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ORIGINAL ARTICLE

The effects of combining erbium, chromium: Yttrium-scandium-gallium-garnet laser irradiation with fluoride application in controlling the progression of enamel erosion

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Enamel microhardness

Abstract *Objective:* Increasing enamel resistance to acid may be useful for preventing cavitation and could reduce changes in the enamel's microhardness. Topical fluoride application and laser irradiation promote acid resistance of dental substrates. The aim of the study was to assess the efficacy of erbium, chromium: yttrium-scandium-gallium-garnet laser irradiation in combination with fluoride application to control enamel erosion.

Design: Sixty human premolar specimens were prepared ($N = 60$) and were randomly assigned to 5 groups, twelve specimens in each group ($n = 12$/group) according to surface treatment. The groups were as follows: group 1 (C): control with no treatment; group 2 (F): application of 1.23% acidulated phosphate fluoride gel alone; group 3 (L): laser irradiation alone; group 4 (F + L): acidulated phosphate fluoride gel followed by laser irradiation; group 5 (L + F): laser irradiation followed by acidulated phosphate fluoride gel. All the specimens were eroded 10 min in citric acid. Baseline measurements were performed using a Vickers microhardness tester before surface treatment. Subsequently, all specimens were subjected to a 60 min erosion-remineralization cycle for five days followed by measurements of the final surface microhardness. Statistical comparisons were performed by a one-way analysis of variance and Tukey's *post hoc* analysis.

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Results: The control, laser, and fluoride + laser groups showed a statistically significant decrease in microhardness values between baseline and post-erosion measurements ($P < 0.05$), indicating that these treatments could not prevent erosion progression. However, the fluoride and laser + fluoride groups showed a significant increase in microhardness values compared to baseline.

Conclusions: Our results suggest that compared to that of the control group, acidulated phosphate fluoride application as well as laser irradiation prior to fluoride application increased enamel surface microhardness and prevented the progression of enamel erosion.

1. Introduction

Dental erosion is the irreversible loss of dental hard tissue, caused by acids from sources other than bacteria (Zero, 1996). It is challenging to diagnose enamel erosion, as it is typically caused by very fine changes on tooth surfaces (Carvalho et al., 2018). Moreover, the ability to protect against damage caused by continuous exposure to acids might be associated with treatments that increase dental resistance and prevent demineralization (Magalhães et al., 2009). Fluoride-containing products play an important role in enamel remineralization and fluorapatite formation; compared to hydroxyapatite, these products are better at making the enamel surface more resistant to caries (Rošin-Grget et al., 2013). Topical application of fluoride results in the formation of a calcium fluoride-like material, which is more resistant to demineralization. Fluoride and calcium are released upon dissolution, and they contribute to the remineralization of the demineralized surfaces (Magalhães et al., 2008). In preventive dentistry, the most commonly used fluoride product is acidulated phosphate fluoride (Valério et al., 2015).

The Er,Cr:YSGG (erbium, chromium: yttrium-scandium-gallium-garnet) laser with a wavelength of 2780 nm, can prevent the progression of enamel erosion (Ceballos-Jiménez et al., 2018). The attraction between water and hydroxyl ions in the tooth structures results in chemical changes in the mineral content of the enamel as temperature increases due to laser irradiation, which promotes the formation of structures with high acid resistance (Bachmann et al., 2004). It is used to prevent caries, reduce the loss of hardness associated with their development, and can produce a cariostatic potential comparable to that produced by fluoridated dentifrice (Altinok et al., 2011; Jorge et al., 2015).

The use of a combination of fluoride and laser irradiation has a synergistic effect in preventing enamel erosion, improving the resistance of the enamel to demineralization, formation of larger ablation areas, and increased surface roughness of the enamel (Altinok et al., 2011). This results in greater fluoride uptake and retention of calcium fluoride-like products, which prolongs and maintains the resistance of enamel to erosion (Zamataro et al., 2013). Moslemi et al. found that the use of fluoride with laser irradiation resulted in less dissolution of calcium into the acid solution and higher acid resistance of the enamel to erosion than those found with fluoride or laser treatment alone. In addition, no difference in acid resistance of the enamel has been found if the laser treatment was used before or after fluoride exposure (Molsemi et al., 2009).

Assessment of surface microhardness it is widely used to measure tooth hardness and has been reported to have a high sensitivity when measurements are taken at the very early stages of erosion (Abad-Gallegos et al., 2009). The microhardness test assesses material hardness by applying a known load to the surfaces of the specimens and sample imprinted with a diamond indenter (Knoop or Vickers); the resulting diagonal indentations are measured with an optical microscope (Petta et al., 2017).

Although the effect of fluoride treatment on the prevention of enamel erosion is widely recognized, few studies have assessed fluoride's ability to prevent the progression of erosion when used in combination with irradiation from an Er,Cr: YSGG laser, and there is little information on the best time to perform this application to prevent the erosion process. Thus, the present study aimed to assess the efficacy of this combined treatment in controlling and preventing erosion of tooth enamel. The null hypothesis was that the combined application of fluoride and laser irradiation would have no effect on the progression of enamel erosion.

2. Materials and methods

2.1. Specimen preparation

This *in vitro* study was approved by the College of Medicine and the University Hospital Institutional Review Board of King Saud University (E-19-4226) and the College of Dentistry Research Center (IR0337) of King Saud University in Riyadh, Saudi Arabia. Human premolars extracted for orthodontic or periodontal reasons were selected. An ultrasonic scaler was used to clean all teeth after which non-fluoridated pumice was used, with a rubber cup mounted on a slow speed handpiece (Kavo EWL, No. 6412500, Germany, Biberach) and stored in a 0.05% thymol solution in distilled water. A digital microscope (HIROX, KH-7700, Digital microscope system, Tokyo, Japan) at 50x magnification was used to check and exclude any specimen with caries, restorations, or structural enamel defects or cracks. Each crown was cut 1 mm apical to the cemento-enamel junction using a slow-speed diamond saw (Isomet Low Speed saw, Buehler, Lake Buff, Illinois, USA), which included a water-coolant spray to remove the root. Further, mesio-distal sectioning was performed to prepare the buccal and lingual surfaces of each crown. A total of sixty specimens were prepared ($N = 60$), twelve of which were assigned to each group ($n = 12$/group). A polyvinyl mold with an external diameter of 20 mm and a height of 5 mm was filled with self-curing acrylic resin (Techno sin, FAMADENT S.L.U. Garrotxa, Vilamalla – España) for tooth mounting. Each tooth surface was placed in the resin so that the buccal or lingual surfaces were facing upward. After the resin had been set, the surfaces of all teeth were sequentially flattened with a silicon carbide sandpaper of 120, 400, and 600 grits (Buehler, São Paulo, Brazil). Finally, each specimen was rechecked to confirm the absence of any cracks or fractures under the digital microscope at 50x magnification.

2.2. Study groups

The 60 collected samples ($N = 60$) were randomized to one of five groups, with each group receiving a different surface treatment (Table 1). Information about all the materials used in this study and the procedures are summarized in Table 2.

2.3. Initial erosion

Each specimen was immersed in 1% citric acid solution (anhydrous citric acid, pH ~ 2.3) for 10 min at room temperature (~24 °C) to induce an initial erosive lesion *in vitro*, after which the specimens were rinsed with distilled water (da Silva et al., 2019).

2.4. Enamel surface microhardness tests

A Vickers microhardness tester (NOVA 130 INNOVA TEST, Borgharenweg, Netherlands) was used to measure the hardness of each enamel surface, with the indenter placed perpendicular to the surfaces. Measurements were taken twice during the study, once at baseline and once at the time of the post-erosive challenge. At each measurement timepoint, a force of 100 g was applied for 10 s, and three indentations, 100 µm apart, were performed at the surface. The first baseline indentations were made in the center of the specimen; the others were placed 500 µm to the right of the first indentation at the time of the post-erosion challenge. The microhardness value for each sample was determined as the mean of these three measurements.

2.5. Surface treatments

For Group 1 (control group), specimens were stored in artificial saliva (Artificial Saliva for Medical and Dental Research, Pickering Laboratories, Inc., Mountain View California, USA). This ready-to-use solution can be stored at room temperature and has a pH of 6.8 (Pokrowiecki et al., 2019). For group 2 (F), acidulated phosphate fluoride (1.23%, pH: 3.6–3.9; Gelato Prophy Paste, Keystone Industries, USA) was applied with a cotton bud for 4 min according to the manufacturer's instructions then the gel was removed with cotton rolls. For group 3 (L) the samples were irradiated using a pulsed Er, Cr:YSGG laser (Waterlase iPlus; Biolase, Irvine, CA, USA) at a wavelength of 2,780 nm, with the following parameters: 0.5 W of power, 20 Hz repetition frequency, a pulse duration of 60 µs, and a 10 s exposure time (11% and 0% of air pressure and water level, respectively). Based on the calculations by the manufacturer, the energy density was determined to be 8.8 J/cm^2. In this study, an MZ6 Zirconia (Biolase, MD, USA) laser with a length of 6 mm and a diameter of 600 µm was employed. The tip was placed 1 mm from and perpendicular to the surface of the enamel. The samples were irradiated by passing the laser over the entire surface area. Throughout the procedure, an endodontic file was fixed at the handpiece and kept 1 mm away from the surface of the enamel. For group 4 (F + L), fluoride gel was applied to each specimen for 4 min followed by laser irradiation for 10 s. Finally, for group 5 (L + F), the samples were irradiated with the laser for 10 s followed by the application of the fluoride gel for 4 min. All specimens were kept in artificial saliva for 24 h in an incubator at 37 °C.

Table 1 Groups used in the study.

Group	Description
Group 1 (C)	Untreated control
Group 2 (F)	Treatment with 1.23% acidulated phosphate fluoride gel alone
Group 3 (L)	Treatment with an erbium, chromium: yttrium-scandium-gallium-garnet laser alone
Group 4 (F + L)	Treatment with 1.23% acidulated phosphate fluoride gel followed by laser irradiation
Group 5 (L + F)	Laser irradiation followed by treatment with 1.23% acidulated phosphate fluoride gel

2.6. Erosive challenge

Following surface treatment, all specimens were subjected to an erosion-remineralization cycle, which consisted of immersion in 0.3% citric acid solution (pH 2.6) for 5 min followed by 60 min of artificial saliva exposure (da Silva et al., 2019). The procedures were repeated four times a day for five days. During the night, all specimens were placed in an incubator at 37 °C. After five days, all the specimens were washed using distilled water and the final surface microhardness of each specimen was measured.

2.7. Statistical analysis

It is found that at least 12 samples were needed for each group to detect a medium effect size ($d = 0.50$) between groups at the level 5% type I error rate and 95% power. The main dependent variable was the mean value of the enamel surface microhardness before and after the erosive challenge. Data obtained from the microhardness testing were compared using Statistical Package for the Social Sciences version 24.0 for Windows (SPSS Inc., Chicago, IL, USA). P-values ≤ 0.05 were considered to be statistically significant. Descriptive data, presented as mean values and standard deviations, were analyzed to assess changes in the microhardness of the enamel surface. The normality of data distributions was confirmed by a Shapiro-Wilk test. For within-group comparisons, the changes in microhardness between the baseline and the erosive challenge time points were assessed using paired two-tailed t-tests. To determine whether there were statistically significant differences between the means of the five groups, data were compared by a one-way analysis of variance followed by a Tukey's *post hoc* analysis when appropriate.

3. Results

The mean enamel surface microhardness values of the five study groups are shown in Table 3. There was a statistically significant difference between the measures at baseline and the post-erosive challenge for all groups (all P-values < 0.05).

Table 2 Summary of the treatments applied to each group and the material characteristics.

Group and treatment	Manufacturer	Composition of the material	Mode of application
Group 1 (control group): Artificial saliva	Pickering Laboratories, Inc., South California, USA	Water, distilled water, deionized water, potassium phosphate, potassium chloride, magnesium chloride, carboxymethyl cellulose sodium, methyl 4-hydroxybenzoate, sodium phosphate (dibasic), calcium chloride dihydrate	• No treatment • Kept in artificial saliva • Erosive cycle exposure for 5 days
Group 2 (F): 1.23% acidulated phosphate fluoride	Gelato Prophy Paste, Keystone Industries, USA	Sodium fluoride, hydrofluoric acid, citric acid monohydrate, magnesium aluminum silicate, phosphoric acid, Polysorbate 20, sodium benzoate, Saccharin Sodium, Water, Xanthan Gum, Xylitol	• 4 min application to the surface of the enamel using cotton buds, then removed with a cotton roll • Stored for 24 h in artificial saliva. • Erosive cycle exposure for 5 days
Group 3 (L): Laser irradiation (erbium, chromium: yttrium-scandium-gallium-garnet laser with 2780 nm wavelength	Waterlase iPlus; Biolase, Irvine, CA, USA	N/A	• The laser tip was placed 1 mm away from the enamel surface with set parameters: - frequency of 20 Hz - pulse duration of 60 µs - 0.5 W output power - 11% air and 0% water • MZ6 Z-Glass tip was utilized. • Stored for 24 h in artificial saliva. • Erosive cycle exposure for 5 days
Group 4 (F + L): (fluoride application followed by laser irradiation)	Acidulated phosphate fluoride: Same as Group F; laser irradiation: Same as Group L	Acidulated phosphate fluoride: Same as Group F; laser irradiation: Same as Group L	• Acidulated phosphate fluoride application, then laser irradiation • Stored for 24 h in artificial saliva. • Erosive cycle exposure for 5 days
Group 5 (L + F): (Laser irradiation followed by fluoride application)	Acidulated phosphate fluoride: Same as Group F; laser irradiation: Same as Group L	Acidulated phosphate fluoride: Same as Group F; laser irradiation: Same as Group L	• Laser irradiation, then acidulated phosphate fluoride application. • Stored for 24 h in artificial saliva. • Erosive cycle exposure for 5 days

Group 5 (L + F) had the highest surface microhardness value compared to that of other groups after the erosive challenge (339.93 ± 22.22). A significant decrease in microhardness values was observed after the erosive cycle in Groups C, L, and F + L (278.35 ± 19.97, 303.12 ± 35.74, 266.52 ± 22.83, respectively); indicating that these treatments were not able to prevent the progression of erosion. However, both Group F and Group L + F showed a significant increase in microhardness values compared to that at baseline, suggesting erosion was prevented to some degree.

The results showed statistically significant differences between and within the groups after treatment (post-treatment compared to baseline measures) ($P < 0.001$). A Tukey's *post hoc* analysis was performed to compare differences in the mean microhardness values between groups (Table 4). The mean microhardness in Group C was significantly lower than those in Groups F and L + F ($P = 0.007$ and $P < 0.001$, respectively). The mean microhardness in Group F and Group L were significantly higher than that in Group F + L ($P < 0. 001$ and $P = 0.009$, respectively). Finally, the mean microhardness in Group L + F was significantly higher than that in Groups C, L, and F + L ($P < 0.001$, $P = 0.009$, and $P < 0.001$, respectively). The data suggest that, in general, the application of fluoride alone and the combination of laser irradiation followed by fluoride application were the only surface treatments that increased the resistance of the enamel to the progression of erosion.

4. Discussion

Laser irradiation has been shown to improve the effects of fluoride on preventing tooth demineralization (Correa-Afonso et al., 2010; de Freitas et al., 2010). However, no consensus has been reached in the dental literature in regards to the efficacy of combined treatment with laser irradiation and fluoride

Table 3 Enamel microhardness values measured at baseline and after the application of surface treatment.

Group	Microhardness at Baseline (Mean ± SD) (kgf/mm^2)	Microhardness After Treatment (Mean ± SD) (kgf/mm^2)	Mean difference	P-value
C (control)	301.56 ± 27.20	278.35 ± 19.97	–23.21	0.004
F (fluoride only)	286.41 ± 28.19	315.93 ± 26.02	29.52	0.009
L (laser only)	342.10 ± 10.53	303.12 ± 35.74	−38.98	0.002
F + L (fluoride + laser irradiation)	303.77 ± 19.28	266.52 ± 22.83	−37.25	0.001
L + F (laser irradiation + fluoride)	308.32 ± 33.52	339.93 ± 22.22	31.61	0.023

Table 4 Level of significance of enamel microhardness.

Group (a)	Versus Group (b)	Mean Difference (a-b)	P-Value
C: Control	F	−37.58	0.007
	L	−24.77	0.149
	F + L	11.83	0.797
	L + F	−61.58	0.000
F: Fluoride treatment only	C	37.58	0.007
	L	12.81	0.746
	F + L	49.41	0.000
	L + F	−24.00	0.172
L: Laser irradiation only	C	24.77	0.149
	Fl	−12.81	0.746
	F + L	36.60	0.009
	L + F	−36.81	0.009
F + L: Fluoride treatment followed by laser irradiation	C	−11.83	0.797
	F	−49.41	0.000
	L	−36.60	0.009
	L + F	−73.41	0.000
L + F: Laser irradiation followed by fluoride treatment	C	61.58	0.000
	Fl	24.00	0.172
	L	36.81	0.009
	F + L	73.41	0.000

administration in increasing enamel hardness (Ana et al., 2012; Anaraki et al., 2012). In the present study, fluoride treatment and laser irradiation were performed only once to simulate a standard, single clinical application. Laser parameters were chosen in accordance with those used by da Silva et al., with a 0.5 W average power output and a repetition rate of 20 Hz for 10 s, as these parameters were previously shown to be capable of controlling the progression of enamel erosion (da Silva et al., 2019).

According to the results of this study, there was an increase in the surface microhardness of the enamel following the single application of fluoride or the combined laser irradiation and fluoride application with mean microhardness differences (P = 0.007 and P < 0.001, respectively) compared to that in the control group. These two surface treatment methods were the only ones able to halt the progression of enamel erosion. Therefore, the null hypothesis was rejected. The combined application of fluoride after laser irradiation resulted in the highest microhardness values measured at the end of the experiment, although that group did not significantly differ from the group treated with fluoride alone. The fact that the use of fluoride significantly increased the microhardness of the enamel erosion is consistent with the results reported by previous studies (Molaasadolah et al., 2017; Tavassoli-Hojjati et al., 2012). However, studies combining irradiation with Er,Cr:YSGG laser and the application of fluoride have found that this combination reduces demineralization of enamel better than any treatment that uses a laser or fluoride alone (de Freitas et al., 2010; Moslemi et al., 2009). Laser irradiation causes chemical and morphological changes at the surface. More specifically, the chemical changes occur as a result of the removal of carbonated apatite, whereas morphological changes result from the increase in the surface temperature. These changes increase fluoride uptake at the surface of the tooth after the application of fluoride gel, increasing the protection of the enamel. Ana et al. noted an increase in the formation of calcium fluoride-like material on the enamel surface after exposing the tooth to Er,Cr:YSGG laser irradiation prior to fluoride application, which could explain why this combination treatment was able to control the progression of enamel erosion in the present study (Ana et al., 2012). Fekrazad and Ebrahimpour also observed a high calcium content in the groups treated with either fluoride alone or a combination of fluoride and irradiation with the same type of laser used in this study and these two groups had lower solubility of tooth enamel than laser irradiation alone (Fekrazad & Ebrahimpour, 2014).

However, it must be noted that in this study, fluoride application before laser irradiation did not prevent the progression of erosion; in fact, it decreased the microhardness of the enamel surface. A previous study found that when fluoride was applied to enamel before laser irradiation, there was no observable increase in the uptake of calcium fluoride-like material (Zancope et al., 2016). This might be due to the mechanical barrier formed by fluoride, which reduces the temperature and energy applied at the surface of the enamel and would prevent any alteration in the enamel surface.

The single laser irradiation could not control the progression of enamel erosion or prevent the reduction in the microhardness of the enamel in the present study. It is possible that during the first days of cycling the laser-modified layer may have been removed by an erosive challenge, minimizing the effects of changes that would have been expected to protect the enamel. This finding is in agreement with the results of other studies, which found that single irradiation with the type of laser employed here was not capable of preventing the progression of enamel erosion (da Silva et al., 2019; Dionysopoulos et al., 2019). However, discrepant results were reported by de Olivera et al.; they found that irradiation with this type of laser alone might maintain enamel microhardness after erosive challenge (de Oliveira et al., 2017). The discrepant results could be explained by the differences in laser parameters and irradiation protocols. For example, in the present study, we irradiated an eroded enamel surface rather than an unaffected surface.

Microhardness measurements can be used to assess the extent of protection afforded by different surface treatments for the prevention of enamel erosion. Hence, in the present study, the surface microhardness measurement was performed using the Vickers hardness test to quantify changes in the mineral content of the enamel during erosion. A force was applied repeatedly within a specific time frame, with each indentation performed at a different location, and the surface microhardness was measured accordingly. However, in each group, the hardness measured at any point could be considered an accurate indication of the hardness of the enamel surface. Depending on a previous study (Molaasadolah et al., 2017) as they found, there was no significant difference observed in the microhardness values between the initial and later indentations because of the proximity of the measured points to one another. In this study, we employed a previously used erosion-remineralization cycle method (Pereira et al., 2017) to simulate the conditions commonly observed in clinical cases, wherein individuals who consume highly acidic beverages are at a higher risk of developing enamel erosion. It should be mentioned that a perfect simulation of clinical enamel erosion is not possible due to the variability in biological factors, such as saliva flow rate, composition, and buffering capacity. These factors play an important role in the remineralization of tooth structure and should be taken into consideration in future studies.

There are several limitations to the present study, namely, enamel specimens were smoothed and polished to produce a flat surface and allow standardized microhardness measurements, and the specimen's preparation might have influenced the results and the conclusions of the *in vitro* studies. Therefore, these *in vitro* results cannot be fully extrapolated to the clinical setting.

5. Conclusions

The results showed that application of fluoride alone as well as the combination of laser irradiation followed by fluoride application were the only surface treatments that increased the resistance of the tooth enamel to the progression of erosion and improved the microhardness of the enamel surface. Further studies must be conducted to determine whether these changes are preserved over time through regular monitoring of a patient's oral health.

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CRediT authorship contribution statement

Ahoud AlShamrani: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Writing - review & editing, Visualization, Supervision. **Alhanouf AlHabdan:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Writing - review & editing, Visualization, Supervision. **Malak AlDaweesh:** Conceptualization, Methodology, Software, Investigation, Writing - original draft. **Rahaf bin Hamdan:** Conceptualization, Methodology, Software, Investigation, Writing - original draft. **Randa AlRajhi:** Conceptualization, Methodology, Software, Investigation, Writing - original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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