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Influence of Er, Cr: YSGG (2780 nm) and Nanosecond Nd: YAG Laser (1064 nm) Irradiation on Enamel Acid Resistance: Morphological and Elemental Analysis

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Keywords: Enamel demineralisation; Er, Cr: YSGG laser; Nanosecond Nd: YAG laser; Enamel acid resistance

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Abstract

BACKGROUND: Enamel demineralisation is an initial step of the serious dental problem including dental caries, white spot lesions and dental erosion.

AIM: Compare the effect of Er, Cr: YSGG (λ = 2780 nm) and nanosecond Nd: YAG (λ = 1064 nm) laser on enamel acid resistance.

MATERIAL AND METHODS: Thirty non-carious human premolars, extracted for orthodontic reasons, were used. The experimental groups (n = 10 each group) were: Group I, untreated (control); Group II, Er,Cr:YSGG laser irradiation (0.75 W, 20 Hz, 140 µs, 10 s); Group III, nanosecond pulsed Nd:YAG laser irradiation (0.8 W, 10 Hz, 7 ns, 10 s). Scanning electron microscope and Energy Dispersive X-ray Spectroscopy (EDX) were used to assess acquired enamel resistance to PH cycling.

RESULTS: After subjecting the three experimental groups to PH cycling, scanning electron microscopic examination revealed irregular porous dissolved enamel surface in group I. However, groups II and III demonstrated partially dissolved enamel surface. EDX analysis demonstrated the lowest mean percentage decrease in calcium and phosphorus content in group II followed by group III, then the highest mean percentage decrease was observed in untreated group I. One-way ANOVA revealed significant differences ($p < 0.0001$) between the tested groups.

CONCLUSIONS: Both Er, Cr: YSSG and nanosecond Nd: YAG laser irradiation were able to improve the acid resistance of enamel. However, enamel surface treated with Er, Cr: YSSG laser showed the lowest mean percentage decrease of calcium and phosphorus (highest acid resistance).

Introduction

Dental caries is a biofilm-mediated, sugar driven multifactorial, preventable, dynamic disease that occurs as a result of two interacting processes; demineralisation and remineralisation of dental hard tissues [1]. Enamel demineralisation is not only the initial step dental caries and white spot lesions, but also it is the first stage of another serious dental problem, which is dental erosion [2].

Several approaches have been made to increase enamel resistance to caries; among them, laser irradiation seems to be very promising [3] due to its strong interaction with dental hard tissues [4]. Lasers that have a higher absorption by dental enamel, such as CO_2 (9.6 µm and 10.6 µm) [5]. Er: YAG (2.94 µm) [6] and Er, Cr: YSGG (2.79 µm) [3] were demonstrated to promote acid resistance of enamel. The low absorbed lasers such as Nd: YAG (1.064 µm) [7] and Argon [8] have also been employed with success.

Er, Cr: YSGG laser emitted at 2780 wavelength is well absorbed by water and hydroxyl radical in the hydroxyapatite. Thus, this type of laser has the potentiality to improve enamel acid resistance and prevent mineral loss by inducing chemical and morphological changes in enamel without an excessive increase of heat [9], [10].

Although the role of longer pulsed Nd: YAG laser on the acid resistance of enamel have been well established, the application of nanosecond pulsed Nd: YAG laser on dental enamel surface still needs to be improved [11]. The Q-switched Nd: YAG laser system with nanosecond pulses offers a significant advantage over long pulsed Nd: YAG laser as regard to pulpal heating. Previous studies showed that the temperature rise associated with nanosecond Nd: YAG irradiation is inferior to 2.5°C, which justifies its use without pulpal damage [12], [13]. It was reported that nanosecond pulsed Nd: YAG laser can be used to obtain minimal morphological alteration associated with a chemical reorganisation enhancing the microhardness values and consequently inhibiting the enamel acid dissolution [11].

Since the effect of laser irradiation on acid resistance of enamel has been observed, the studies on comparisons of acid resistance of enamel when irradiated with Er, Cr: YSGG and nanosecond pulsed Nd: YAG lasers are scarce.

The present work aims to evaluate and compare the influence of Er, Cr: YSGG and nanosecond Nd: YAG laser irradiation on acquired enamel acid resistance through scanning electron microscope examination and elemental analysis.

Material and Methods

This experimental *in vitro* study was held in the labs of the National Research Centre and dental laser Centre Ain Shams University, after the approval of the local ethical committee of the National Research Centre. Thirty non-carious human premolars, extracted for orthodontic reasons, were selected randomly from unknown persons.

Specimen Preparation: Each enamel surface was rinsed with deionised water and examined under a stereomicroscope (Leica Microsystem S, Switzerland). Teeth with any defects, erosions, micro-cracks or visible stains were excluded from the study. A window (4×4 mm) was measured by calibre and marked with two layers of an acid-resistant varnish. The window was located in the middle of the middle third of the buccal enamel surface [14]. All the teeth were stored in deionised water for not more than 1 week.

Experimental design: The specimens were randomly assigned to three groups of (10 specimens each) as follows: Group 1: No treatment (control), Group 2: Treated with Er, Cr: YSSG laser and Group 3: Treated with nanosecond pulsed Nd: YAG laser.

The specimens from all groups were then subjected to PH cycling (demineralisation).

Experimental procedures

a) Er,CR:YSGG laser irradiation conditions: A pulsed Er,CR:YSGG laser (Waterlase, Biolase, USA) at 2780 nm wavelength was used with the following parameters: 0.75 W, power, 12.5 mJ, pulse energy, 20 Hz, repetition rate, 140 μs, pulse width and 10 s average exposure time. The air pressure and water level were set at 11 % and 0 % respectively. Laser tip type used in the study was MZ6 Zirconia (Biolase, MD, USA) with a length of 4 mm and diameter 600 μm. The tip was positioned perpendicular 1 mm from the enamel surface, and the samples were irradiated by scanning once in each direction, horizontal and vertical, to promote homogeneous irradiation and to cover the entire sample area. An endodontic file was fixed at the handpiece and kept a distance of 1 mm from the surface during all the procedures.

b) Nd: YAG laser irradiation conditions: A pulsed nanosecond Nd-YAG laser (Continuum laser, Powerlite, DLS 9000, USA) at 1064 nm wavelength was used with the following parameters: 0.8 w power, 10 Hz Repetition rate,7 ns pulse width and 10 seconds average exposure time.

c) PH cycling model: For the acid challenge, samples were submitted to a pH cycling procedure, according to previously described protocols [15], [16]. The demineralisation solution (pH 4.3) consisted of 2.0 mmol/L of Ca, 2.0 mmol/L of phosphate in a buffer solution of acetate 0.075 mol/L. Remineralization solution (pH 7.0) consisted of 1.5 mmol/L of Ca, 0.9 mmol/L of phosphate, 150 mmol/L of potassium chloride. PH was measured by a pH meter to verify the required pH accurately.

Each specimen was individually immersed in 5.0 ml of demineralising solution for 6 hours and was then washed with deionised water. This was followed by immersing the samples again in 5 ml of remineralising solution for 18 hours, which was then washed off with deionised water. This procedure was carried out for 14 days. At the end of each 5 consecutive days of cycling, the samples were kept in the remineralising solution for 2 days.

Scanning electron microscope analysis

Scanning electron microscope analysis was performed to analyse the surface morphology of the specimens at the baseline, and after pH cycling. The specimens were air-dried then examined under SEM (Quanta FEG 250/ EDS, Octane Pro, USA) at x 1000 and x5000 magnification.

Energy Dispersive X-ray Spectroscopy (EDX) analysis

Elemental analysis of enamel surface in all groups was performed at baseline of untreated sound enamel then after exposure to pH cycling. The EDX analysis system works as an integrated part of SEM Quanta FEG 250, attached with EDX unit. The quantitative amount of elements in the studied surfaces was determined by an X-ray microanalyser EDX using spot measurements, EDX line scans and element mapping. Weight percentage values of Calcium and Phosphorus elements were expressed as mean values.

Statistical analysis

The data obtained from the elemental analysis were subjected to statistical analysis using the SPSS software. The Shapiro-Wilk test was carried to assess the normal distribution of data. The results were analysed by ANOVA. Multiple comparisons between groups were performed by paired t-test and post-hoc Tukey test. For the entire evaluation, p was <0.05 considered to be statistically significant.

Results

SEM analysis

SEM observation showed a smooth homogenous surface at the baseline for all the experimental groups (Figure 1).

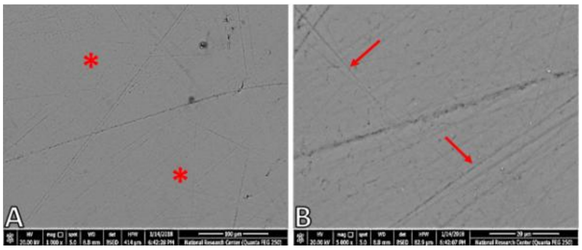

Figure 1: Scanning electron microscopic photomicrograph of sound enamel surface showing: A) smooth homogenous surface of rodless enamel (asterisks); B) the surface contained multiple scratches (red arrows); A) SEM x 1000; B) SEM x 5000)

After exposure to pH cycling, a group I, showed irregular porous dissoluted enamel surface (Figure 2).

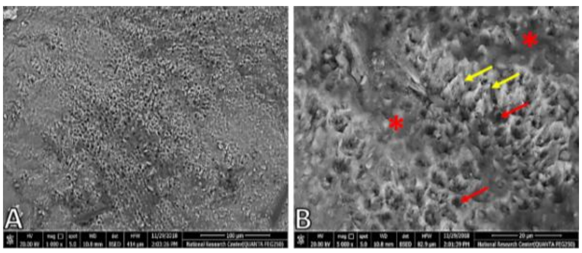

Figure 2: Scanning electron microscopic photomicrograph of group I (untreated) showing: A) irregular dissolution porous enamel surface, B) hollowing of rod core (red arrows), raised peripheral region (yellow arrows), indistinct rod morphological appearance (asterisks); (A; SEM x1000, B; SEM x5000)

In contrast, a partially dissolved enamel surface was observed in both group II (Figure 3) and group III (Figure 4).

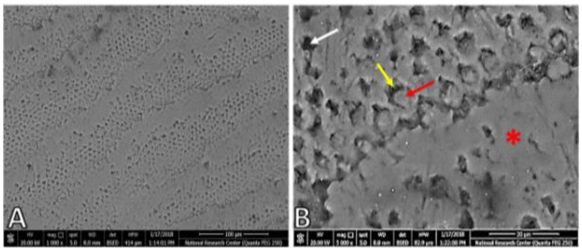

Figure 3: Scanning electron microscopic photomicrograph of group II (enamel treated with Er,Cr:YSGG laser) showing: (A) partially porous enamel structure intermixed with areas of rodless enamel, (B) intact rod core (red arrow), loss of rod peripheries (yellow arrow), loss of rod core (white arrow), smooth rodless enamel (asterisk). (A; SEM x 1000, B; SEM x 5000)

Energy Dispersive X-ray Spectroscopy (EDX) analysis

Comparison between the Ca and P weight percentage (mean±standard deviation) values at baseline and after demineralisation cycle within each group using paired t-test was displayed in (Table1 and 2). In all group's values of Ca and P weight percentage were significantly lower after exposure to demineralisation cycle ($p < 0.0001$).

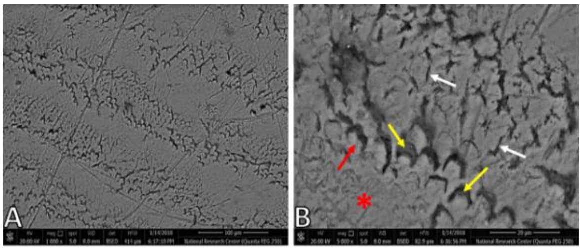

Figure 4: Scanning electron microscopic photomicrograph of group III (enamel treated with Nd:YAG laser) showing: (A) partially dissoluted enamel surface, (B): eroded rod peripheries (yellow arrows), intact rod core (red arrow), fine cracks (white arrows), smooth enamel surface with no enamel rod exposure (asterisk). (A; SEM x1000, B; SEM x5000)

The mean difference of change in Ca and P weight percentage at baseline and after demineralisation within each group was calculated. Mean percentage decrease in Ca and P within each group was obtained. One-way ANOVA revealed significant differences ($p < 0.0001$) between the tested groups. The lowest mean percentage decrease values were recorded in group II, followed by group III, then the highest mean percentage decrease was observed in the control group (group I) (Table 1 and 2). Multiple pairwise comparisons were then made between the three groups. For Ca, group II demonstrated a significant percentage decrease as compared to group I and III. Also, group III showed a significant decrease as compared to group I (Table 1). Regarding P, the mean percentage decrease in group II differed significantly from the group I but not with group III. However, a significant decrease was recorded in group III as compared to the control group (Table 2).

Table 1: Descriptive analysis for weight percentage of calcium

Groups		Mean ± SD	Within-group comparison Paired t-test p-value	Mean difference ± SD	Mean percentage decrease ± SD	ANOVA p-value
Group I (Untreated)	Baseline	56.26 ± 2.23	< 0.0001*	41.21 ± 2.84[a]	73.19 ± 3.11	< 0.0001*
	DC	15.06 ± 1.55				
Group II (Er,Cr:YSSG laser)	Baseline	56.25 ± 1.84	< 0.0001*	13.51 ± 1.51[b]	24.00 ± 2.48	
	DC	42.75 ± 1.89				
Group III (Nanosecond Nd:YAG laser)	Baseline	55.58 ± 3.83	< 0.0001*	23.03 ± 2.95[c]	41.51 ± 2.65	
	DC	32.56 ± 3.18				

**Significance level at p-value ≤ 0.05. DC: Demineralization cycle; Post-hoc Tukey's Test mean with the different superscript letter are significantly different* (p < 0.0001).

Discussion

Overall management of dental caries and erosion involves consideration of methods of preventing demineralisation and also methods of encouraging remineralisation of existing lesions [17]. Researchers often use *in vitro* studies to study the demineralisation-remineralisation process in cariology research. The *in vitro* studies are simple and easy to control to meet the research requirements with more reliable assessment methods that cannot be used for in vivo experiments [1]. Based on this information, this study was carried on *in vitro* conditions.

Table 2: Descriptive analysis for weight percentage of phosphorus

Groups		Mean ± SD	Within-group comparison Paired t-test p-value	Mean difference ± SD	Mean percentage decrease ± SD	ANOVA p-value
Group I (Untreated)	Baseline	25.94 ± 2.15	< 0.0001*	14.84 ± 2.17[a]	57.14 ± 5.62	< 0.0001*
	DC	11.09 ± 1.59				
Group II (Er,Cr:YSSG laser)	Baseline	25.74 ± 0.79	< 0.0001*	8.40 ± 0.87[b]	32.68 ± 3.73	
	DC	17.34 ± 1.33				
Group III (Nanosecond Nd:YAG laser)	Baseline	24.93 ± 0.91	< 0.0001*	10.15 ± 1.28[b]	40.70 ± 4.74	
	DC	14.78 ± 1.28				

**Significance level at p-value ≤ 0.05. DC: Demineralization cycle; Post-hoc Tukey's Test: means sharing the same superscript letter are not significantly different* (p = 0.2442), *means with the different superscript letter are significantly different* (p < 0.0001).

There are still many conflicts concerning the effect of laser irradiation on the enamel structure. This is most probably due to the high number of variables involved as it is a multifactorial process: power, pulse frequency and duration of irradiation [18]. Thus, the choice of laser parameters for different applications is very important. Regarding irradiation parameters used in the present study, all the irradiation conditions in both Er, Cr: YSGG or nanosecond Nd-YAG lasers aimed to be below the ablation threshold to avoid mechanical damage to the enamel.

Although the use of water can control the temperature increase, the water sprayed directly onto the surface of irradiated tissue can lead to greater enamel demineralisation and more ablation during an acid challenge [19]. Geraldo-Martins et al., [14] concluded that, the presence of water during Er, Cr: YSGG laser irradiation makes it difficult to obtain an enamel surface more resistant to acids. Therefore, we did not utilise the water spray in the present study.

The Er, Cr: YSGG power used in the present study was (0.75 W). This was based on previous studies which compared this power (0.75 W) with other Er, Cr: YSGG power values or with other types of lasers. Results revealed that 0.75 W power gave the best results regarding acid resistance enhancement [20], [21], [22]. The power of nanosecond Nd: YAG laser used in the herein study was 0.8 W. It was selected according to a previous pilot study done with 3 samples with different powers; 0.5 W, 0.8 W and 1.2 W. The power of 0.8 W revealed the best results concerning the morphological and elemental analyses. On the other hand, the power of 0.5 W did not produce any apparent effect, and 1.2 W produced an ablative effect on the enamel surface.

In the current study, several methods were used to evaluate enamel acquired acid resistance. Structural analysis was performed using a scanning electron microscope. It is used widely for studying the morphological changes of enamel and the effect of different treatment methods on its surface [23], [24]. Therefore, SEM was employed in this investigation. SEM analysis in the present study showed a smooth homogenous surface with a uniform rodless enamel surface layer at the baseline for all the experimental groups. This was by Huang et al., [25] and El Moshy et al., [26] who demonstrated a smooth surface of untreated sound enamel. In Group I (untreated enamel) after pH cycling, SEM revealed irregular porous dissoluted enamel surface. Same findings were conducted by Zorba et al., [27] who demonstrated multiple pores in the etched enamel surface.

After subjecting enamel treated with Er, Cr: YSSG laser in group II to pH cycling, partially porous enamel structure intermixed with areas of rodless enamel that covered the underlying structure was observed by SEM. This was in agreement with Hossain et al., [28], Moslemi M et al., [29], and Geraldo-Martins et al., [14] who in their individual studies reported a significant decrease in enamel demineralization after surface treatment with Er, Cr: YSGG laser compared to the control group. This was attributed to the well-known thermal effect of erbium laser and its positive role in enhancing the enamel acid resistance by modification of the chemical and physical structure of enamel [21].

In Group III (enamel treated with nanosecond pulsed Nd: YAG laser), SEM after pH cycling revealed partially dissoluted enamel surface intermixed with some relatively smooth areas indicating enhanced resistance to acid dissolution. This result was in agreement with Al Jedani et al., [30] who found that enamel surface treated with nanosecond Nd: YAG showed acquired acid resistance.

In the present study, EDX was used as an elemental analytic method for both Ca and P since the main components of enamel hydroxyapatite are Ca and P [31]. This method permits fast and quantitative microanalysis estimating the number of minerals in a given tooth sample in a nondestructive manner [32].

The mean difference of change in Ca and P at baseline and after demineralisation within each group was calculated. This was used to obtain Ca and P percentage decrease within each group. Acquired enamel acid resistance was estimated in terms of percentage decrease values in Ca and P, along with all the experimental groups.

Results in the current work showed that the lowest mean percentage decrease in Ca and P was recorded in Er, Cr: YSSG group (II) followed by nanosecond Nd: YAG group (III), then the highest mean percentage decrease was observed in control untreated group (I). This indicates that the highest acquired enamel acid resistance was achieved upon Er, Cr: YSSG laser irradiation. In this study, upon pairwise comparison for mean percentage decrease values, the difference between both group II and III as compared to the control group was statistically significant. This indicates that both types of laser treatments improved the enamel acid resistance significantly. Our findings were in agreement with many previously reported results concerning acquired enamel acid resistance following treatment with Er, Cr: YSGG laser application [14], [21] or nanosecond Nd: YAG laser [11], [30].

Going through pairwise comparison results in the current work, the difference between group II and III was statistically significant regarding Ca percentage decrease. However, no significant difference was recorded with P. This showed that nanosecond Nd: YAG had a similar effect as Er, Cr: YSSG as regard to percentage decrease of P.

From the present study, it could be concluded that under pH, cycling conditions, both Er, Cr: YSSG and nanosecond Nd: YAG laser irradiation improved the acid resistance of enamel. However, enamel surface treated with Er, Cr: YSSG laser showed the lowest mean percentage decrease of calcium and phosphorus (highest acid resistance). Although Er, Cr: YSSG and nanosecond Nd: YAG laser seems to be a promising treatment in preventive dentistry, further researches are needed to verify the role of laser irradiation parameters to take better benefits of laser potential in enhancing enamel acid resistance.

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