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Morphological changes in root dentin after irradiation with diode laser 980 nm in two directions: an *in-vitro* study

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Background/aim

Laser technology is an essential aid tool for endodontic treatment procedures. The most common type of laser used in the endodontic field was diode laser 980 nm. The present work aimed to assess the morphological variations and chemical changes in root canal dentin treated with diode laser 980 nm in two directions parallel and perpendicular by scanning electron microscope (SEM) and energy dispersive X-ray spectroscopy (EDS).

Materials and methods

Thirty extracted human single-rooted lower premolars teeth with mature apex were chosen from Dental Department of Research Institute of Ophthalmology, Egypt. Their crowns were resected at the cement-enamel junction (CEJ), and their roots were mechanically prepared. The roots were categorized into two groups (each = 15): according to the direction of laser application. In group A the laser was directed parallel to the root canal, while in the group B it was directed perpendicular to the root canal. All groups were analyzed by SEM and EDS examination. Statistical analysis was done by Kolmogorov-Smirnov and Shapiro-Wilk tests and comparison between two groups was performed by independent t-analysis, while in qualitative data Chi-square test was used.

Results

The results of group A where the laser was directed parallel to the root canal exhibited score 1 (86.7%) in the coronal section, score 3 (80%) in the middle section, and score 4 (80%) in the apical section. The results of group B where the laser was directed perpendicular to the root canal represented score 1 (86.7%) in the coronal section, score 1 (73.3%) in the middle section, and score 3 (73.3%) in the apical section.

Conclusion

Diode laser 980 nm in a perpendicular direction to root canal dentin surface produced more morphological changes than when used in a parallel direction, however regarding the chemical changes, there were no chemical changes detected between the two directions.

Keywords:

diode laser, energy dispersive X-ray spectroscopy, parallel, perpendicular, scanning electron microscope

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Introduction

Endodontic treatment was designed to clean and disinfect the root canals, but anatomical complexities such as accessory canals and ramifications retard this process [1]. New techniques and devices have recently been developed to cope with these difficulties [2,3]. It also simplifies endodontic treatment by having a faster, easier, safer and more efficient treatment [4].

Laser technology is an essential aid tool for endodontic treatment procedures [5–7]. Laser makes simultaneous modifications in wall morphology and removes the smear layer from the root canals [8,9]. It also can seal the apical foramen [10] and modify dentin permeability [11].

The most common type of laser used in the endodontic field was high intensity diode laser 980 nm. Its many

delightful specifications made it common among endodontists: tiny size, low expense and simple usage. These properties were related to its consistency as it was formed from fiber delivery system [12]. It also has an important advantage, which is flexibility that makes it able to enter easily into curved canals [13]. Furthermore it has antimicrobial properties which related to its capability to enter deeply into dentinal tubules and reach areas cannot be reached by irrigant solutions [14].

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It is substantial to reflect that laser's action on dental tissues, like enamel or dentin, varies according to numerous factors, such as revival mode, wavelength, stroke duplication ratio, capacity density, tissue water content, strength, and irradiation direction [15].

Scanning electron microscope (SEM) was especially well eligible for the survey of dental enamel and dentin structure because of its capacity to produce high-resolution images of hard tissues [16]. The energy dispersive X-ray spectroscopy (EDS) microanalysis was correlated to an electron microscope. It analyzes the elements by producing particular X-rays that detect ingredient existence in the samples. It is utilized in various medical domains in many pieces of research due to its high vulnerability in detecting various elements in tissues [17].

Thus, the study was aimed to assess the morphological variations and chemical changes in root canal dentin treated with diode laser 980 nm in two directions parallel and perpendicular by SEM and EDS.

Materials and methods

Calculated sample size

It was determined based on Azmy and colleagues [18] research as a reference. According to this research, the least reasonable specimen size was 12 for each category, as a restraint in every category was normally dispensed with a standard deviation of 2.6. If the assessed variation was 3, while the probability was 0.8, the type I error eventuality accompanied by this test was 0.05. The overall specimen size was raised to 15 for dropout recovery. The sample size was calculated using P.S Power 3.1.6. Software.

Study design

Thirty extracted human single-rooted lower premolars teeth with mature apex were chosen. They were possessed from patients who extracted their teeth due to orthodontic treatment in Dental Department of Research Institute of Ophthalmology, Giza, Egypt. The picked teeth were tested to exclude cracks, resorption, or fractures. They were categorized into two groups (each = 15), as follows:

Group A: The picked teeth irradiated with laser parallel to the root canals.

Group B: The picked teeth irradiated with laser perpendicular to the root canals.

Ethical approval

The 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 3/1/2023 and the approval reference was: NILES-EC-CU 23/3/6.

Teeth preparation

The crowns of all teeth were cut at their cement-enamel junction (CEJ) by a diamond stone mounted in a high-speed handpiece under water coolant. The length of the roots was standardized to 15±1 mm. The REVO-S (MICRO MEGA) rotary system mounted on an endodontic motor (Motor X smart E-Cube, Korea) was used for mechanical preparation. 2 ml of distilled water irrigant were used after every file. At the end of preparation, all root canals were irrigated with 10 ml of distilled water to remove dentin chips.

Treatment of the roots

Diode laser 980 nm with a 200 μ m fiber optic tip was inserted into the apical portion of the roots of group A. Then after the laser was activated, it was withdrawn slowly to the coronal area in a helicoid motion and reintroduced to the root apex. The laser irradiation period was 10 s.

Diamond disk mounted in a low-speed handpiece was utilized in making a longitudinal sulcus on the labial and lingual surfaces of the roots of group B without touching inside the root canals. Then they were sectioned longitudinally with a chisel and an endodontic hammer. Diode laser 980 nm was directed perpendicular to the canal surface and away from it by 1 mm for 10 s.

All groups were irradiated with diode laser 980 nm of 1.5 W for 10 s and 5 s rest for 3 repetitions.

Evaluation by SEM and EDS

Samples of groups A were sectioned longitudinally in the same way as group B. All samples were rinsed with 5 ml of filtered water for 1 min. They were then dried in a hot oven for 30 min at 120°C. They were fixed on aluminum stubs and covered with a layer of gold in a vacuum evaporator.

All samples were assessed by SEM (Inspect S50, Czech Republic). Standardized photomicrograph sets were taken at various magnifications, 400X, 1000X, and 2000X. It was taken at 2 mm (apical region), 8 mm (middle region), and 14 mm (cervical region) from the root apex.

Two examiners assessed the photos in a double-blind investigation. Scores were instituted based on the

properties of untreated dentin to instruct them during morphological analyses as follows [19]:

Score 1: Clean surface, no smear layer; open dentinal tubules, no modified organic matrix layer.

Score 2: Little smear layer, opened dentin tubules higher than 50%, little modified organic matrix layer.

Score 3: Intermediate smear layer, opened dentin tubules lower than 50%, modified organic matrix layer.

Score 4: Dense smear layer, total effacement of dentinal tubules.

All samples were analyzed by EDS to determine the amount of calcium (Ca), phosphorous (P) and oxygen (O) and also to assess the Ca/P ratio.

Statistical analysis

All data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 23. The quantitative data were presented as mean and standard deviations. Also, qualitative variables were presented as number and percentages. Exploration of the qualitative data was performed using Shapiro-Wilk test and Kolmogorov-Smirnov test for normality which indicated that all data originated from normal data. Comparison between two different groups was performed by using Independent t-test. All the comparisons in qualitative data were done by using Chi square test. The P-values less than 0.05 were considered significant and less than 0.01 were considered highly significant.

Results

Results of scanning electron microscope (SEM)

Comparison between different scores in each section Group A, in which diode laser 980 nm was directed parallel to the root dentin surface, regarding the coronal section, score 1 (86.7%) was significantly the highest, and in the middle section, score 3 (80%) was significantly the highest. However, in the apical section, score 4 (80%) was significantly the highest as shown in Table 1 and Fig. 1.

Group B, in which diode laser 980 nm was directed perpendicular to the dentin surface, regarding the coronal section, score 1 (86.7%) was significantly the highest, and in the middle section, score 1 (73.3%) was significantly the highest. While in the apical section, score 3 (73.3%) was significantly the highest as shown in Table 1 and Fig. 2.

Comparison between different sections in each score

Group A, in score 1, the coronal section (86.7%) was significantly the highest. In score 2, there was an insignificant difference among all sections. In score 3, the middle section (80%) was significantly the highest. In score 4, the apical section (80%) was significantly the highest as shown in Table 1 and Fig. 3.

Group B, in score 1, the coronal section (86.7%) and the middle section (73.33%) were significantly the highest. In score 2, there was an insignificant difference among all sections. In score 3, the apical section (73.3%) was significantly the highest. In score 4, there was 0% in all sections as shown in Table 1 and Fig. 3.

Comparison between different groups

In the coronal section, there was an insignificant difference between both groups at all scores as *P-value* greater than 0.05 as shown in Table 2.

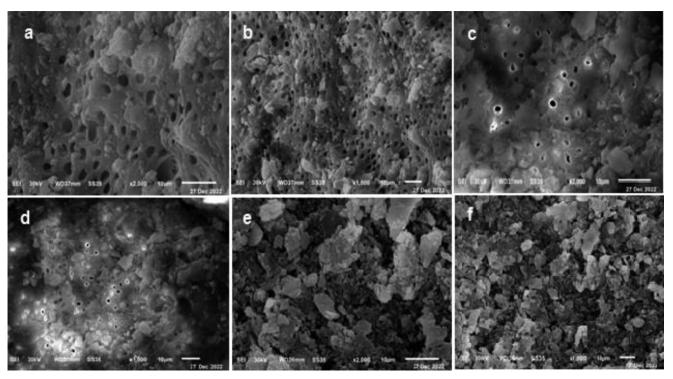
In the middle section, group A was significantly lower than group B in score 1. At the same time, group A was significantly higher than group B in score 3, but there was an insignificant difference between them in score 2 as shown in Table 2.

Table 1 Frequency and percentages of different scores among apical, middle, and coronal sections in both groups regarding SEM and comparison between different sections and different scores

Group	Section	Score 1 N (%)	Score 2 N (%)	Score 3 N (%)	Score 4 N (%)	P-value
Group A	Coronal	13 (86.7)	2 (13.3)	0	0	0.005 **
	Middle	0	3 (20.0)	12 (80.0)	0	0.020*
	Apical	0	0	3 (20.0)	12 (80.0)	0.020*
	P-value	<0.0001**	0.07	<0.0001**	<0.0001**	
Group B	Coronal	13 (86.7)	2 (13.3)	0	0	0.005**
	Middle	11 (73.3)	4 (26.7)	0	0	0.070
	Apical	0	4 (26.7)	11 (73.3)	0	0.070
	P-value	<0.0001**	0.37	<0.0001**		

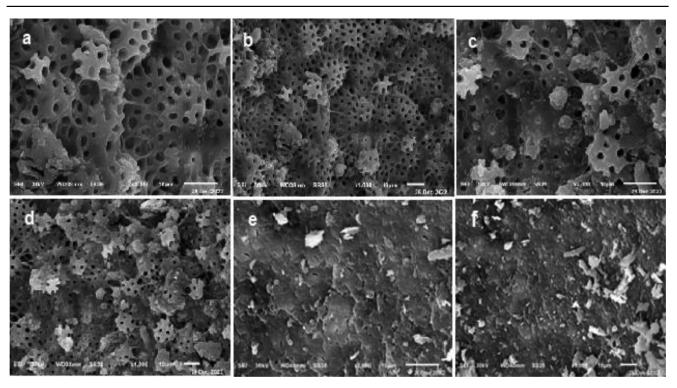
All data are expressed as frequency (N) and percentage (%). *Significant difference as P-value less than 0.05 using Chi square test. **Highly Significant difference as P-value less than 0.01 using Chi square test.

Figure 1



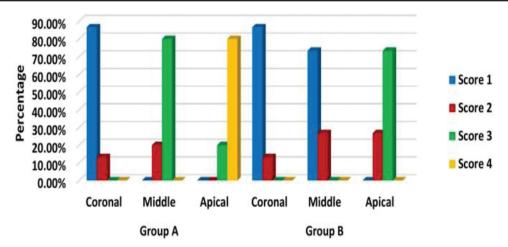
SEM photomicrograph of dentin of group A, where a): coronal third at X 2000, b): coronal third at X 1000, c): middle third at X 2000, d): middle third at X 1000, e): apical third at X 2000, and f): apical third at X 1000.

Figure 2



SEM photomicrograph of dentin of group B, where a): coronal third at X 2000, b): coronal third at X 1000, c): middle third at X 2000, d): middle third at X 1000, e): apical third at X 2000, and f): apical third at X 1000.

Figure 3



Bar chart showing percentages of different scores among apical, middle, and coronal sections in groups.

Table 2 Frequency and percentages of different scores among apical, middle, and coronal sections in both groups regarding SEM and comparison between different groups

Score	Group	Coronal N (%)	Middle N (%)	Apical N (%)
Score 1	Group A	13 (86.70%)	0 (0)	0 (0)
	Group B	13 (86.70%)	11 (73.30%)	0 (0)
	P-value	1.00	<0.0001**	
Score 2	Group A	2 (13.30%)	3 (20.00%)	0 (0)
	Group B	2 (13.30%)	4 (26.70%)	4 (26.70%)
	P-value	1.00	0.71	0.03*
Score 3	Group A	0 (0)	12 (80.00%)	3 (20.00%)
	Group B	0 (0)	0 (0)	11 (73.30%)
	P-value		<0.0001**	0.004**
Score 4	Group A	0 (0)	0 (0)	12 (80.00%)
	Group B	0 (0)	0 (0)	0 (0)
	P-value			<0.0001**

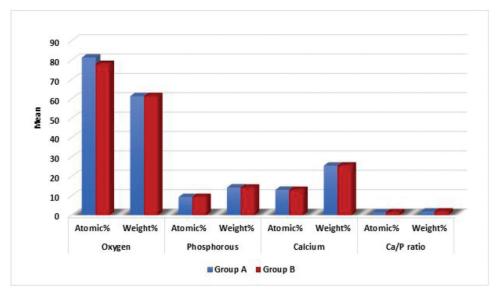
All data are expressed as frequency (N) and percentage (%). *Significant difference as P-value less than 0.05. **Highly significant difference as P-value less than 0.01.

Table 3 Mean and standard deviation of treated groups regarding oxygen, phosphorous, calcium and calcium/phosphorous ratio and comparison between them

				95% CI	
	Group A	Group B	P- value*	Lower	Upper
Oxygen					
Atomic%	81.32±5.80	77.95±6.30	0.13	-1.15	7.90
Weight%	61.39±2.39	61.33±3.26	0.94	-2.07	2.19
Phosphorous					
Atomic%	9.33±1.92	9.29±1.28	0.94	-1.18	1.26
Weight%	14.22±2.21	14.17±1.35	0.93	-1.31	1.41
Calcium					
Atomic%	13,02±2.84	12.97±1.37	0.95	-1.61	1.71
Weight%	25.51±1.98	25.47±2.13	0.95	-1.49	1.58
Ca/P ratio					
Atomic%	1.40±0.18	1.40±0.15	0.99	-0.12	0.12
Weight%	1.79±0.28	1.80±0.25	0.96	-0.20	0.19

All data are expressed as mean±SD. *Insignificant difference as P-value greater than 0.05 using Chi square test. CI, Confidence interval.

Figure 4



Bar chart showing mean of groups regarding oxygen, phosphorous, calcium and calcium/phosphorous ratio.

In the apical section, group A was significantly lower than group B in scores 2 and 3, while group A was significantly higher than group B in score 4 as shown in Table 2.

Results of energy dispersive X-ray spectroscopy (EDS)

Comparison between group A and group B revealed an insignificant difference between them as *P* greater than 0.05 at atomic and weight percentage in oxygen, phosphorous, calcium and calcium phosphorous ratio as shown in Table 3 and Fig. 4.

Discussion

Laser applications have been considered an effective complementary tool in endodontic treatment [20] as it aids in the root canal sterilization process [2] and smear layer removal [8].

The present research aimed to assess morphological variations and chemical changes in root dentin treated with diode laser 980 nm when applied in a parallel and a perpendicular direction.

In our study, water was used as an irrigant during mechanical preparation because water does not produce variations mechanically or chemically in root dentin characteristics or smear layer [21,22]. Therefore, only the morphological variations and chemical changes that occurred by laser on dentin structure could be assessed because Marending and colleagues [23] proved that sodium hypochlorite (NaOCl), when used as an irrigant produces

extensive variations of the dentin matrix, lowering flexural strength and changing the permeability of dentin. These changes were related to the degradation of the organic components of dentin. Subsequently, using any active chemical irrigants may submit fake results.

SEM analysis was used to assess morphological variations in root dentin after laser application because it gives a qualitative assessment of the morphological aspects [24].

In this study we considered the group in which the laser beam was directed parallel to the root canal dentin surface (Group A) as a control for a comparison with the group treated with perpendicular direction of laser beam (Group B) because the parallel direction was matching the condition seen in clinical practice, while the perpendicular direction of the laser beam was selected to assess the maximum impact of its wavelength on root dentin [25].

The results of our study showed that when diode laser 980 nm was used parallel to the dentin root surface in the coronal section, the smear layer was eliminated with opened dentinal tubules, in the middle section; there was an intermediate quantity from the smear layer and less than 50% of dentin tubules were opened. Furthermore, there was a dense smear layer and total effacement of dentinal tubules in the apical section. However, when diode laser 980 nm was used perpendicular to the dentin root surface in the middle and coronal sections, no smear layer was

found with opened dentinal tubules. In the apical section, there was an intermediate quantity of the smear layer, and lower than 50% of dentin tubules were opened. There was a loss of efficiency for the parallel diode laser application compared with the perpendicular application. This may be attributed to the limitations of the laser applicator through the optical fiber. In this case, the laser beams exist from its tip to inside the root canals, some of these laser beam rays have leaked through the whole root length, resulting in a dissimilar distribution of rays along the root [26-28]. These results were in accordance with Marchesan and colleagues [25] results, which proved that the most intense effect of laser beam was obtained when it applied on the root canal dentin in a perpendicular direction. Also with Wang and colleagues [29] who assessed the effect of diode laser 980 nm 5 W in a parallel direction on the morphology of root canal dentin and was concluded that the laser beam opened the dentinal tubules and removed the smear layer. Results of Saraswathi and colleagues [30] proved that the applying of diode laser 980 nm on root dentin produced changes diversified from dentin coalition to smear layer removal.

The smear layer considers as an impediment between root canal dentin and the filling material. This prevents root canal medications, irrigants, and sealer permeation through dentin tubules, which leads to a decrease in the bond strength of the sealer and an increase in microleakage [31]. The results of Alfredo and colleagues [32] proved that applying diode laser 980 nm on dentin after irrigation by 1% NaOCl followed by 17% Ethylenediaminetetraacetic acid (EDTA) improved the bond strength of AH plus sealer. These findings were due to morphological modifications occurred by laser in root canal dentin, as opening of dentinal tubules and removal of smear layer.

The morphological changes of root canal dentin are related to the temperature increase. This is affected by the used laser's frequency, output power and application mode [7,33], and also by photochemical, photothermal, photo disruption, and photoacoustic efficacy [34].

In our study, after application of diode laser 980 nm in both directions, the EDS analysis showed no chemical changes in root canal dentin components. These results may be attributed to the using of distilled water as an irrigant immediately before application of the diode laser 980 nm on the root canal dentin surface, which in turn absorbed the excess heat and thus maintained the inorganic content of dentin without affection [35]. As the increasing in the temperature during laser application leads to dissolving of the dentin apatite crystals, disintegration of its proteins and alteration of the Ca/P ratio [36]. Lopes and colleagues [7] proved that, there were no any chemical variations detected in root canal dentin after treatment with diode laser and also after treatment with distilled water after assessment with EDS analyzer. The results of Gutkneccht [37] were not in the same line with our results as he stated that the laser produced chemical changes on root canal dentin by total removal of organic content of the dentin and hence resulting in obliteration of dentinal tubules. This finding may be attributed to the using of different type of laser with different parameters as he used Nd:YAG laser. And also Azmy and colleagues [18] results, which stated that root canal dentin chemically changed after treatment with diode laser. This may be related to using diode laser with different wavelength.

Understanding the morphological changes in the intracanal dentin produced by laser application helps the dentist choose a suitable laser device to achieve the requirements of each clinical condition during root canal treatment to amend outcomes [25].

Conclusion

The present study concluded that, when used diode laser 980 nm in a perpendicular direction to the surface of root canal dentin, produces morphological changes more than when used in a parallel direction. The morphological changes on the root canal dentin surface ranged from occlusion of dentinal tubules to elimination of the smear layer. Moreover, there were no chemical changes detected in the root canal dentin components after application of laser in a perpendicular direction in comparison with the parallel direction.

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Conflicts of interest

There are no conflicts of interest.

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