

Effect of Combined Application of Er,Cr:YSGG and Sodium Fluoride Varnish in Prevention of Demineralization of Primary Tooth Enamel: an In Vitro Study

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Abstract

Background and Aim: Previous studies revealed improved enamel caries resistance after laser irradiation. Present in vitro study evaluated the effect of Er,Cr:YSGG laser with or without topical fluoride application on microhardness of primary enamel after artificial demineralization.

Materials and Methods: In this in vitro study, 35 primary molars with sound surfaces were bisected and randomly divided into seven groups of 10 specimens. In group F, 5% sodium fluoride varnish was applied while in group L1 and L2, Er,Cr:YSGG laser with the energy level of 0.25 W and 0.5 W was used, respectively. In group F/L1 and F/L2, application of fluoride varnish was followed by utilizing the Er,Cr:YSGG laser with the energy level of 0.25 W and 0.5 W respectively. Whereas, in groups L1/F and L2/F, the order of fluoride varnish and laser was reversed. After treatment, specimens were kept in distilled water for 24 h followed by a pH-cycling. Microhardness of each specimen was measured before treatment and after pH-cycling. The Analysis of Covariance (ANCOVA) was used for statistical analysis.

Results: The impact of different treatments on the microhardness value was not significant among the tested groups ($P=0.89$).

Conclusion: Our findings revealed that combination of Er,Cr:YSGG laser irradiation and application of topical fluoride have no superiority over either laser irradiation or fluoride application alone in increasing microhardness of primary teeth enamel, regardless of order of treatments or laser power.

Key Words: Lasers, Sodium Fluoride, Hardness, Tooth, Deciduous

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Introduction

Despite the substantial decrease in the prevalence of dental caries in many developed countries during recent decades, caries remains one of the most prevalent chronic infections of childhood and

adolescence worldwide [1,2]. Even in the developed countries, the costs of traditional dental treatments impose a heavy burden on community. Meanwhile, the key to promote oral health is an improvement in the prevention measures [2].

In order to reduce the prevalence of dental caries, including Early Childhood Caries (ECC), different efficacious practices have been introduced; for instance establishment a proper diet, twice-daily brushing with fluoridated toothpaste, and professionally-applied topical treatments [3]. Topical fluoride agents in the form of mouth rinse, gel, and varnish have shown protective effects against dental caries [4,5]. However, excessive use of fluoride agents arises concern regarding dental fluorosis in children [6,7]. In addition, topical fluorides seem to show incomplete effects in primary enamel compared to permanent teeth [8]. Therefore, new techniques in combination with fluoride agents have been introduced to prevent dental caries [1,7-10].

The history of using a laser to prevent dental caries dates back to 1971 by a rubbery laser [8-10]. Since that time, various types of the laser such as Argon, CO₂, Nd:YAG, and Erbium family, with various irradiation parameters have been suggested as a supplementary method to conventional preventive treatments in dentistry [9,11,12]. Previous studies have shown that enamel pretreatment with laser irradiation could decrease enamel demineralization against acid attack [1,9,11,13-16].

The most effective type of lasers for dental hard tissue applications are erbium family lasers. Among these lasers, utilization of Er,Cr:YSGG with 2.79 μm wavelength, has been newly developed in dental context. Minimum damage to the pulp and surrounding tissues as well as caries removal and cavity preparation with a lesser need to local anesthesia are some of the advantages of Er,Cr:YSGG utilization in dental treatments [7,15,17,18]. In addition, this laser can serve as a caries prevention measure [15]. For this purpose, laser power must be lower than surface ablation and fracture threshold to prevent unfavorable damages to the surface structure in conjunction with increasing surface temperature. This alteration makes chemical and morphological changes in enamel, in addition to alterations to enamel crystals; and as a result, laser induces its caries prevention effects [13,15].

Few studies exist regarding the combined application of Er,Cr:YSGG laser and fluoride in caries prevention particularly in primary teeth enamel [7,9,13,15,19-22]. Primary and permanent

teeth enamel are dissimilar in many features. For instance, thickness, the degree of mineralization, and amounts of calcium and phosphorus ions are lower in primary enamel compared to the permanent teeth enamel [21,23,24]. These differences lead to faster caries progress in primary enamel compare to permanent enamel.

Since maintaining primary teeth is one of the goals in the pediatric dentistry, and with regard to the structural differences between primary and permanent teeth enamel [23], the aim of the present in vitro study was to evaluate the effect of the Er,Cr:YSGG laser, with or without use of topical fluoride, on caries resistance of primary teeth enamel using artificial demineralization.

Materials and Methods

The present in vitro study was approved by the Ethics Committee of Tehran University of Medical Sciences with the ethical approval number of 92-03-97-21621. The sample size of 10 specimens for each group was calculated according to the analysis of variance formula using Esteves-Oliveira study¹⁶ and considering $\alpha=0.05$, statistical power=80%, the standard deviation of 16, and a minimum difference of 25 units between study groups (effect size=0.52). 35 primary molars, extracted for orthodontic reasons, with sound buccal and lingual surfaces were selected. The teeth were stored in 0.1% thymol solution for one week [1,7,15]. The teeth were entirely cleaned by rubber cap/pumice prophylaxis and were stored in normal saline. The root portion of each tooth was cut at 1 mm below the cemento-enamel junction, and the crown was bisected by a diamond disk in a mesiodistal direction, and as a result, two specimens were prepared from each tooth (70 specimens in total). All specimens were examined under a stereomicroscope ($\times 10$) for any cracks or enamel defects and then embedded in acrylic resin blocks. All specimen surfaces were covered with two layers of nail polish, except for a 3×3 mm window on the flattest portion of enamel surface.

The specimens were randomly divided into seven groups of 10 samples, and the following treatments were applied on the specimens:

Group 1 (F): 5% sodium fluoride varnish (DuraShield®, Sultan Healthcare Inc, Englewood, Calif., USA) was applied on the

surface for four minutes according to the manufacturer's instructions. The specimens then were stored in normal saline for 4 minutes, and then the varnish layer was removed by rubber cap and low-speed handpiece.

Group 2 (L1): Er,Cr:YSGG (Waterlase, Biolase Technologies Inc., San Clemente, CA, USA) laser (0.25 W, 2.8 J/cm², 20 Hz, 11% air, 0% water) was irradiated on the specimens' surface. The laser was irradiated without using water spray in a scanning style with a distance of 2-3 mm from the enamel surface for about 8-10 seconds.

Group 3 (L2): Er,Cr:YSGG laser (0.5 W, 5.7 J/cm², 20 Hz) was irradiated on the surface of specimens as described for group 2 (L1).

Group 4 (F/L1): first, 5% sodium fluoride varnish was applied as described for group1 (F). After 24 hours varnish layer was removed. Then, Er,Cr:YSGG laser (0.25 W, 2.8 J/cm², 20 Hz) was irradiated as explained previously.

Group 5 (F/L2): first, 5% sodium fluoride varnish was applied and afterward Er,Cr:YSGG laser (0.5 W, 5.7 J/cm², 20 Hz) was irradiated in the same procedure as described in group 4 (F/L1).

Group 6 (L1/F): Er,Cr:YSGG laser (0.25 W, 2.8 J/cm², 20 Hz) was irradiated similarly to group L1 followed by application of 5% sodium fluoride varnish with the same detail of group 1.

Group 7 (L2/F): in this group, the same procedure as group 6 (L1/F) was followed only Er,Cr:YSGG laser specifications were 0.5 W, 5.7 J/cm², 20 Hz. All treatments were performed by skillful operators, and the evaluation was done blindly.

The pH cycling model [14,25] was used to produce artificial caries via a demineralization challenge followed by a remineralization period. Specimens were individually immersed in 10 ml of a demineralizing (DE) solution (2mmol/L of calcium, 2mmol/L phosphate and 75mmol/L acetate, pH 4.6) for 6 hours, followed by rinsing with distilled water for 10 seconds. Then, specimens were individually immersed in the same quantity of remineralizing (RE) solution (1.5mmol/L calcium, 0.9mmol/L phosphate, 150mmol potassium chloride and 20mmol/L cacodylate buffer, pH 7.0) for 18 hours. All procedures were carried out at 37°C. The DE and RE solutions were renewed daily. After every five days, the specimens were immersed individually in

the RE solution for two days. The experiment lasted for a total of 14 days.

All specimens were tested for their microhardness on two occasions; first before the treatments (baseline), and then at the end of the pH cycling stage (post-treatment). A Vickers hardness tester (Bareiss Hardness Tester, Bareiss Prüfgerätebau GmbH Corp, Germany) with the load of 50 g for 15 s was used for measuring the microhardness. Three indentations were made on each sample, and the average value was recorded as the microhardness of each specimen.

Statistical analysis

The collected data from baseline indentations and post-treatment indentations in each group were used for statistical analysis. The changes in microhardness of enamel was determined by subtracting post-treatment values from the baseline measurements values. The Analysis of Covariance (ANCOVA) ($\alpha=0.05$) was carried out to determine the statistically significant changes of microhardness between groups by considering the pretreatment microhardness values as the baseline.

Results

The mean value and standard deviation of the microhardness of each group, before and after treatments, are shown in Table 1.

There was no significant difference between the impact of various treatment on the microhardness of primary teeth enamel, $F(6, 50) = 0.36$, $P = 0.89$.

Discussion

The present study investigated the effect of the various interventions on microhardness of extracted primary teeth. The results showed no significant difference in decreasing microhardness of groups after treating teeth with fluoride and/or Er,Cr:YSGG laser irradiation regardless of the type and order of treatments.

Most studies on caries preventive effect of laser, specifically Er,Cr:YSGG lasers, have been performed on permanent teeth and little information is available about their impact on primary teeth enamel.

A few studies have investigated the effect of combined application of fluoride and Er,Cr:YSGG in the prevention of demineralization of permanent teeth enamel [7,9,13,15,19,20]. However, the

Table 1. The mean value of the microhardness (kgf/mm²) in each group before and after treatments; values are presented as mean (standard deviation)

Groups	Before Treatments	After Treatments	Microhardness differences
Fluoride	221.77(60.97)	148.16(83.21)	73.60(48.58)
Fluoride + Laser 0.25 Watt	245.80 (63.80)	152.84(42.02)	92.96(44.43)
Fluoride + Laser 0. 5 Watt	277.95(59.31)	185.65(59.64)	92.30(72.70)
Laser 0.25 Watt + Fluoride	223.85(56.65)	146.42(32.33)	77.43(45.44)
Laser 0. 5 Watt + Fluoride	277.86(71.93)	187.79(40.62)	90.07(36.00)
Laser 0.25 Watt	239.22(66.18)	177.82(76.87)	61.40(64.32)
Laser 0. 5 Watt	225.31(67.44)	146.15(48.46)	79.17(60.18)

pattern of caries development and its prevention are different in primary compared to the permanent teeth [23]. Enamel and dentin of primary and permanent teeth are dissimilar in various aspects; for instance, enamel is thinner in the primary teeth compared to the permanent teeth. Moreover, the degree of mineralization in primary enamel is lower than the permanent teeth enamel. Also, amounts of calcium and phosphorus ions enamel in the deciduous teeth are less than permanent teeth [21,23,24]. These dissimilarities lead to faster progression of initial caries lesions and cavity development in primary teeth compared to permanent teeth. Therefore, to make a practical and clinical decision for primary teeth, referring to studies on permanent teeth seems irrational.

According to previous studies, the use of fluoride therapy and different types of lasers together could result in more resistant enamel compared to employing fluoride or laser alone [1,7,9,10,26].

Among various kinds of lasers, erbium laser family is the most useful laser for ablation of dental hard tissue¹. Er,Cr:YSGG (wavelength: 2.78 μ m) has a high absorption in hydroxyapatite. When a sub-ablative wavelength of this laser is used, the superficial temperature will increase without transferring the heat to the surrounding tissues

which could induce crystallographic changes in enamel toward a more acid-resistant surface in enamel [20].

Moslemi et al. [20] reported that combined application of fluoride and Er,Cr:YSGG leads to significantly less calcium ion release compared to utilizing the laser or fluoride individually. Moreover, the outcome of using laser or fluoride alone were similar and did not have a significant difference [20]. Subramaniam et al. [21] have reported the comparable results since the microhardness value was higher after combined application of CPP-ACP and Er,Cr:YSGG compared to CPP-ACP alone.

Fekrazad and Ebrahimpour [7] reported a significant difference between the calcium content of the groups which treated with fluoride and combination of acidulated phosphate fluoride gel (APF) and Er,Cr:YSGG, with the control group. They also found that combination of Er, Cr:YSGG laser and fluoride or fluoride alone resulted in significantly lower enamel solubility than the application of laser alone. However, the difference between the use of fluoride and the combined treatment of laser and fluoride did not differ significantly. Furthermore, they found no significant difference between treating with laser

alone and no treatment groups [7]. In the present study, various treatment modalities were employed, and there were no significant differences in preventing primary teeth enamel demineralization. However, Fekrazad and Ebrahimpour [7] experimented on permanent teeth enamel and differences in results might be due to the fact that their demineralization solutions and fluoride agent were different from the present study.

In another study, Anaraki et al. [9] assessed the effect of Er,Cr:YSGG laser (Power= 0.25 W) and topical APF gel in comparison with either fluoride or laser alone. They used atomic absorption spectrometry to detect calcium ions. They found no significant differences between the three treatment groups. However, demineralization in control group (with no treatment) was significantly higher than that the other groups. In their study, a laser was directly irradiated through APF gel, and they called it Laser Assisted Fluoride Therapy (LAFT) [9]. Ana et al. [13] have reached the similar results; they learned that although the combination of APF gel and Er,Cr:YSGG laser seemed more effective than their isolated effect, differences were not significant [13].

In the present study, the use of 0.5 W Er,Cr:YSGG with fluoride varnish appeared to be more effective in preventing enamel demineralization; however, the difference was not statistically significant. de Freitas et al. [15] reported a direct relationship between the power of Er,Cr:YSGG laser and resistance of enamel to acid. Nevertheless, sub-ablative parameters of irradiation have been recommended for caries prevention without ablating the enamel [15]. Previous studies have suggested that increase in the laser power, in sub-ablative threshold ($8-13 \text{ J/cm}^2$), raises the surface temperature and make more morphologic and crystallographic changes in enamel [26-29]. In other words, the photothermal effect of laser would melt and fuse hydroxyapatite crystals to produce a more acid-resistant enamel [9,15,27]. Besides, researchers have also attributed surface resistance changes to the photochemical effect of the laser through reduction of carbonate content, or partial decomposition of the organic matrix [7,13,19,20,22]. However, to prevent ablation of enamel, sub-ablative parameters should be used to

preserve the pulp from the possible thermal damages [9]. Therefore, we have chosen the power of 0.25 and 0.5 watts in the present study.

A combined application of laser and fluoride could prevent caries and decrease demineralization through different mechanisms. Laser irradiation produces microscopic spaces which could trap free fluoride ions. Moreover, it could change hydroxyapatite to fluorapatite that makes a stronger structure against demineralization [9]. There are some inconsistencies in the literature regarding the order of fluoride application and laser irradiation. Tagomori and Morioka [30] found that laser irradiation followed by fluoride application leads to a higher fluoride uptake in enamel compared to the reverse order [30]. Alternatively, it has been suggested that laser irradiation on the fluoride coated enamel could melt the outer layer of enamel and result in bonding fluoride ion to hydroxyapatite and formation fluorapatite crystals which are more acid resistance than hydroxyapatite⁹. However, another study has found no significant differences regarding the order of treatments [20], which is similar to our findings. It seems that the type of laser and laser parameters, such as power and fluency, are more important than the order of fluoride application and laser irradiation [20].

Overall, the diversity of findings from various studies is probably due to differences in parameters of irradiation, laser settings, demineralization solutions, and methods of measuring the demineralization that used in different studies.

Further studies are needed to fully ascertain the surface changes and chemical alterations of enamel after treating by laser and/or fluoride. Also, more investigations on the impact of different demineralization solutions and various methods of evaluating the resultant demineralization are required. In addition, more studies on the effect of Er,Cr:YSGG laser on caries prevention could be useful.

Conclusion

According to our findings, a combination of Er,Cr:YSGG laser irradiation and application of topical fluoride showed no superiority over either laser irradiation or fluoride application alone in increasing microhardness of primary teeth enamel,

regardless of the laser power and/or the order of treatments.

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