologic and structural changes in tooth structure, changing the tooth permeability, which in turn increases the resistance of tooth enamel against acid attack. The extent of the changes depends on the laser beam parameters (wavelength, output power, and the exposure time) and the optical properties of the tissue (hydration conditions, the presence of camphors, and absorption coefficient).[4] Topical fluoride ion (F) leads to remineralization of

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incipient caries. Considering the progressive course of caries in children, one of the methods to overcome such a problem is to use topical F. When F is used correctly, its different forms can improve the oral health status of toddlers and children. Similarly, in too many other cases, the incorrect use of F will give rise to overt complications. Therefore, the dentist is responsible for instructing patients and the correct prescription of these products.[5] Despite a decrease in the prevalence of caries in Western countries, unfortunately in our country the caries rate is still very high among children.[6] A few studies have been done with regard to fluoride in Iran.[7-11] Although the role of the systemic ingestion of F from drinking water, as compared to its topical use, is an area in which differing views are held, in many parts of Iran, the F content of drinking water is below the level considered by some to be optimal.[12] As a result, it appears that, apart from an emphasis on instructing about oral health and correcting the children's dietary habits, local F therapy can have a greater role in decreasing the caries rate. Efforts are underway now to determine the factors that can increase and lengthen the effect of F on tooth structures. Furthermore, since caries are more common in primary teeth, which in itself is one of the most important factors in transferring caries to newly erupted permanent teeth, the present study was undertaken to determine techniques to increase the efficacy of F, to decrease the dose and frequency of F therapy, and to prevent fluorosis because the incorrect and excessive use of F increases the risk of fluorosis.[2] Er:YAG lasers are solid-state lasers whose lasing medium is erbium-doped yttrium aluminium garnet. Several studies have shown the beneficial effects of laser beams on the prevention of caries. However, the precise parameters of erbium laser beams that bring about such an effect have not been mentioned in references.[13] In addition, the efficacy of laser in combination with F has not been completely elucidated.[1] Therefore, the aim of the present study was to evaluate the extent of uptake of local F in enamel with the use of erbium laser irradiation in primary teeth.

MATERIALS AND METHODS

Preparation of the samples: Ninety extracted primary canine teeth were selected. The protocol of the study was approved by the Ethics Committee, Faculty of Dentistry, Kerman University of Medical Sciences, Kerman, Iran, under the code K/92/470. The sample size was 15 teeth based on similar studies.[2] Having used the formula to compare means, the minimum estimated sample size was 10 in each group; in order to adjust for the multiple comparison effect, we recruited 15 samples. The assumptions for sample size calculation were: alpha error=0.05, power=0.9; d=1.5 SD.

Before the study commenced, the teeth were stored in 1% NaCl solution which was refreshed weekly. A 3×3-mm square was drawn on the crown on each tooth and the square was cut and separated from the rest of the crown with the use of a disk in a handpiece. The thickness of the enamel block removed was 1 mm and consisted of enamel only. Before undertaking the study procedures, the enamel surface was evaluated with a magnifying glass for the presence of any cracks.

Samples with cracks were excluded. The samples were randomly assigned to 6 groups (Table 1).

Table 1. Experimental groups

Group	Surface Treatment	No. of samples
G1	No surface treatment	15
G2	Varnish #1	15
G3	Varnish #2	15
G4	Er:YAG laser	15
G5	Er:YAG laser + varnish #1	15
G6	Er:YAG laser + varnish #2	15

Surface treatments: All the samples were placed on a wooden block during the procedures. In group 1 (G1), no surface treatment was carried out and this group was considered the negative control group. In group 2 (G2), F varnish #1 (Durasheild, Sultan, USA) was applied to the exposed enamel surfaces using a microbrush. The varnish consisted of 5% sodium fluoride, 2.26% F ion, 20–25% propanol, and less than 45% of synthetic resin. First, the sample surface was cleaned with a brush using the specific tooth prophylaxis paste and dried with an air syringe. A soft microbrush was used to apply the varnish to the sample surface. The sample was once again dried with the air syringe. The varnish was kept in contact with the sample surface and no moisture was allowed to reach the sample surface. Then the samples were stored in 98% ethanol for 6 hr to eliminate the colour of the absorbed varnish. In group 3 (G3), the samples were treated with F varnish #2 (Pascal, USA) in a manner similar to that in G2. Similar to the F varnish #1, the varnish in this group also contained 5% sodium fluoride. The samples in group 4 (G4) were irradiated with Er:YAG (Er:YAG and Nd:YAG, Fotona, Slovenia) laser beam with the following irradiation parameters:

- Peak power (Pulse energy/Pulse duration: 80–90 mJ)
- Repetition rate: 2 Hz
- Handpiece: RO2
- The beam diameter at the focal area for RO2 handpiece: 300 µm
- The distance between the fiber tip and the target tissue: 1–2 mm
- Irradiation time: 60 sec/cm^2
- Mode: SP

The tip of the handpiece was placed perpendicular to the enamel surface and to achieve homogeneous irradiation, the laser beam was delivered in two vertical and

horizontal directions. Irradiation was carried out manually with one uniform movement. In group 5 (G5), the samples underwent Er:YAG laser irradiation (similar to the conditions in G4) and then F varnish #1 was applied to the enamel surfaces. The varnish was applied with a disposable microbrush for 4 min and the samples were immersed in ethanol for 6 hr to eliminate colour changes due to the varnish.

In group 6 (G6) as well, the samples were irradiated with Er:YAG laser beams (similar to that in G4), followed by surface treatment with F varnish #2 (under conditions similar to those in G5).

pH cycling: After the surface treatments, all the samples in the 6 groups underwent a pH cycling procedure.[14,15] The demineralizing and remineralizing solutions were prepared similarly to that in a study by Raymond.[16]

The samples in each group were immersed, for 3 hr, in a demineralizing solution (consisting of 1.0 mM of lactic acid solution, 6% hydroxyethylcellulose at a pH value of 4.5). A total of 10 mL of the solution was used for each block. Then the samples were placed, for 20 hr, in a remineralizing solution consisting of 0.03 g/L of $MgCl_2.6H_2O$, 3.85 g/L of calcium lactate, 0.121 g/L of KH_2PO_4, 0.625 g/L of potassium chloride, 0.05 ppm of F, 2.0 g/L of methylp-hydroxybenzoate, and 0.4 g/L of sodium carboxymethyl cellulose at a pH value of 6.7. A total of 10 mL of the solution was used for each block. The cycles were repeated daily for 5 consecutive days and the enamel blocks were stored in deionized water after these cycles until they were analyzed. The demineralizing and remineralizing solutions contained 99% methyl hydroxybenzoate to prevent fungal growth. After all the procedures above, the F content of each block was determined by the potentiometric technique with a F ion specific electrode in the Faculty of Pharmaceutics, Kerman University of Medical Sciences. To this end, after retrieval of the samples from the buffer solution, the samples were rinsed for 24 hr in 1 mol/L potassium hydroxide solution to eliminate loosely bonded F. In the next stage, the samples were biopsied using the acid-etch technique by 0.5 mol/L perchloric acid ($HClO_4$) for 30 sec. At this stage, the samples within the acidic solution were shaken gently to prevent the F ions released from the samples from returning to the samples. Then the enamel samples were rinsed with 2 mL of 0.2 mol/L potassium hydroxide (KOH). The enamel surfaces were dried up with small cotton pellets. The cotton pellets were transferred into the test tubes containing the liquid resulting from the biopsy procedure so that the dissolved enamel would be completely collected. At the end of this stage, the biopsies of the samples were diluted to determine their F content and pH values of the solutions were adjusted at around 6–6.5 with the use of TISAB3 solution (Total Ionic Strength Adjusting Buffer, MERCK, Germany). In order to eliminate any ionic interferences or any other possible interference, 1 mL of each biopsy sample was selected to determine the F concentration and diluted 10 fold. In addition, in order to eliminate any interference by the OH ions during the F concentration determination procedures by the potentiometric technique, the pH value was adjusted at the weak acid range of approximately 6–6.5 and the appropriate TISAB buffer solution was added

during the dilution procedures. After preparation of the potentiometer, the electric potential of each concentration was read with the use of the specific electrode of the F ion (Methton CO., Switzerland). The test was carried out twice for each sample and the mean of the two tests was recorded as the F content of those samples after drawing the standard curve (Figure 1).

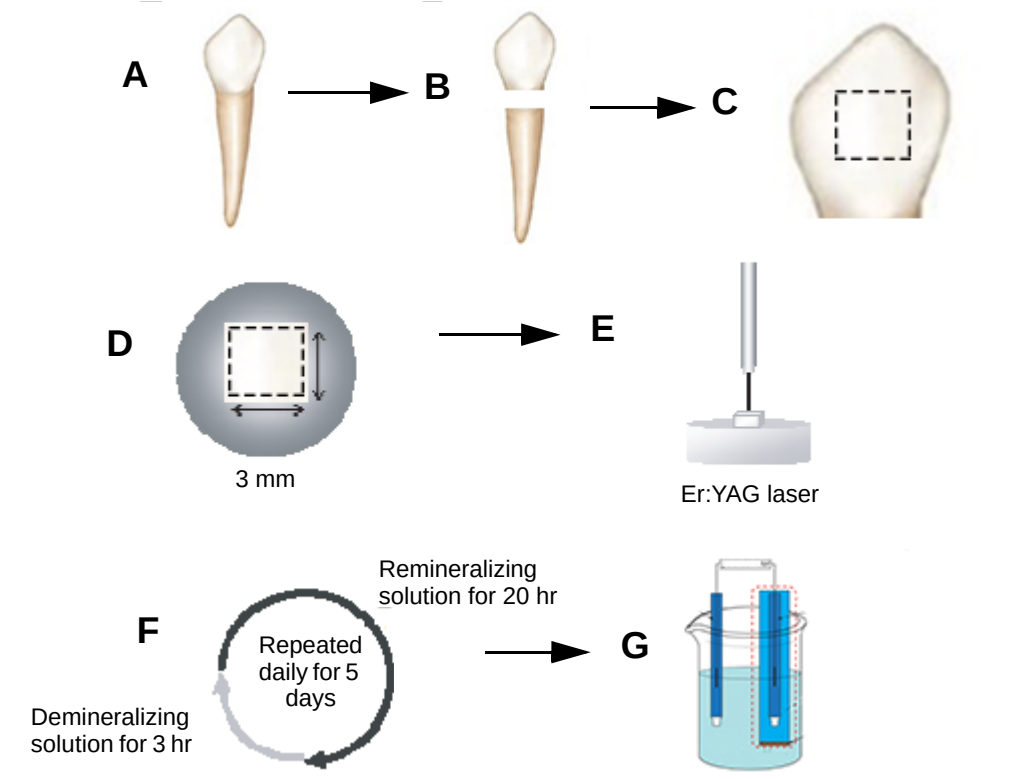

Figure1. Diagram of the experimental phase. (A) Primary canines were collected and stored in saline solution. (B) The teeth were sectioned at the enamel junction interface. (C) The crowns were longitudinally sectioned, and samples with a standardized area of 3×3 mm were obtained. (D) The enamel blocks. (E) Samples from groups G4 and G5 and G6 were irradiated with the Er:YAG. G2 was subjected to fluoride treatment and G1 was kept without surface treatment. (F) Acid challenge for 3 hr with demineralizing solution followed by 20 hr with remineralizing solution, daily for 5 days. (G) F electrode potentiometry.

Statistical analysis: The main variables were described in each group and the means, standard deviations (SDs), standard errors (SEs), and percentages were calculated. One-way ANOVA was used to compare the numeric variables, followed by post hoc Tukey tests. The chi-squared test was used for the classified variables. Data analyses were performed by the SPSS 20 software (SPSS Inc, Chicago, IL, USA). Statistical significance was defined as $p<0.05$.

RESULTS AND DISCUSSION

The chi-squared test showed significant differences between the different study groups. The analyses carried out with the specific electrode of the F ion showed significant differences in F absorption between the control group and the other

groups ($p<0.05$) apart from the G4 (Er:YAG laser) group. The means of the F absorption in the G1 group (no treatment, negative control), the G2 group (treated F varnish #1) and in the G5 group (F varnish #1 + laser) were 0.191, 0.872, and 1.433 µg/mL, respectively (Figure 2).

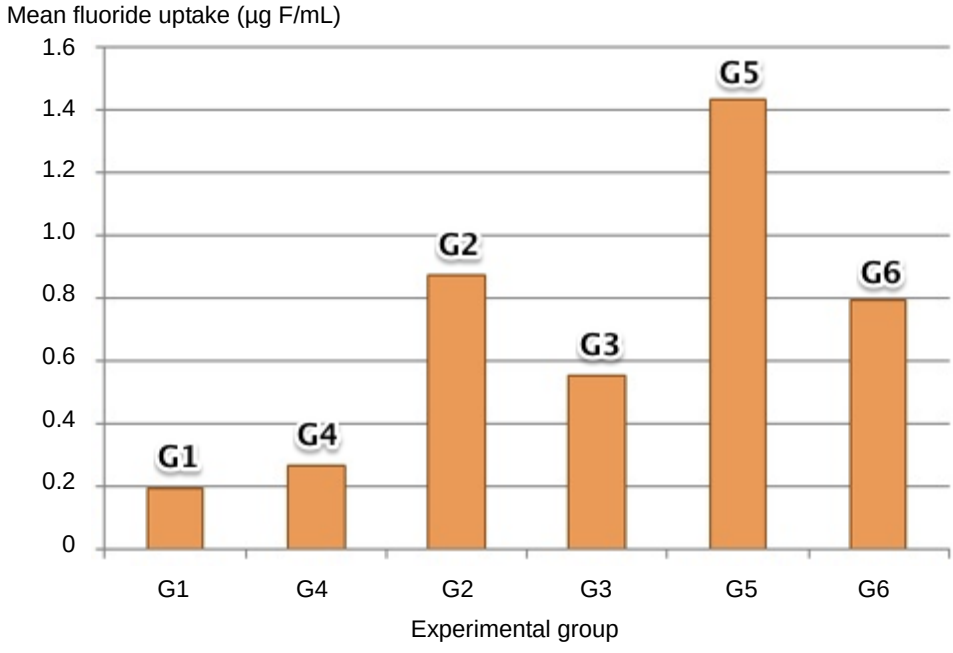

Figure 2. Graphic representation of the mean F uptake (µg/mL) in the experimental groups. G1: no treatment (negative control); G4: Er:YAG laser; G2: F varnish #1; G3: F varnish #2; G5: F varnish #1 + Er:YAG laser; and G6: F varnish #2 + Er:YAG laser.

Table 2 presents the descriptive data for the F uptake in the 6 groups. As the data in the table indicate, the maximum mean F uptake occurred in the laser group, G5, with the use of the Sultan varnish, varnish #1 (Table 2).

Table 2. Fluoride uptake in the experimental groups (µg F/mL)

(SD=standard deviation of mean, SE=standard error of mean)

Groups	N	Mean (µg F/mL)	SD (µg F/mL)	SE (µg F/mL)	Minimum (µg F/mL)	Maximum (µg F/mL)
G1	15	0.191	0.121	0.031	0.07	0.52
G2	15	0.872	0.267	0.069	0.24	1.22
G3	15	0.555	0.180	0.046	0.22	1.02
G4	15	0.267	0.103	0.026	0.08	0.40
G5	15	1.433	0.630	0.162	0.34	2.41
G6	15	0.793	0.128	0.033	0.56	0.98
Total	90	0.685	0.510	0.053	0.07	2.41

There were significant differences in F uptake in the enamel samples in different groups (Table 3).

Table 3. Statistical significance of fluoride uptake by human primary tooth enamel with and without the use of the Er:YAG laser. (NS=not significant)

Groups	G1	G2	G3	G4	G5	G6
G1	–	p<0.05	p<0.05	NS	p<0.05	p<0.05
G2	p<0.05	–	p<0.05	p<0.05	p<0.05	NS
G3	p<0.05	p<0.05	–	p<0.05	p<0.05	p<0.05
G4	NS	p<0.05	p<0.05	–	p<0.05	p<0.05
G5	p<0.05	p<0.05	p<0.05	p<0.05	–	p<0.05
G6	p<0.05	NS	p<0.05	p<0.05	p<0.05	–

The results showed significant differences in the amount of F absorbed by the primary tooth enamel samples, in both the F alone groups (G2 and G3) and in the groups in which the F therapy was combined with laser beams (G5 and G6). Based on the results of the present study and the studies evaluating the combined use of laser and F, laser beams increase the precipitation of F ions on enamel surfaces. Such an increase in F precipitation is more than that when teeth undergo only local F treatment. Similar studies with different lasers and different wavelengths (such as CO_2, Argon, Nd:YAG, and diode lasers) have shown the same results.[17,18]

Different studies have reported different reasons for increasing the resistance of enamel after laser irradiation. It is not clear how the enamel traps more F ions after the combined use of F and laser. However, two possible mechanisms have been proposed:

1. Binding of F to the enamel due to the thermal effect of laser beams.

2. Binding of F to the enamel due to changes in the enamel surface after laser irradiation, including an increase in enamel surface roughness.

Apparently, the mechanism of thermal effect has a more important role. Previous studies have shown that heat induces trapping of F ions within the enamel by synthesizing hydroxyapatite. On the other hand, the F content of the enamel increases by the synthesis of calcium F crystals on the enamel surface [17, 19] and by decreasing the loss of the mineral contents of the tooth structure.[20, 21] Er:YAG laser beams prevent caries predominately through the induction of chemical changes in dental hard tissues.[2] Additionally, *in vitro* studies have shown that laser use decreases the enamel dissolution pH point from 5.5 to 4.8. In the presence of

even minor amounts of F as low as 0.1 ppm, the laser-irradiated enamel will not be dissolved until a pH of 4.8 is reached. There is a synergism between the effects of laser beams and F in decreasing the enamel solubility.[22, 23] Furthermore, laser beams increase the resistance of enamel against acid attacks [24, 25] and decrease the depth of the carious lesions.[26] Irradiation by low-power laser beams (10–20 j/cm^2) changed the enamel surface, decreasing its demineralization.[27] Other studies showed that laser beams are effective in increasing enamel hardness, decreasing the effect of acid attack,[28, 29] and decreasing the incidence rate of caries.[30] However, some studies have reported no effect of laser irradiation before F therapy.[31, 32] In the present study, since there were no significant differences in the enamel F content between the control groups and the Er:YAG laser-irradiated group, it might be inferred that laser irradiation alone does not have any effect on strengthening tooth enamel through an increase in F uptake, consistent with the results of a similar study.[33] Additionally, some studies have shown that low-power erbium laser beams decrease enamel solubility without significantly changing the enamel structure.[34] Some studies have shown that Er:YAG laser beams, alone or in association with F, decrease enamel solubility. However, combined therapy (laser in association with F) had no additional effect.[35] Bahar et al. showed that Nd:YAG laser beams before F therapy resulted in the absorption of more F ions by enamel through the elimination of debris from the tooth surfaces, making it more effective than the use of F in association with mechanical or chemo-mechanical tooth surface cleaning techniques.[36] In the present study, the F concentrations in the two F varnish groups were significantly different, with a significantly higher F content in varnish #1 group. Considering the identical sodium fluoride content of the two varnishes used, with both containing 5% sodium fluoride, it appears that the differences between them might be explained by differences in the viscosity of the two varnishes. Different amounts of F uptake have been reported with the application of different materials. One of the possible etiologic factors is the non-homogeneous distribution of F in different production series of the products. In addition, the presence of abrasives and their type are effective in deactivating F ions. Furthermore, the presence of interfering metallic ions in the chemical composition of some products, such as flavouring agents, might prevent the activity of F ions, finally decreasing the amount of F absorbed.[37] Considering the availability of various F varnishes on the market, it is expected that different products will yield different results. In this context, F varnish #1 appeared to be preferable to F varnish #2. Further studies are recommended to overcome the shortcomings of the present study, by including a larger sample size, the use of more of the F products available on the market including those with different F concentrations, and the use of different lasers to determine more accurately which F product and at what concentration in association with which laser yields the best results. Further long-term *in situ* and *in vivo* studies are required to collect more data for a better understanding of primary tooth enamel after exposure to laser beams. It is expected that under *in situ* and *in vivo* conditions laser irradiation will give rise to more F uptake. It should be pointed out that one of the disadvantages of F is the fact that it should be used frequently to exert its protective effects on

tooth structures. The combined application of laser beams and F therapy is recommended in children with a high risk of caries only if the frequent application of laser has positive effects and can improve the efficacy of topical F. Because of the critical role of pediatric dentists in the prevention of dental caries, they should have up to date information of efficacy of each preventive strategy and choose the best method for each patient. Pedodontists should be aware of the caries preventive strategies and the correct use of high-intensity lasers as an acceptable, new and pain-free therapeutic tool.

CONCLUSIONS

This paper provides information on a caries preventive method in pediatric dentistry. Overall, the results of the present study showed that the combined use of F-laser had a positive effect on F uptake by the enamel in primary teeth. Therefore, this technique is recommended as a promising and effective clinical treatment to combat dental caries. Given the higher efficacy of this technique, it might be possible to decrease the dose of F at each stage of F therapy to avoid the risk of fluorosis and other risks of F use. It might also be possible to increase the time intervals between the F therapy sessions. These measures would improve the cost-effectiveness of the treatment and make it possible to offer such treatment to more community members.

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