The Effect of Er:YAG Laser Irradiation Combined With Fluoride Application on the Resistance of Primary and Permanent Dental Enamel to Erosion

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Abstract

Introduction: Erosion is an important cause of tooth mineral loss. The combined use of lasers and fluoride has been introduced as a novel modality for the prevention of enamel demineralization. This study aimed to assess the effect of Er:YAG laser combined with fluoride application on primary and permanent enamel resistance to erosion.

Methods: Eighty enamel specimens of permanent (n=40) and primary (n=40) molars were prepared and randomly assigned to 4 groups: C—control (no pretreatment), F—acidulated phosphate fluoride (APF) gel, FL—APF gel application followed by Er:YAG laser irradiation, and LF—Er:YAG laser irradiation followed by the application of APF gel . The specimens were then submitted to pH cycling using Coca-Cola (pH = 2.4). Enamel micro-hardness was measured using the Vickers micro-hardness tester before pretreatment and after the erosive process. The collected data were analyzed using the Kolmogorov-Smirnov test, two-way ANOVA and repeated measures ANOVA.

Results: The micro-hardness of both permanent and primary enamel specimens significantly decreased after the erosive process ($P < 0.05$). In the permanent enamel specimens, the greatest reduction in micro-hardness was noted in groups C and F, while the least reduction was noted in group FL. However, these differences were not statistically significant ($P > 0.05$). In the primary enamel specimens, the greatest reduction in micro-hardness was noted in groups C and LF, while the least reduction was noted in group F. These differences were not statistically significant ($P > 0.05$).

Conclusion: Within the limitations of this study, Er:YAG laser irradiation combined with fluoride application could not prevent erosion in permanent and primary enamel during the erosive process.

Keywords: Tooth Erosion; Er:YAG Laser; Fluoride; Dentition; Permanent; Tooth; Primary.

Introduction

The prevalence of dental erosive wear has increased in recent years due to the change in lifestyle and the increased use of acidic beverages.[1,2] Erosive tooth wear is an important cause of tooth mineral loss in children and adolescents.[3] Dental erosion refers to progressive and irreversible loss of tooth structure during a chemical process without the involvement of bacteria.[4] Erosion is caused by intrinsic and extrinsic acids that dissolve the hydroxyapatite crystals of enamel and cause tooth hypersensitivity, change in tooth morphology, unaesthetic appearance, and pulp exposure in severe cases. External factors causing erosion include the use of acidic foods and carbonated beverages, fruit juices, citrus and to a lesser extent, occupational exposure to acidic environments.[5] The most common intrinsic factors include chronic gastrointestinal diseases such as gastrointestinal reflux, anorexia and bulimia (due to frequent vomiting).[5-7] Hydrochloric acid in gastric juice has a low pH (1.5 to 3) and can cause severe erosion if it is refluxed into the mouth.[8]

Enamel demineralization is the initial phase of the erosive process, which is characterized by the initial softening of enamel and increased surface roughness.[9]

In the advanced stages of dental erosion, dentin is increasingly exposed and tooth hypersensitivity and change in tooth anatomy may occur.[10]

Considering the increased prevalence of dental erosion and its irreversibility, early detection and proper measures to prevent and control it are important.[11,12] Erosive lesions repeatedly require preventive and restorative treatments.[13] However, restorative treatments cannot prevent further progression of erosive/abrasive wear.[14] Preventive strategies such as controlled diet and nutrition, stimulation of salivary flow, fluoride application, limiting the consumption of erosive drinks, using buffering agents and improving oral hygiene can decrease demineralization due to the acids of a nonbacterial origin.[14,15] However, controlling all etiologic factors is difficult if not impossible because it requires patient cooperation.[12] For this reason, other modalities such as fluoride therapy and laser therapy that do not require patient cooperation, were introduced to prevent or stop the process of erosion and subsequent tooth hypersensitivity.[16]

The application of fluoride-containing products such as 1.23% acidulated phosphate fluoride (APF) gel is reportedly effective in the prevention of erosion since it decreases the penetration depth of acids into the enamel.[2,7] This protective act of fluoride is due to the formation of a superficial CaF_2 layer that prevents the exposure of enamel to acids and its dissolution.[18] However, some studies have shown that the efficacy of fluoride therapy in controlling dental erosion is limited.[19,20] This highlights the need for novel modalities such as laser therapy for the prevention and control of dental erosion.

Some studies have shown that the application of Nd:YAG laser along with APF gel is effective in the prevention of erosion.[21,22] CO_2 laser has been used for the prevention of erosion in many previous studies.[3,9,12,23-27] Ramalho et al[3,27] and Esteves-Oliveira et al[28] showed that CO_2 laser irradiation with 0.3 J/cm^2 energy density, 5 µs pulse width, and 226 Hz frequency decreased enamel loss during the erosive process. However, these parameters are not available in clinical laser devices.[29]

Although some studies have reported the positive efficacy of Er:YAG laser irradiation in the prevention of tooth decay,[30,31] information is limited on its efficacy in the prevention of erosive demineralization. Azevedo et al[32] reported that Er:YAG laser irradiation combined with the application of APF gel effectively decreased bovine enamel dissolution during erosive processes. However, Scatolin et al[33,34] indicated that Er:YAG laser irradiation alone could not slow down the progression of erosive lesions caused by citric acid.

Considering the fact that Er:YAG laser irradiation can reinforce the crystallinity of the enamel[35] and enhance the deposition of CaF_2 on the enamel surface,[36] this study aimed to assess the effect of Er:YAG laser irradiation combined with fluoride application on primary and permanent enamel resistance to erosion.

Materials and Methods

This in vitro experimental study was conducted on the extracted primary molars and the permanent third molars. The inclusion criteria were sound human primary molar and permanent third molar teeth. The exclusion criteria were the presence of caries, cracks, wear and enamel hypoplasia.

The sample size was calculated to be 10 in each group (40 in total) according to a study by Esteves-Oliveira et al,[28] assuming the effect size of 0.6, power of 80%, and alpha = 0.05 by using the one-way ANOVA feature of PASS software. Thus, 20 primary and 20 permanent teeth were included using convenience sampling. The teeth were then randomly divided into 4 groups and stored in 0.1% thymol solution until the experiment. The teeth were first cleaned of calculus, dental plaque and debris using a scaler and a non-fluoride prophylaxis paste with low-speed handpiece. The teeth were then sectioned into buccal and lingual halves using a low-speed saw (Isomet, Cuehler Ltd., USA). Eighty samples were prepared as such. The samples were embedded in transparent auto-polymerizing acrylic resin (Acropars, Tehran, Iran) using a mold, with the enamel surfaces (buccal or lingual surface) facing upwards. The enamel surfaces were ground flat and polished using the following sequence of silicon carbide abrasive papers: 400, 600, 800, 1000, 1500, 2000 and 2500-grit.

Adhesive tapes measuring 3×3 mm^2 were placed on the samples and the rest of the surface was coated with 2 layers of nail varnish. After drying the varnish and removing the tapes, the samples were rinsed with deionized water. The enamel windows were created as such. The samples were then coded with a marker and a bur placed in a high-speed hand-piece, and stored in deionized water.

Measurement of Baseline Surface Micro-hardness

The baseline surface micro-hardness (SMH) was measured using a Vickers micro-hardness tester (V-test, Bareiss Prüfgerätebau GmbH, Oberdischingen, Germany). According to a previous study,[37] 100 g load was applied to three points on the surface of each sample (with the minimum distance of 120 µm) for 10 seconds, and the micro-hardness was measured at the three indentations. The mean of the three values was calculated and recorded as the micro-hardness of the respective sample.

Grouping of the Samples and Their Pretreatment

The enamel samples in each of the primary molar (n = 40) and permanent molar (n = 40) groups were divided into 4 groups (n = 10) as follows:

- Group 1: Control group with no pretreatment (C)
- Group 2: APF gel application (F)
- Group 3: APF gel application followed by Er:YAG laser irradiation (FL)
- Group 4: Er:YAG laser irradiation followed by APF gel application (LF)

In groups 2, 3 and 4, 1.23% APF gel (Dentonics, Bristol, UK) was applied over the samples by a micro-brush and removed after 4 minutes using cotton rolls.

Er:YAG laser (Smart 2940D plus, Deka, Calenzana, Italy) was irradiated on the samples in groups 3 and 4 with 100 mJ maximum energy, 1.59 J/cm^2 energy density, 1 W power, 10 Hz frequency, 250 μs pulse width, 10-second duration, 1 mm spot diameter and 50% water, using swiping motion at 2 mm distance from the surface. The irradiated surface area was 16 mm^2.

The pH Cycling

All samples were submitted to pH cycling. For this purpose, the samples of each group were immersed in 200 mL of Coca-Cola (Khoshgovar Co, Tehran, Iran) containing phosphoric acid with a pH of 2.4 for 2 minutes, rinsed with deionized water, dried and immersed in artificial saliva (2.91 g NaCL, 0.12 g CaCl$_2$, 0.13 g NaH$_2$PO$_4$, 5 cc NaF, 5 cc NaN$_3$ per 0.5 L, pH ≅ 6.5) for 2 hours in an incubator at 37°C. This process was repeated 4 times a day for 5 days. The pH of Coca-Cola was measured using a pH meter. After the last daily erosive process, the samples were stored in the artificial saliva in an incubator during the night. Artificial saliva was renewed daily before the beginning of the erosive process.

Measurement of Final Micro-hardness

The final micro-hardness of the samples was measured by applying 100 g load for 10 seconds as previously described for the baseline micro-hardness.

Statistical Analysis

The data were analyzed using SPSS version 22 (SPSS Inc., IL, USA). The normal distribution of the micro-hardness data in each group before and after the erosive process was assessed using the Kolmogorov-Smirnov test. The mean, standard deviation (SD), 95% confidence interval (95% CI), minimum and maximum micro-hardness values were reported. Two-way ANOVA was utilized to assess the effect of pretreatment in the 4 groups and the type of tooth (permanent/primary) on micro-hardness. The repeated measures ANOVA was applied to compare the baseline and final micro-hardness values in each group. $P < 0.05$ was considered statistically significant.

Results

The mean SMH values of primary and permanent samples at the baseline and after the erosive challenge are described in Tables 1 and 2.

The Kolmogorov-Smirnov test showed that the micro-hardness data had a normal distribution. Thus, one-way ANOVA was applied and showed no significant difference between primary and permanent enamel samples or the 4 subgroups in terms of baseline micro-hardness.

Repeated measures ANOVA showed that the mean enamel micro-hardness in all subgroups of the permanent and primary teeth after the erosive process decreased compared to baseline micro-hardness and this reduction was statistically significant ($P < 0.0001$).

Comparing the 4 subgroups of the permanent teeth showed that the greatest reduction in the mean SMH values occurred in the control and F groups and the least reduction occurred in the FL group, although these differences were not statistically significant ($P > 0.05$).

Comparing the 4 subgroups of the primary teeth showed that the greatest reduction in the mean SMH values occurred in the FL and control groups while the least reduction occurred in the fluoride group. However, these

Table 1. Mean Surface Micro-hardness of the Permanent Teeth Samples at the Baseline, After pH Cycling and Changes in Surface Micro-hardness (ΔSMH)

Groups	Mean Surface Micro-hardness Values		
	Baseline	After pH Cycling	ΔSMH (Baseline −After pH Cycling)
	Mean ± SD	Mean ± SD	Mean ± SD
Control	352.29 ± 15.51	259.64 ± 21.16	92.64 ± 21.20
F	349.07 ± 37.75	256.51 ± 25.26	92.56 ± 31.04
FL	348.64 ± 24.05	264.42 ± 31.84	84.21 ± 36.80
LF	345.68 ± 28.78	257.23 ± 42.55	88.45 ± 48.73

Table 2. Mean Surface Micro-hardness of the Primary Teeth Samples at the Baseline, After pH Cycling and Changes in Surface Micro-hardness (ΔSMH)

Groups	Mean Surface Micro-hardness Values		
	Baseline	After pH Cycling	ΔSMH (Baseline −After pH Cycling)
	Mean ± SD	Mean ± SD	Mean ± SD
Control	340.49 ± 41.56	242.98 ± 55.77	97.50 ± 57.83
F	338.49 ± 32.44	250.90 ± 55.89	87.59 ± 54.40
FL	351.09 ± 48.28	251.36 ± 36.49	99.73 ± 21.26
LF	323.98 ± 37.83	232.62 ± 36.60	91.35 ± 46.39

differences were not statistically significant ($P > 0.05$).

The pairwise comparison of the subgroups separately in each of the primary and permanent groups was done using two-way ANOVA. Although no significant difference was noted between the subgroups ($P > 0.05$), in the permanent teeth, change in micro-hardness in FL and LF groups was less than that in the control group. In the primary teeth, change in micro-hardness of the fluoride and LF groups was less than that in the control group.

The pairwise comparison of the primary and permanent enamel subgroups was also performed (Figure 1), which showed that the percentage of reduction of micro-hardness in the control, FL and LF subgroups of the permanent teeth was less than that of the primary teeth, but this difference was not statistically significant ($P > 0.05$).

Discussion

At present, dental erosion is an important factor responsible for the loss of tooth structure in children and adults.[3] Considering the confirmed efficacy of fluoride in the prevention of tooth decay,[38] it has also been recommended for the prevention of dental erosion. Many studies have assessed the efficacy of fluoride alone and in combination with other protective measures in the prevention of erosion.[3,24,39-42] Laser irradiation alone or combined with fluoride application has also been suggested to increase enamel acid resistance,[31,43] and some studies demonstrated the positive efficacy of Er:YAG laser in increasing enamel resistance against acid attacks and dental caries.[30,31,44]

Although both caries and erosion are caused by the demineralization of enamel, their main etiologic factors and mechanism of development are totally different. Considering the need for further evaluation of the efficacy of Er:YAG laser in the prevention of erosion, this study assessed the effect of Er:YAG laser irradiation combined with fluoride application on primary and permanent enamel resistance to erosion. The results showed that the micro-hardness of both permanent and primary enamel significantly decreased after the erosive process.

Considering the relatively high rate of consumption of carbonated beverages and their significant role in causing dental erosion,[1,2] Coca-Cola was used in this study as a demineralizing agent. Evidence shows that the consumption of acidic beverages 4 times a day may cause dental erosion,[42] The low pH of the oral environment caused by the consumption of these beverages remains for about 2 minutes.[45] We took these factors into account when simulating frequent acid attacks.

Considering the high absorption of erbium lasers by the tooth structure and its use in the previous studies to minimize enamel demineralization,[32,40,44] Er:YAG laser was used in this study. To prevent mechanical injury to the enamel, Er:YAG laser with sub-ablative fluency was irradiated on the enamel surfaces.
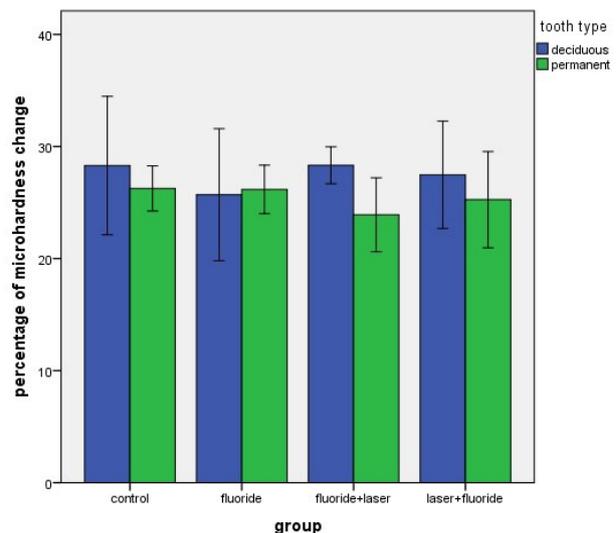

Figure 1. The Percentage of Reduction in Enamel Micro-hardness of The Subgroups of the Primary and Permanent Teeth.

Our results showed that despite the use of different pretreatment measures, enamel micro-hardness of both primary and permanent teeth significantly decreased by the erosive process. In other words, the tested measures could not prevent the erosive effect of Coca-Cola on enamel micro-hardness. This result was in line with that of dos Reis Derceli et al.[40] They also showed that the Er:YAG laser irradiation, APF gel application or combination of both could not prevent enamel erosion caused by Coca-Cola. Rocha et al[25] indicated that the application of stannous fluoride gel alone or combined with CO_2 laser irradiation could not protect the primary teeth against the erosive action of hydrochloric acid. However, Azevedo et al[32] and Rios et al[22] applied Er,YAG laser and Nd:YAG laser respectively and showed laser irradiation combined with fluoride application minimized enamel loss during erosive processes. Ramalho et al[3,27] showed that AmF/NaF fluoride gel alone and combined with CO_2 laser minimized enamel loss due to exposure to citric acid compared to the control group. The difference between our results and theirs may be due to the use of different types/parameters of lasers or fluoride compounds.

In our study, the reduction in micro-hardness of permanent enamel following pretreatment with fluoride followed by laser irradiation was less than that of the control and fluoride groups. Azevedo et al[32] obtained similar results using Er:YAG laser (80 mJ, 2 Hz) and APF gel but with significant differences between the combined groups and the control and fluoride groups. This difference between their findings and ours (in terms of statistical significance) can be due to different laser parameters or the use of water spray during laser irradiation. The absorption of laser energy by water might have decreased the efficacy of combined treatment.

Dos Reis Derceli et al[40] reported that the enamel wear (measured by profilometry) during the erosive process

with Coca-Cola following treatment with Er:YAG laser and APF gel was similar to that in the fluoride group. Although the demineralization of enamel following combined treatment with fluoride followed by the laser was less than that following fluoride application alone, it could not completely stop the erosive enamel wear. However, they did not have a negative control group.

In contrast to our study, Belcheva et al[46] and Esteves-Oliveira et al[28] showed that reduction in enamel micro-hardness following the combined use of CO_2 laser and fluoride was significantly smaller than that in the control and APF gel groups. This difference may be due to the use of different laser devices and parameters or the difference in the pH cycles and the quantitative method for the assessment of erosion.

Our study showed that the reduction in primary enamel micro-hardness in the use of fluoride alone was less than that in the combined treatment groups. Ramos-Oliveira et al[9] evaluated permanent molar enamel samples and obtained similar results using CO_2 laser. They showed that the protective effect of the laser irradiated before the application of APF gel for the prevention of erosion was less than the efficacy of fluoride application alone.

In the current study, the reduction in enamel micro-hardness in the FL group was similar to the LF group. Rios et al[22] and Azevedo et al[32] reported that enamel loss in the FL and LF groups was not significantly different, which is in agreement with the findings of our study.

Controversy exists regarding the susceptibility of primary and permanent teeth to erosion.[47] Our study showed that the mean micro-hardness in the primary teeth was slightly lower than that of the permanent teeth but not significantly. This was also true for the secondary micro-hardness (after the erosive process). This finding was in line with that of Lussi et al,[48] and Murakami et al[2] showed that the application of APF gel decreased permanent enamel loss during the erosive process with Coca-Cola but it was not effective in the primary teeth. In the control groups, reduction in micro-hardness of the primary teeth was significantly greater than that in the permanent teeth.[2] These results regarding the permanent teeth are not in accord with our findings. In our study, enamel micro-hardness of the permanent teeth in the fluoride group significantly decreased after the erosive process. Jordao et al,[24] Sobral et al,[21] and Scatolin et al[33] showed that APF gel decreased the erosive loss of bovine enamel. This controversy in the results may be due to the difference in the study design in terms of the type of tooth, pH cycling and the quantitative method of erosion assessment. Similar to our study, Azevedo et al[32] showed that the application of APF gel alone could not prevent the reduction in enamel micro-hardness during the erosive process.

Further similar studies with a larger sample size are required using profilometry and chemical assessment of the release of calcium and phosphate ions.

Conclusion

Within the limitations of this study, Er:YAG laser irradiation combined with fluoride application could not prevent erosion in permanent and primary enamel during the erosive process.

Ethical Considerations

Ethical approval was obtained from the research ethics committee of Shahid Beheshti University (IR.SBMU.RETECH.REC.1396.1120).

Conflict of Interests

The authors declare no conflict of interest.

Acknowledgements

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