Comparative assessment of antibacterial effect of two types of laser and their effect on morphology and mineral content of dentin

Soha Adel Abdou[a], Haythem S Moharrum[b], Elsayed Abdallah Eltayeb[b]

[a]Dental Department, Research Institute of Ophthalmology (RIO), Giza, Egypt, [b]Medical Applications of Laser Department, National Institute of Laser Enhanced Sciences, Cairo University, Egypt

Correspondence to Soha Adel Abdou, MD, Researcher of Endodontics, Dental Department, Research Institute of Ophthalmology (RIO), Giza, Egypt. Mob: +01000535482; e-mail: soha.adel@rio.sci.eg

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Background/aim
Scientists have proven that the main cause of endodontic diseases and failures is the existence of micro-organisms in the root canals and inside the dentinal tubules. So the aim of the study was to compare the antibacterial effect of Erbium, chromium-doped yttrium, scandium, gallium, and garnet (Er,Cr:YSGG) laser and diode laser 980 nm alone and in combination with 5.25% sodium hypochlorite (NaOCl) irrigant against *Enterococcus faecalis* biofilm and also compare their effect on the morphology and mineral content of root canal dentin by scanning electron microscope (SEM) and energy-dispersive X-ray spectroscopy (EDX) analysis.
Materials and methods
The specimens used in our study were fifty four extracted human single-rooted teeth. They were collected from the Dental Department of the Research Institute of Ophthalmology, Egypt. Mechanical preparation was done to all teeth after their crowns were removed. They were autoclaved, and after that, they were inoculated with *Enterococcus faecalis* for 30 days. Roots were divided into six groups (each=9) according to type of treatment. Group A: 5.25% NaOCl, group B: Er, Cr:YSGG laser, group C: 5.25% NaOCl and Er,Cr:YSGG laser, group D: diode laser 980 nm, group E: 5.25% NaOCl and diode laser 980 nm, in addition to control group: saline. Their antibacterial effect was assessed before and after treatment. Changes in the morphology and mineral content of root canal dentin were assessed by SEM and EDX. Statistical analysis was done.
Results
Group C had significantly the highest percentage of *Enterococcus faecalis* reduction (98.95%), followed by group E (98.04%), then group D (95.27%), then group A (94.65%), then group B (92.99%), while the control group had significantly the lowest percentage of *Enterococcus faecalis* reduction (0.22%). Regarding SEM results, group B and group D exhibited score 1 (88.9%), control group and group C score 3 (77.8%), group A score 4 (88.9%), and group E score 5 (88.9%). Regarding EDX results, group B was significantly the lowest in Ca/P ratio, while there was an insignificant difference between other groups.
Conclusions
Er,Cr:YSGG laser, and diode laser, when used without 5.25% NaOCl irrigant, produced less morphological changes to dentinal tubules, but when combined with 52.5% NaOCl irrigant, produced more antibacterial effect against *Enterococcus faecalis*. Lasers alone or combined with 5.25% NaOCl irrigant produced changes in the mineral content of root canal dentin.

Keywords:
biofilm, diode laser, energy dispersive X-Ray spectroscopy, *Enterococcus faecalis*, Er, Cr: YSGG laser, scanning electron microscope

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Introduction

Scientists have proven that the main cause of endodontic diseases and failures is the existence of micro-organisms in the root canals and inside the dentinal tubules [1]. Hence, it is necessary to get rid of these micro-organisms and inhibit their reentry to the root canals in order to increase the success rate of endodontic treatment [2]. In order to achieve this, several procedures must be done together, including mechanical preparation and chemical disinfection by using endodontic irrigants and intra-canal medicaments [3,4]. Many studies have stated that they are not adequate for the elimination of the micro-organisms from the root canals because of the anatomical complexities of the root canals and the existence of resistant micro-organisms like *Enterococcus faecalis* [5]. It is one of the most popular micro-organisms isolated from cases of endodontic failures and secondary infections. It has the ability to form bacterial biofilm and enter deeply through the dentinal tubules of the root canals [6]. In addition, it is not affected by high PH, which is the reason for the antibacterial effect of calcium hydroxide ($Ca(OH)_2$) intra-canal medicament, and also not affected by the absence of nutrition or elevated salts concentration [7].

Sodium hypochlorite (NaOCl) is one of the most utilized irrigants, with many concentrations. It has several benefits that qualify it for that, which are low price, easy obtainable, antibacterial properties, capability to dissolve organic tissues, and removal of smear layer [8]. But it has many drawbacks, such as high cytotoxicity and the ability to produce chemical and morphological changes in root canal dentin [9,10].

Root canal irrigants and intra-canal medications cannot reach the narrow and curved areas of the root canals and also cannot penetrate deeply into dentinal tubules. Therefore, we tended to use the laser in addition to the irrigants in order to increase their efficiency and be able to reach the areas that the irrigants couldn't reach on their own [11].

Erbium, chromium-doped yttrium, scandium, gallium, and garnet (Er,Cr:YSGG) laser is a kind of infra-red laser that was used in the past in cases of surgery in dental field, as it excelled over the old traditional surgery by avoiding complications after surgery and causing a lack of feeling of pain [12]. It has many advantages when it is used in the endodontic branch, as it has the ability to activate the endodontic irrigants, which results in the removal of the smear layer, especially in the apical part of the root canal. Also, it has antibacterial properties [13].

Recently, diode laser has been used a lot in the dental field owing to its multiple advantages. It has a thin optical fiber, which is why it can get into tight, curved canals and inaccessible areas of the root canals. Furthermore, its price is low and it has multiple wavelengths as 810 nm, 830 nm, 940 nm, and 980 nm [14].

The main inorganic components of tooth hard tissue are calcium (Ca) and phosphorus (P). The solubility, microhardness, and permeability of root canal dentin can all be negatively impacted by changes in the calcium phosphorus (Ca/P) ratio, which can also change the amount of organic and inorganic components that were originally present [15,16]. It could also negatively impact the sealing properties and root canal sealer adherence [17,18].

The aim of the study was to compare the antibacterial effect of Er,Cr:YSGG laser and diode laser 980 nm alone and in combination with 5.25% NaOCl irrigant against *Enterococcus faecalis* biofilm and also compare their effect on the morphology and mineral content of root canal dentin by scanning electron microscope (SEM) and energy-dispersive X-ray spectroscopy (EDX) analysis.

Materials and methods

Calculation of specimen size

Size of the specimen was determined using a prior study by Afkhami *et al.* [19] as a guide. If the control group's mean and standard deviation are $2.07\times10^5\pm1.25\times10^5$ and the estimated mean is 4.5×10^5 with an effect size of 1.75, when power is 80% and α error probability is 0.05, the study only required a minimum of 7 subjects in each group; however, the total specimen size was increased to 9 subjects per group to account for 20% dropout. Specimen size was calculated using an Independent t test with G. power 3.1.9.7.

Ethical approval

Our study was conducted in accordance with the Declaration of Helsinki and was approved by the Research Ethical Committee (REC) of National Institute of Laser Enhanced Sciences on 18/7/2023 and the approval reference was: NILES-EC-CU 23/7/13.

Study design

The specimens used in our study were fifty four single-rooted human teeth extracted due to periodontal diseases from the Dental Department of the Research Institute of Ophthalmology, Egypt. Their roots were inspected for the absence of fractures or cracks. They were randomly categorized into six main groups (each=9) according to treatment as follows:

Group A: 5.25% NaOCl irrigant
Group B: Er,Cr:YSGG laser.
Group C: 5.25% NaOCl irrigant then Er,Cr:YSGG laser.
Group D: diode laser 980 nm.
Group E: 5.25% NaOCl irrigant then diode laser 980 nm.
Control group: saline irrigant.

Preparation of the teeth

Teeth were cleaned of any calculus or debris. They were kept in a saline solution until the test was done.

The crowns of all teeth were removed at the cementoenamel junction using a fissure bur at highspeed motor, and the roots length were adjusted to be 15 mm±2 mm. Mechanical preparation was done to all roots by the ProTaper rotary system (Maillefer – Dentsply, Baillagues, Switzerland) to size F4. Two ml of 2% NaOCl irrigant was used between every file. At the end of preparation, the roots were irrigated with three ml of 17% ethylenediaminetetraacetic acid (EDTA) then three ml of 2% NaOCl. Finally, all roots were flushed with 6 ml of saline solution. Varnish was used to coat the external surfaces of the roots, and the flowable composite was used to close the apices of the roots. Each root was placed in an Eppendorf tube and sterilized at 121°C for thirty minutes.

All the next steps were done inside the laminar air flow.

Preparation of bacterial suspension and infection of the roots

The bacterial suspension of *Enterococcus faecalis* ATCC 29212 in brain heart infusion (BHI) broth was prepared and standardized to 1 McFarland. Each root was inoculated with 8 μl of infected broth and inserted in the incubator for 30 days at 37°C to form a bacterial biofilm. The broth changed every three days.

Treatment of the roots

The roots of group A were treated with five ml of 5.25% NaOCl irrigant for two minutes.

The roots of group B were treated with Er,Cr:YSGG laser (Waterlase iPlus, Biolase, USA) at a 2780 nm wavelength with a power of 1.5 W and a repetition rate of 20 Hz. After activation of the laser, the optical fiber was slowly introduced into the root canals. The laser was applied in three cycles; the duration of each is 20 seconds, as shown in Fig. 1. There is also a 10-second rest period between each cycle.

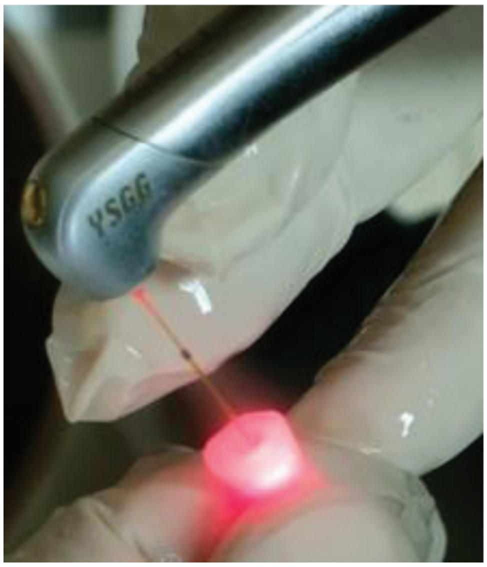
In group C, the roots were treated with five ml of 5.25% NaOCl irrigant for two minutes and then activated by Er,Cr:YSGG laser as described in group B.

While in group D, the roots were treated with a diode laser 980 nm wavelength and a power of 1.5 W, as described in group B.

The roots of group E were treated with five ml of 5.25% NaOCl irrigant for two minutes and then activated by a diode laser 980 nm wavelength as described in group B.

In the control group, the roots were irrigated with five ml of saline irrigant for two minutes.

Figure 1



Application of Er,Cr:YSGG laser.

During the application of the laser in groups Er,Cr: YSSG laser (Groups B and C), water and air cooling were applied.

After all the teeth were treated in the previous way, they were irrigated with 6 ml of sterile saline.

Microbial assessment

Two samples were taken from each root canal: one before treatment application and after 30 days of root canal infection, while the second sample was taken immediately after treatment application. Samples were taken using paper points size 40. Infected paper points were inserted separately in sterile Eppendorf tubes containing 2 ml of BHI broth and vortexed for 25 seconds. Serial dilution was done till 1/10000, and then fifteen microns of infected broth were cultured on bile esculin agar plates and incubated at 37 °C for two days. The numbers of colonies on agar plates were counted, and the colony forming units (CFUs) were calculated.

Morphological variations assessment by scanning electron microscope

All tested roots were sectioned longitudinally into two halves by making a longitudinal groove on the lingual and labial aspects of the roots with a diamond disc of 0.5 mm. The sectioning process was completed with a chisel and mallet. Sectioned roots were irrigated with 10 ml of distilled water. After that, they were desiccated in a hot oven. They were fixed on aluminum stubs and sprayed with gold in the vacuum evaporator. Photomicrograph sets were taken by SEM (Quanta 250 FEG) at multiple magnifications: 1000X, 3000X, and 5000X. The morphological variations were assessed from the photomicrographs by two examiners in a double-blind investigation. The following scores were instituted to guide the examiners in the morphological analysis process [20]:

Score 1: Clean root canal wall, no smear layer, and open dentin tubules more than 95%.
Score 2: Slight quantity of smear layer and open dentin tubules more than 60%.
Score 3: Moderate quantity of smear layer and open dentin tubules less than 40%.
Score 4: Dense smear layer with complete effacement of dentin tubules.
Score 5: Slight or no smear layer, sealed dentin tubules, melting and fusion.

Mineral content assessment by energy-dispersive X-ray spectroscopy

All samples were analyzed by EDX to determine the amount of carbon (C), oxygen (O), sodium (Na), magnesium (Mg), P, and Ca and also to assess the Ca/P ratio.

Statistical analysis

All qualitative data were provided as frequency and percentages, while all quantitative data were presented as mean and standard deviation. SPSS 16® (Statistical Package for Scientific Studies), GraphPad Prism, and Windows Excel were used to conduct the statistical analysis. Shapiro-Wilk and Kolmogorov-Smirnov tests were used to examine the quantitative data for normality and the results showed that the significant level (*P* value) was insignificant because *P* value >0.05, which meant that all of the data came from a normal distribution (parametric data). Accordingly, a Paired t-test was used to compare two different intervals, a One Way ANOVA test was used to compare all groups, followed by a Tukey's Post Hoc test, which was used for multiple comparisons. A Chi square test was used for comparison between different scores regarding SEM in all groups.

Results

Bacterial evaluation

Intra-group comparison (comparison between before and after treatment)

Comparison between mean values of *Enterococcus faecalis* count before and after treatment in all groups was performed using the Paired t test, which revealed a significant decrease in mean values of *Enterococcus faecalis* count after treatment in all groups as $P<0.05^*$

Inter-group comparison (comparison between different groups)

Comparison between different groups was performed by using the One Way ANOVA test, which revealed a significant difference between them in all as $P<0.05$, followed by Tukey's Post Hoc test as shown in Table 1.

Before treatment: group D (598.33±9.01) was significantly the lowest mean value of *Enterococcus faecalis* count, but group E (612.67±9.54) was significantly the highest mean value of *Enterococcus faecalis* count, while there was an insignificant difference between other groups.

After treatment: there was a significant difference between all groups. Control group (600±1.73) was significantly the highest mean value of *Enterococcus faecalis* count, followed by group B (42±1.73), then group A (32.33±2.18), then group D (28.33±2.65), then group E (12±1.73), while group C (6.33±1.32) was significantly the lowest mean value of *Enterococcus faecalis* count.

Difference between before and after treatment: group C (598.67±7.76) and group E (600.67±8.00) had significantly the highest mean values of difference in *Enterococcus faecalis* count between before and after treatment, followed by group A (572±5.68) and group D (570±7.79), then group B (557.33±10.9), while control group (1.33±1.32) had significantly the lowest mean value of difference in *Enterococcus faecalis* count between before and after treatment.

Table 1 Comparison between mean values of *Enterococcus faecalis* count before and after treatment in all groups

Bacterial count	Control group	Group A	Group B	Group C	Group D	Group E
Before	601.33±1.32 ^{a}	604.33±3.50 ^{ab}	599.33±11.69 ^{ab}	605±7.70 ^{ab}	598.33±9.01 ^{a}	612.67±9.54 ^{b}
After	600±1.73 ^{a}	32.33±2.18 ^{b}	42±1.73 ^{c}	6.33±1.32 ^{d}	28.33±2.65 ^{e}	12±1.73 ^{f}
P value	0.010*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*
Difference	1.33±1.32 ^{a}	572±5.68 ^{b}	557.33±10.90 ^{c}	598.67±7.76 ^{d}	570±7.79 ^{b}	600.67±8.00 ^{d}
% reduction	0.22±0.21 ^{a}	94.65±0.39 ^{b}	92.99±0.25 ^{c}	98.95±0.22 ^{d}	95.27±0.40 ^{e}	98.04±0.26 ^{f}

All data are expressed as mean±SD. *Significant difference between before and after groups at $P<0.05$, using Paired *t*-test. All data with different superscript letters (a, b, c, d, e, f) per raw were significantly different as *P value* <0.05, using ANOVA test.

Percentage of *Enterococcus faecalis* reduction: there was a significant difference between all groups. Group C (98.95±0.22) was significantly the highest mean value of *Enterococcus faecalis* reduction, followed by group E (98.04±0.26), then group D (95.27±0.40), then group A (94.65±0.39), then group B (92.99 ±0.25), while control group (0.22± 0.21) was significantly the lowest mean value of *Enterococcus faecalis* reduction.

Results of scanning electron microscope

Frequency and percentages of different scores regarding SEM in all groups were presented in Table 2 and Figs. 2 and 3.

A comparison between them was performed using the Chi square test, which revealed that:

(1) Control group and group C: score 3 (77.8%) was significantly the highest.
(2) Group A: score 4 (88.9%) was significantly the highest.
(3) Group B and group D: score 1 (88.9%) was significantly the highest.
(4) Group E: score 5 (88.9%) was significantly the highest.

Results of energy-dispersive X-ray spectroscopy

Mean and standard deviation of the percentage weights of C, O, Na, Mg, P, and Ca, and Ca/P ratio for all groups were presented in Table 3.

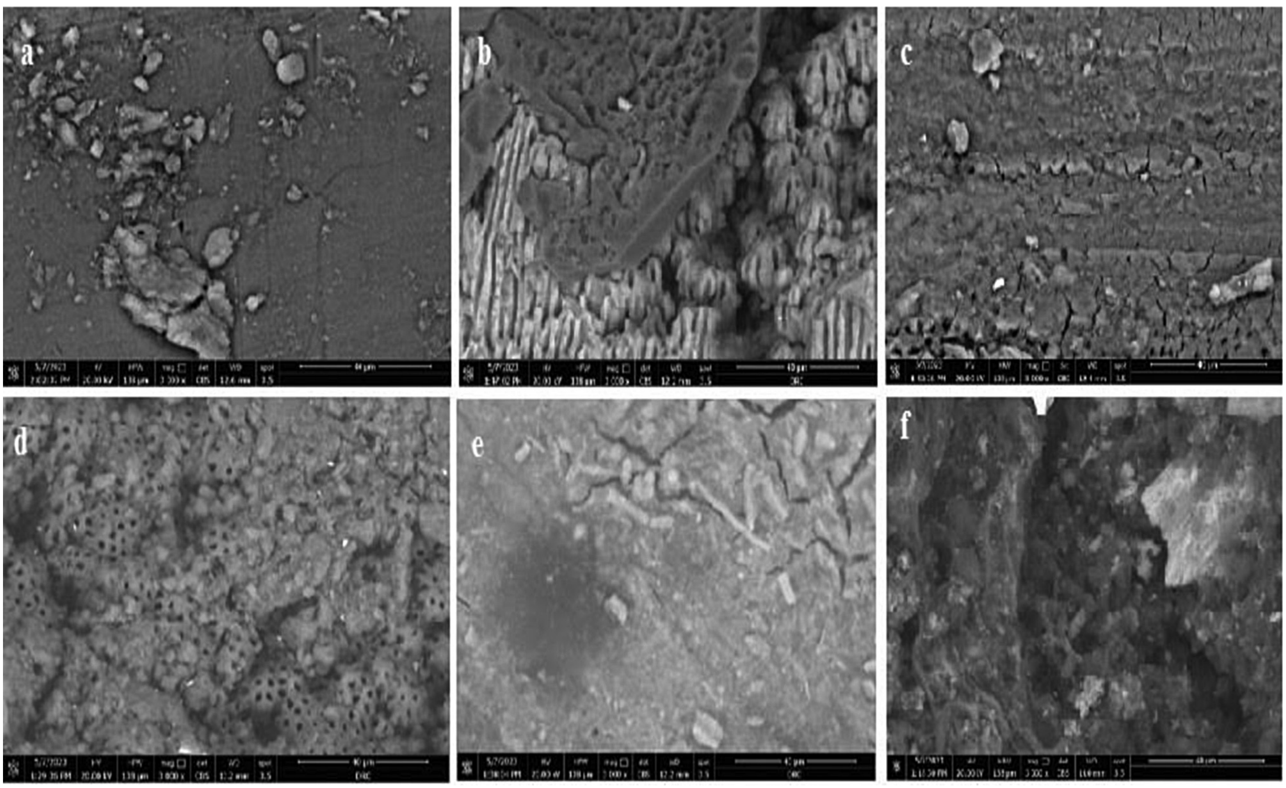
Comparison between different groups was performed by using the One Way ANOVA test, which revealed a

Table 2 Frequency and distribution of different scores in all groups using scanning electron microscope

Groups	Score 1 *N* (%)	Score 2 *N* (%)	Score 3 *N* (%)	Score 4 *N* (%)	Score 5 *N* (%)	*P value*
Control group	0 (0)	0 (0)	7 (77.8%)	2 (22.2%)	0 (0)	0.001*
Group A	0 (0)	0 (0)	0 (0)	8 (88.9%)	1 (11.1%)	0.0002*
Group B	8 (88.9%)	1 (11.1%)	0 (0)	0 (0)	0 (0)	0.0002*
Group C	0 (0)	0 (0)	7 (77.8%)	0 (0)	2 (22.2%)	0.001*
Group D	8 (88.9%)	1 (11.1%)	0 (0)	0 (0)	0 (0)	0.0002*
Group E	0 (0)	0 (0)	0 (0)	1 (11.1%)	8 (88.9%)	0.0002*
P value	0.0002*	0.31	0.001*	0.0002*	0.0002*	

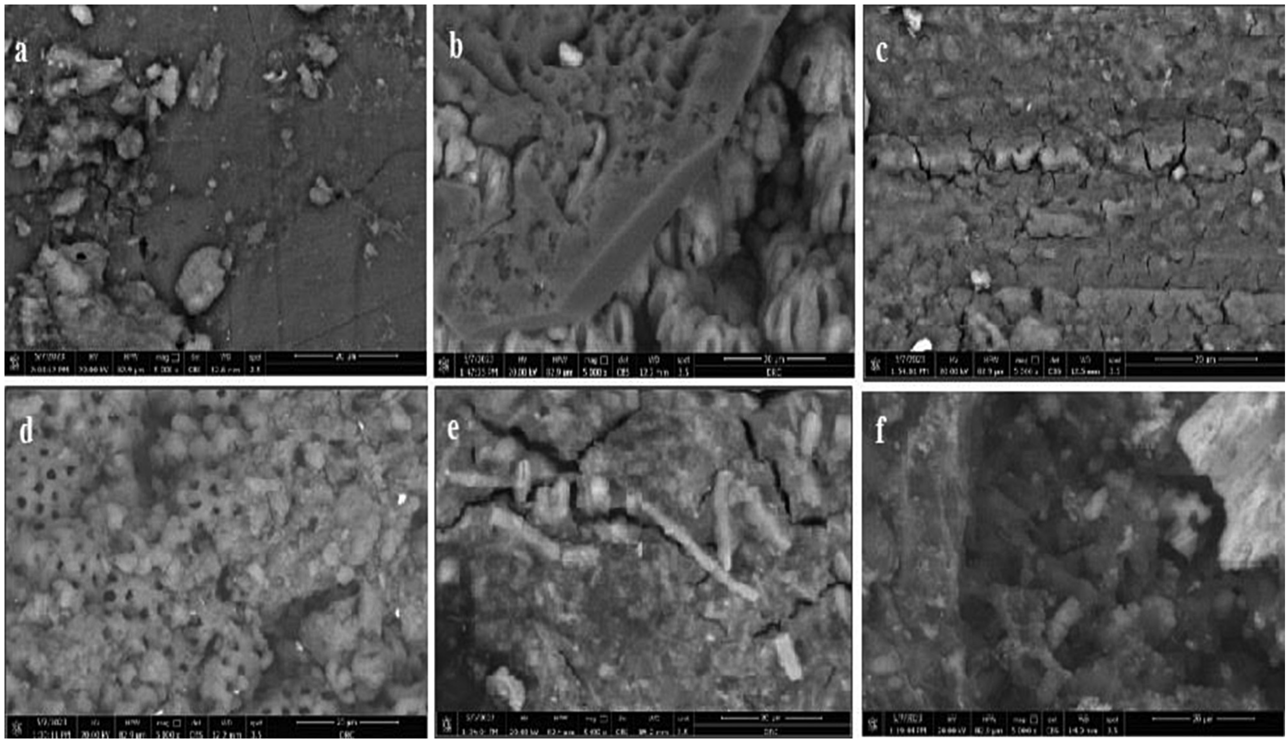
All data are expressed as frequency (N) and percentage (%). *Significant difference at $P<0.05$, using Chi square test.

Figure 2



SEM photomicrograph of dentin at X 3000, where (a): group A, (b): group B, (c): group C, (d): group D, (e): group E, and (f): control group.

Figure 3



SEM photomicrograph of dentin at X 5000, where (a): group A, (b): group B, (c): group C, (d): group D, (e): group E, and (f): control group.

Table 3 Comparison between percentage weights of minerals of all groups

EDX Weight	Control group	Group A	Group B	Group C	Group D	Group E
C	18.10±0.77 ^{a}	5.37±0.74^{b}	4.17±0.84^{c}	5.17±1.18^{bc}	8.23±0.22^{d}	11.23±0.93^{e}
O	36.80±1.20 ^{a}	36.77±1.13^{a}	56.27±3.38^{c}	37.93±2.82^{a}	42.87±0.74^{b}	45.37±1.13^{b}
Na	0.80±0.09 ^{a}	1.00±0.09^{b}	0.43±0.13^{c}	1.13±0.18^{b}	1.10±0.09^{b}	1.47±0.13^{d}
Mg	0.67±0.13 ^{a}	0.80±0.09^{a}	5.90±1.50^{b}	1.07±0.13^{a}	1.03±0.13^{a}	1.30±0.15^{a}
P	13.80±0.43 ^{a}	17.53±0.18^{b}	14.70±0.69^{c}	17.13±1.07^{b}	14.30±0.35^{ac}	13.33±0.13^{a}
Ca	29.87±0.28 ^{a}	38.40±0.48^{b}	19.87±2.28^{c}	37.27±3.29^{b}	32.63±0.70^{d}	27.27±0.79^{e}
Ca/P	2.17±0.08 ^{a}	2.19±0.03^{a}	1.36±0.19^{b}	2.17±0.06^{a}	2.28±0.09^{a}	2.05±0.08^{a}

All data are expressed as mean±SD. All data with different superscript letters (a, b, c, d) per raw were significantly different as *P value* $<$0.05.

significant difference between them in all groups as $P<0.05$, followed by Tukey's Post Hoc test, which revealed that:

Carbon weight, the control group (18.10±0.77) was significantly the highest, while group B (4.17±0.84) and group C (5.17±1.18) were significantly the lowest.

Regarding the weight of Oxygen, group B (56.27±3.38) was significantly the highest, while the control group (36.80±1.20), group A (36.77±1.13), and group C (37.93 ±2.82) were significantly the lowest.

Sodium weight, group E (1.47±0.13) was significantly the highest, while group B (0.43±0.13) was significantly the lowest.

Magnesium weight, group B (5.90±1.50) was significantly the highest, while there was an insignificant difference between all other groups.

Regarding the phosphorus weight, group A (17.53 ±0.18) and group C (17.13±1.07) were significantly the highest, while the control group (13.8±0.43) and group E (13.33±0.13) were significantly the lowest.

Calcium weight, group A (38.4±0.48) and group C (37.27±3.29) were significantly the highest, while group B (19.87±2.28) was significantly the lowest.

However, the Ca/P ratio in group B (1.36±0.19) was significantly the lowest, while there was an insignificant difference between other groups.

Discussion

Successful endodontic treatment demands total cleaning and getting rid of the harmful micro-organisms from the root canal system [21]. So the aim of our study was to compare the antibacterial effect of Er,Cr:YSGG laser and diode laser 980 nm alone and in combination with 5.25% NaOCl irrigant against *Enterococcus faecalis* biofilm and also compare their effect on the morphology and mineral content of root canal dentin by SEM and EDX analysis.

Since *Enterococcus faecalis* is very resistant to many disinfectants and is also crucial in recurrent endodontic infections and unsuccessful root canal procedures, it has been employed in numerous studies to assess the disinfectant capability of antibacterial irrigants or different types of lasers [22]. *Enterococcus faecalis*, on the other hand, is very heat-resistant, and since the main antibacterial impact of the laser is mostly a result of the heating effect, it is advised to use it to examine the heat-independent antimicrobial effect of laser [23].

The results of our research showed that the highest percentage of *Enterococcus faecalis* reduction was found in the 5.25% NaOCl irrigant with Er,Cr:YSGG laser group, which was 98.95%, followed by the 5.25% NaOCl with diode laser group, which was 98.04%. This may be due to the combination of the antibacterial effect of 5.25% NaOCl irrigant and the antibacterial effect of laser [24,25]. Also, activation of the NaOCl irrigant by a laser beam increases the temperature of the NaOCl irrigant and hence increases its antibacterial efficacy [23]. Furthermore, the Er,Cr:YSGG laser has the affinity to absorb water, and water-based irrigants lead to the formation of vapor bubbles, which are called cavitation. This bubble enlarges during the activation process of the irrigant by the laser beam. Once the activation process ends, this bubble contracts until it rupture. Subsequently, many small cavitation bubbles are created and are known as secondary cavitation bubbles [26]. This process created pressure waves inside the irrigant called shock waves and acoustic waves. These waves produce shear stress in the root canals, facilitating the arrival of the irrigant in all inaccessible areas of the root canals and entering deeply into dentinal tubules [27]. As well, these movements of NaOCl irrigant in root canals increase the contact of chlorine particles to the root canal dentin, thereby increasing its antibacterial efficacy [28]. These results were in line with Betancourt *et al.* [27] and Seet *et al.* [29] results, which stated that the combination between NaOCl irrigant and Er,Cr:YSGG laser was more effective against *Enterococcus faecalis* biofilm than NaOCl irrigant alone with the same concentration. Also with the results of Castelo-Baz *et al.* [30] and Preethee *et al.* [31] which stated that the combination of NaOCl irrigant with diode laser has a higher antibacterial effect than NaOCl irrigant alone.

NaOCl is the most usually used irrigant during endodontic treatment. The antimicrobial effect of NaOCl is due to the presence of hypochlorous acid. It acts on sulphydryl groups in the enzymes of microorganisms through its oxidative effect [3]. Our results stated that the percentage of *Enterococcus faecalis* reduction in the group treated with 5.25% NaOCl irrigant was 94.65%, while it was 95.27% in the group treated with diode laser alone and 92.99% in the group treated with Er,Cr:YSGG laser alone. These results were in agreement with the results of Yavari *et al.* [32] who stated that NaOCl irrigant was more effective than Er,Cr:YSGG laser against *Enterococcus faecalis*. These results were in disagreement with the results of Sohrabi *et al.* [33] and the results of El-Tayeb and Nabeel [34], which stated that the percentage of *Enterococcus faecalis* reduction in the group treated with 5.25% NaOCl irrigant was higher than the group treated with diode laser. This may be related to using different parameter and wavelength of used laser.

The percentage of *Enterococcus faecalis* reduction in groups treated with laser alone, even diode or Er,Cr: YSGG, was lower than groups treated with a combination of 5.25% NaOCl irrigant and laser. This may be attributed to the laser's property of water absorption. So the absence of irrigant in the root canal makes the effect of the laser beam limited to the superficial area of dentinal tubules and less enter deeply into dentinal tubules, which results in a decrease in the antibacterial effect of the laser [25]. This result was in coincidence with the results of EL-Gendy *et al.* [35], who stated that the antibacterial effect of NaOCl irrigant in combination with Er,Cr:YSGG laser was more effective than Er,Cr:YSGG laser alone against *Enterococcus faecalis*.

SEM was chosen in this study to assess the ultrastructural changes that occurred on the dentin surface after the application of the tested treatments due to its ability to evaluate the morphological aspects qualitatively and quantitatively [36].

Our results showed that, the application of laser alone, even Er:Cr,YSGG or diode, on root canal dentin removed the smear layer and kept all dentinal tubules open. These results were in agreement with Lopes *et al.* [20] results, which stated that the Er:Cr, YSGG laser removed the smear layer completely and reserved the dentinal tubules open. And also with Wang *et al.* [37] results, which stated that diode laser 980 nm was effective in removing dentin smear layer and debris while keeping the dentinal tubules open. In contrast, Moura-Netto *et al.* [38] and Camargo *et al.* [39] stated that dentin fusion and resolidification were caused by the laser, along with some smear layer and debris clearance. This controversy in the results may be due to using different type of laser and different parameters.

And also, when the Er:Cr,YSGG laser was combined with 5.25% NaOCl irrigant, more than 60% of dentinal tubules were sealed, and there was a moderate quantity of smear layer on the dentin surface. However, when diode laser was combined with 5.25% NaOCl irrigant, it caused the sealing of dentinal tubules and the melting of them. Additionally, there was a dense smear layer on the dentin surface with complete effacement of dentin tubules after treatment with 5.25% NaOCl irrigant alone. Marending *et al.* [40] stated that NaOCl irrigant produced breakdown of organic dentin components, which results in a significant alteration of the peripheral dentin matrix, a decrease in elastic modulus and flexural strength, and a change in intertubular dentin permeability. Also, Mohammadi [41] stated that NaOCl's weak physicochemical activity, which is restricted to organic particles, may be the cause of its ineffectiveness in removing the smear layer from the dentin surface. Our results were in accordance with the results of Rathakrishnan *et al.* [42], Kandil *et al.* [43], and Ulusoy and Görgül [44], which stated that there was a heavy smear layer on the dentin surface after irrigation with NaOCl irrigant. In contrast, Saraswathi *et al.* [45] stated that using irrigation of NaOCl and EDTA in addition to diode laser irradiation of root canal dentin improved smear layer elimination. This may be attributed to using diode laser with different wavelength.

EDX was chosen in our study to measure the mineral content of dentin. The primary benefit of this technology is its ability to analyze materials accurately and without causing any damage. The technique is based on irradiating the sample with a high-voltage electron beam, which produces emission at a specific wavelength for each mineral. The variations in the wavelengths of the light emitted from the sample reveal variations in the concentration of a particular mineral on the sample surface [13]. The primary inorganic components of dentin hydroxyapatite are Ca and P [46]. Our results showed that, compared to the control group, the mean percentage weight of Ca increased in the 5.25% NaOCl irrigant group, the diode laser group, and the 5.25% NaOCl irrigant with Er,Cr:YSGG laser group. And they were decreased in the Er,Cr:YSGG laser group and the 5.25% NaOCl irrigant with diode laser group.

Whereas the mean percentage weight of P increased in the 5.25% NaOCl irrigant group, the Er,Cr:YSGG laser group, and the 5.25% NaOCl irrigant with Er,Cr: YSGG laser group, while it did not change in the diode laser group or in the 5.25% NaOCl irrigant with diode laser group. The mean percentage weight of O increased in the Er,Cr:YSGG laser group, the diode laser group, and the 5.25% NaOCl irrigant with diode laser group. But it did not change in the other tested groups. The mean percentage weight of C decreased in all tested groups, while the mean percentage weight of Na increased in all tested groups, except in the Er,Cr: YSGG laser group, where it decreased.

When a 980 nm diode laser is used on the dentin surface, some of the energy is absorbed by the mineral elements of the dentin, disrupting the arrangement of the dentin crystals and causing the dentin to dissolve [47]. However, when an Er:Cr, YSGG laser is used on the dentin surface, the elements that are bound to the water in the dentin react, causing the crystalline dentin structure to enlarge. Peritubular dentin and dentin tubules are destroyed, resulting in a different pattern [48]. Our findings supported the findings of Sazak *et al.* [49], which showed that dentin surface imperfections and differences in the mineral composition were caused by laser application. Additionally, Secilmis *et al.* [50] findings, which demonstrated that the application of Er,Cr:YSGG laser had an impact on the amount of Ca, Na, and P levels in the dentin of permanent teeth. And also with the findings of Kazeminejad *et al.* [51] that demonstrated that laser use had a substantial impact on P removal from dentin. However, Guler *et al.* [52] findings contrasted with our findings. They stated that the dentin's mineral content, which was Ca, Na, and P was unaffected by the application of a laser to its surface. This discrepancy in results may be caused by the fact that primary teeth were employed in their study and that they used a different kind of laser. And with the findings of Malkoc *et al.* [53], which stated that the levels of Ca, P, and Na in dentin were not affected by the application of the Er,Cr:YSGG laser, this may be due to using different testing method. Furthermore, Gurbuz *et al.* [54] stated that the percentage of Ca and P decreased in the group treated with 5.25% NaOCl irrigant.

Changing the inorganic and organic components of root canal dentin leads to an alteration in the Ca/P ratio [55]. All the tested groups in our study didn't affect the Ca/P ratio of dentin except the Er,Cr:YSGG laser group, which decreased the ratio. Ari and Erdemir [56] stated that the use of large concentrations of NaOCl as an irrigant may impact the inorganic material that limits further dentin breakdown, or it may disintegrate the organic components and produce a layer of mineralized tissue. Because of this mineralized layer, irrigation with 5.25% NaOCl does not affect the Ca/P ratio of root canal dentin. Owing to its low molecular weight, it is dispersed throughout the collagen matrix of dentin [18].

The results of Alhadi *et al.* [57] proved that the application of laser to root canal dentin increased the erosion degree caused by irrigants. This erosion resulted from the removal of Ca ions from the inner dentin wall. These results were similar to the results of Saghiri *et al.* [58] and De Moura-Netto *et al.* [38].

Saraswathi *et al.* [45] proved that NaOCl and EDTA irrigation alone or combined with diode laser did not have any effect on the Ca/P ratio of root dentin. In contrast, Doğan and Çalt [59] stated that NaOCl irrigant alone changed the Ca/P ratio of root canal dentin. This might be related to using different concentration of NaOCl.

The mineralization process, especially crystal development, has been thought to be influenced by a little amount of Mg that is always visible in the mineralized tissues in addition to Ca and P, and a lack of it can lower the number of odontoblasts and impede the production of dentin. Dentin is strengthened as a result [60]. In our study, all the tested groups didn't affect the mean percentage weight of Mg in dentin except the Er,Cr:YSGG laser group, which increased the percentage. This has been said to be related to the substitution of Mg for Ca [61].

The relative Ca or P content of dentin may rise as a result of the evaporation of organic substances during laser irradiation [62]. These results are consistent with those of an experiment conducted by Altundasar *et al.* [63], who showed that Er,Cr:YSGG laser irradiation increased Ca, P, and Mg levels.

The outcomes of the in vitro investigations conducted by Doğan and Çalt [59] showed that the Mg level significantly increased following the application of a chelating agent in combination with NaOCl irrigant, and Gurbuz *et al.* [54] showed that teeth treated with NaOCl irrigant had a high Mg level. In contrast, Ari and Erdemir [56] confirmed that Mg level in dentin were not affected after using NaOCl irrigant.

Conclusions

The combination of Er,Cr:YSGG laser or diode laser with 5.25% NaOCl irrigant had an antibacterial effect against *Enterococcus faecalis* more than the laser alone or 5.25% NaOCl irrigant alone. Er,Cr:YSGG laser and diode laser, when used without 5.25% NaOCl irrigant, produced less morphological changes to dentinal tubules and also removed the smear layer. Lasers alone or combined with 5.25% NaOCl irrigant produced changes in the mineral content of root canal dentin.

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Conflicts of interest

There was no conflict of interest.

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