

# Comparative SEM Study on the Effect of Irrigating the Radicular Dentine with NaOCl and EDTA Through Conventional Techniques and Diode Laser

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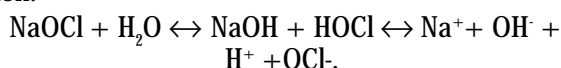
*Since there are not many references in literature about the action of diode laser 940nm on the dentin and root smear layer, the aim of this study is to evaluate and compare, through SEM analysis, the efficiency of the Sodium hypochlorite 2% (CERKAMED) - EDTA 17% (CERKAMED) combination, operated in the middle and apical third of the root, by conventional syringe needle EndoEze and diode laser 940nm treatment. The study was performed on 40 single-root teeth, which were divided into two groups: group A consisting of 20 teeth, for which irrigation was performed only with the syringe and a top open side needle EndoEze Irrigator, resulting in a turbulence moving upward which efficiently irrigates the root canal and prevents periapical water solution; and group B consisting of 20 teeth, which benefited from irrigation associated with diode laser 940nm treatment. In the middle third and apical third smear layer removal was more effectively achieved when teeth benefited from irrigation associated with the laser diode treatment. In Endodontics, Diode laser is a good decontamination system, with biostimulation effect, which is important in healing the periapical area. Significantly better results were obtained using 940nm laser diode associated with successive irrigation with 2% NaOCl and 17% EDTA, as compared to the conventional technique performed by syringe and lavage needle EndoEze Irrigator (Ultradent).*

**Keywords:** 940nm laser diode, irrigation, SEM analysis, Irrigator

Instrumentation of root canal during endodontic treatment leads to the formation of the smear layer (SL), which covers the dentin walls and has a thickness of approximately 1-2 microns. At the entrance of dentin tubules, this layer forms dentin plugs with a depth of approximately 40 microns. SL is comprised of inorganic and organic particles (vital or necrotic pulp debris, odontoblast prolongations, bacteria, red blood cells).

The 3-D root filling can only be performed once the SL layer has been removed. The most common method used in endodontic practice in order to remove SL consists in the combined irrigation of EDTA, which prevents formation and deposition of SL and NaOCl, which has an antibacterial role.

Sodium hypochlorite (NaOCl), the most common irrigant used in Endodontics has clinical efficacy due to its oxidative properties, to hydrolysis and osmotic tissue removal by fluid flow. Following contact with water, Sodium hypochlorite decomposes into sodium hydroxide and hypochlorous acid (HOCl), which in turn dissociate into ions, according to the reaction:



Aminoacids are neutralized by hydroxyl ions, while the hypochlorous acid and the hypochlorite ions degrade them and cause their hydrolysis by releasing chlorine, which, in combination with the protein amino group, form chloramine, which, in turn, interferes with cell metabolism.

Since NaOCl does not act on inorganic components, hence on SL, irrigation with chelating agents like EDTA should be associated. EDTA is synthesized from ethylenediamine, formaldehyde (methanol) and sodium cyanide.

EDTA is a carboxylic -poly -amino- acid with the formula:  $[\text{CH}_2\text{N}(\text{CH}_2\text{CO}_2\text{H})_2]_2$ .

Moreover, a 5 min irrigation with EDTA has the greatest benefits in chemical debridement and removal of the root canal SL [1].

Research studies have shown that the combination of NaOCl and EDTA is not as effective in removing SL from apical third as it is in coronal or medium third. This means that other more efficient therapeutic techniques need to be found. The use of lasers in Endodontics seems to be the key to getting better results by significantly reducing the risk of relapse.

Studies show that successful endodontic treatment is determined by the synergistic effect of laser irradiation and chemical root canals irrigation. However, using lasers without a previous sterilization protocol reduces the chances of increasing the therapeutic performance.

Laser diode (from 810 nm to 1.064nm) is used in therapy owing to its small size tips, low cost and easy operating settings, on the continuous wave or pulsed power.

Laser diode interacts mainly with soft tissue by diffusion and has a penetration depth of up to 3 mm. Laser diode 940nm (gallium aluminium arsenide Biolase) Epic Biolase emits continuous wave energy in the power range of up to 10W, has a fiber diameter of 400 µm and different tips; the most commonly used one in Endodontics is 200 µm in diameter and 14 -22 mm in length. The practitioner may randomly change the operation time.

Root canal instrumentation up to ISO 30 is needed in order to allow easy circular motion of the tips. The small tip size gives it easy access to the apical third.

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Since there are not many references in literature about the action of diode laser 940nm on the dentin and root smear layer, the aim of this study is to evaluate and compare, through SEM analysis, the efficiency of the Sodium hypochlorite 2% (CERKAMED)-EDTA 17% (CERKAMED) combination, operated in the middle and apical third of the root, by conventional syringe needle EndoEze and diode laser 940nm.

### Experimental part

The study was performed on 40 *single-root* teeth, extracted for orthodontic or periodontal reasons. All the teeth received the same endodontic treatment, which consisted in mechanical instrumentation by means of rotary instruments, associated with irrigation with sodium hypochlorite 2% (CERKAMED) and EDTA 17% (CERKAMED).

The teeth were then divided into two groups: group A consisting of 20 teeth, for which irrigation was performed only with the syringe and a top open side needle EndoEze Irrigator, resulting in a turbulence moving upward which efficiently irrigates the root canal and prevents periapical water solution; and group B consisting of 20 teeth, which benefited from irrigation associated with diode laser 940nm.

#### Teeth preparation protocol for the experiment

After extraction, teeth were decontaminated in 0.5% hypochlorite solution for 60 min, then they were kept 30 min under running water to remove tissue debris. The teeth were sterilized in a short cycle and then rehydrated for 24 h in saline.

#### Protocol of endodontic treatment

The preparation protocol included the following steps: performing access cavity with no. 2 round bur LA Axxess with long neck and diamond bur LA Axxess (Kerr Endodontics); accessing the root canals by means of dental probe; manual permeation by means of no. 10 K-file (Kerr Endodontics) and rotary instruments with the Reciproc R25 needle attached to a VDW Silver Reciproc (VDW Dental) low speed piece.

Irrigation was performed with 2% Sodium hypochlorite (CERKAMED) in a 5ml luer-lock syringe with 27 Ga (0.361 mm) diameter fitted with a lavage needle EndoEze Irrigator (Ultradent), as well as with 17% EDTA (CERKAMED) in a 2 mL luer-lock syringe fitted with a lavage needle EndoEze Irrigator (Ultradent).

#### Protocol of laser irrigation

Epic Bioase Diode laser 940 nm and endodontic tips of 200  $\mu$ m diameter and 22 mm length have been used. First,

sterile, single use tip needs to be checked to correctly transmit the light wave concentrated in one point. Working length minus 1 mm is measured on it, the mark is recorded with a stopper, easy movement of the tip into the channel is checked, throughout its entire length to avoid the fiber optic breaking into the channel (fig.1). The channel is irrigated with 2% NaOCl solution and 17 % EDTA.

The work technique consists in the apical-crown-wise introduction of the uninitialized inactive tip which will be activated when it has been inserted into the root canal. With slow circular motions, conducted on the entire length of the canal for 10 s, the entire canal is irradiated, with respect to the previously stopper-recorded mark which is 1mm less than the working length (fig. 2). It is to be emphasized that, before laser action, the root canals were dried with paper points. The irradiation procedure was repeated four times, with alternating succession of 2% NaOCl and 15% EDTA lavages, then drying and laser action again. The tip is then carefully cleaned with a sterile compress soaked with saline. Any debris resulting from preparation may be stored on the optical fiber, thus preventing the correct dissemination of energy. Accidental activation of the tip results in its replacement.

After mechanic and chemical preparation of the canal and laser therapy, one R 25 gutta point was inserted to protect the dentin. Under the magnifying glass, with diamond bur and under cooling, the root was transversally sectioned from medium third to the apex, and then longitudinally sectioned.

For SEM analysis, the samples were dehydrated, fixed on a STAB type aluminium support by pasting on a conductive copper tape (fig. 3 a, b). The new format was coated with a 9 nm gold layer, after having been introducing in the Quorum coater type, in order to carry out the conductivity test for 60 s (fig.4).

Each specimen from the examined groups was evaluated according to the 4-score system used by Ahmad and co-workers (1992).

- 0 = without smear layer and open dentinal tubules;
- 1 = reduced smear layer and 50% open dentinal tubules;
- 2 = mild smear layer and less of 50% open dentinal tubules;
- 3 = smear layer on large areas which locks dentinal tubules.

Apical and mid third were examined, data was statistically analyzed through Chi-square, statistical significance was  $p < 0.05$ .

### Results and discussions

According to the established scores, SEM analysis of the two groups is shown in figures 5-8.

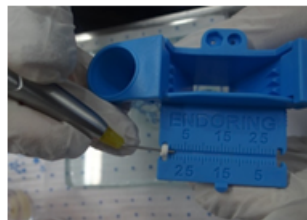


Fig.1 Measurement of the working length on the optical fiber of the laser

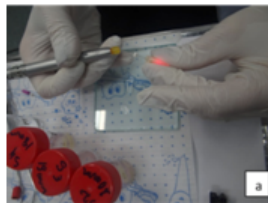


Fig. 2 Laser irradiation

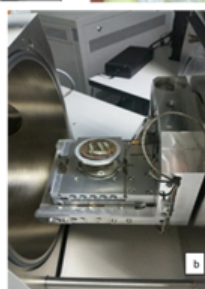


Fig. 3 (a, b) Sample investigation was performed by QUANTA INSPECT F scanning electron microscopy, equipped with a field emission gun (FEG), with a resolution of 1.2 nm, and energy dispersive X-ray spectrometer (EDS) with a 133eV resolution to MnK

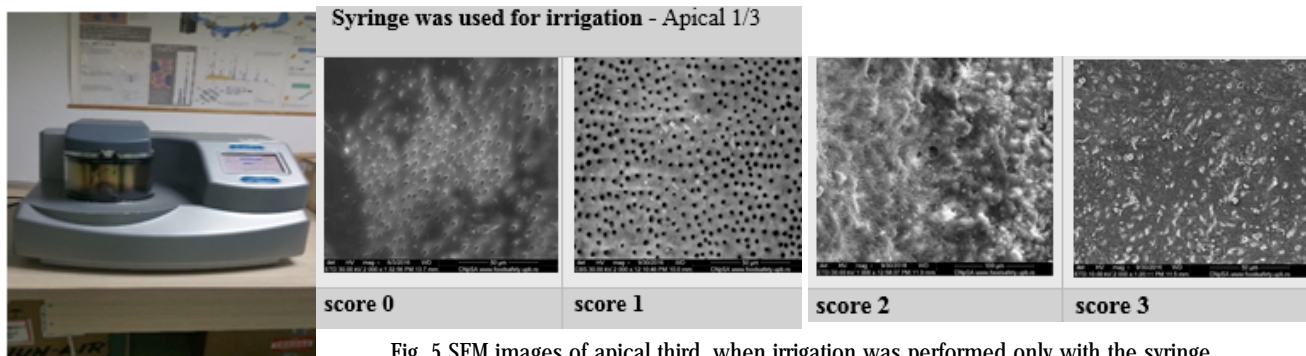


Fig.4 QUORUM type covered device, with a gold layer for sample conductivity test. It has variable time, depending on the layer type that should cover the sample

Fig. 5 SEM images of apical third, when irrigation was performed only with the syringe

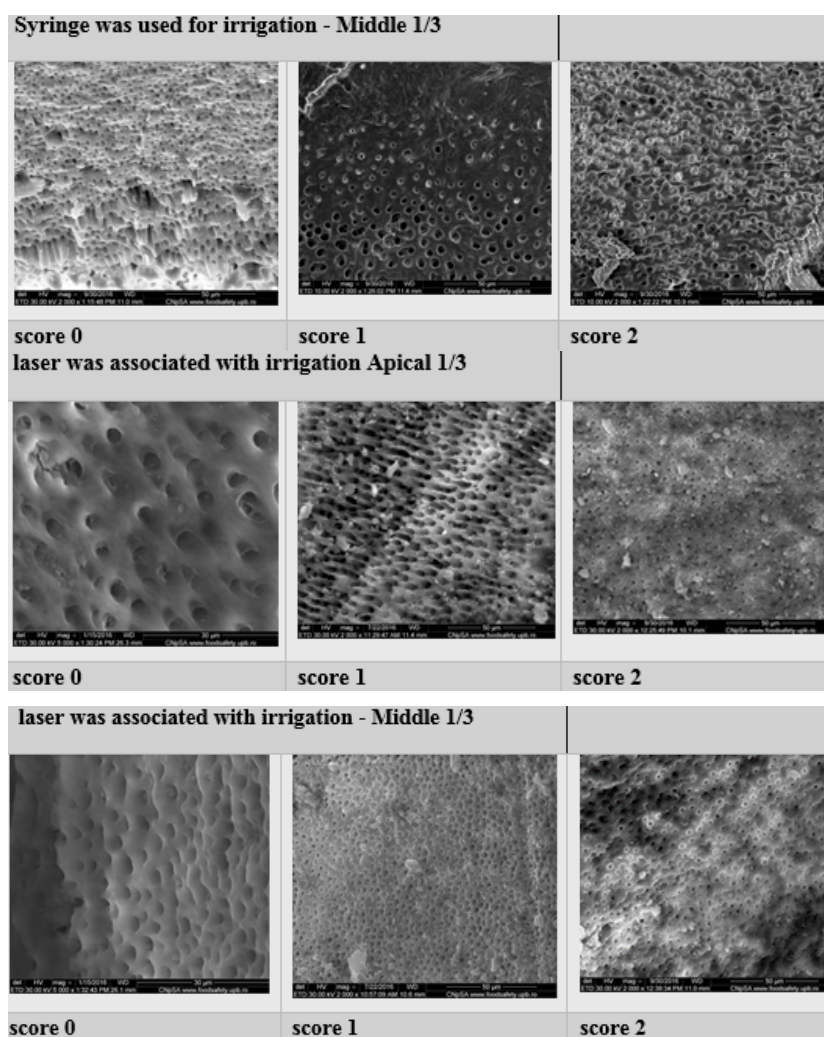


Fig. 6 SEM images of middle third, when irrigation was performed only with the syringe

Fig. 7 SEM images of apical third, when teeth benefited from irrigation associated with diode

Fig.8 SEM images of middle third, when teeth benefited from irrigation associated with diode laser 940nm.

Scores frequency distribution with regard to the smear layer presence and root canal opening in the 2 analyzed groups, is shown in table 1.

In the middle third, higher value for score 0 (40%) is observed in the group treated with laser and score 3 is absent in both groups. In the apical third, score 3 about 35% in group A prevails.

Chi-square test and statistical significance calculation showed that, in the middle third ( $P = 0.006$ ) and apical third ( $P = 0.04$ ) SL removal was more effectively achieved when teeth benefited from irrigation associated with the laser diode.

This study presents the association of Diode laser 940 nm and irrigation solutions in endodontic treatment. In

Endodontics, Diode laser is a good decontamination system, due to its ability to penetrate dentin walls (up to  $750\mu$  with laser 810 nm diode) and to destroy bacteria by means of the photo-thermal effect. Laser action on hydroxyapatite and water is almost zero, and that is why, in order to permeate dentinal tubules, lavage with EDTA and sodium hypochlorite solutions are needed. Application of laser emission in wet canals may dramatically affect permeation, because water is not absorbed. In addition, moist environment causes the endodontic tip to be initialized, thus concentrating the energy on its top.

Diode laser 940 nm has a biostimulation effect, which is important in healing the periapical area, by stimulating



**Table 1**  
SCORES FREQUENCY DISTRIBUTION

MIDDLE 1/3		No.	%	Medium intensity
Group A n=20	Scor 0	2	10.0	1.50±0.688
	Scor 1	6	30.0	
	Scor 2	12	60.0	
	Scor 3	0	0.0	
Group B n=20	Scor 0	8	40.0	1.00±0.918
	Scor 1	4	20.0	
	Scor 2	8	40.0	
	Scor 3	0	0.0	
Chi-Square		$\chi^2 = 14.583$		
Probability		0.006<0.05		

APICAL 1/3		No.	%	Medium intensity
Group A n=20	Scor 0	2	10	1.90±1.021
	Scor 1	5	25	
	Scor 2	6	30	
	Scor 3	7	35.0	
Group B n=20	Scor 0	7	35.0	1.50±1.318
	Scor 1	10	50.0	
	Scor 2	3	15.0	
	Scor 3	0	0.0	
Chi-Square		$\chi^2 = 2.8$		
Probability		0.0423<0.05		

cell proliferation and by inhibiting the propagation of the enzymes involved in the destruction of tissues.

Significantly better results were obtained using 940nm laser diode associated with successive irrigation with 2% NaOCl and 17% EDTA, as compared to the conventional technique performed by syringe and lavage needle EndoEze Irrigator (Ultradent).

The results are in agreement with those reported in the literature by other authors [2-5].

Recently, Lagemann et al. (2014) have shown that, when using the 940nm laser diode associated only with EDTA, the SL elimination is significantly superior compared with the conventional irrigation syringe technique. The risk of apical penetration by the irrigation solution is greatly reduced when using the laser diode which produces fluid movement by 4-5mm/s, compared to the erbium laser which causes a speed of about 30 mm/s [6-8].

Due to the small size of 940nm diode laser fiber, radiation can penetrate the apical third, an area that cannot be often accessed by conventional techniques.

The present study has shown that almost half of the studied cases, which have received laser treatment in mid third, show lack of the smear layer and opening of dentinal tubules, while, in the apical third, no case of the same category shows SL on extended areas (score 3).

The superiority of the laser diode as compared to syringe irrigation, especially in the apical third, has been reported in other studies [9-12].

The increased effect of the lasers on SL in the apical third is explained by the proximity of the tip to the tooth structure, resulting in the melting and evaporation of the SL. This effect is influenced by power of the laser employed. Some authors report that dentine melts at 3W as an effect of overheating [13].

The association of EDTA with diode laser 808 nm for 40 s may cause an increased reduction of dentin hardness compared to using EDTA alone [14].

In the present study, no morphologic changes of the dentin have been noticed, although the action took 40 s, because it has been performed with a power of only 1W.

The data agrees with Di Vito, who following microscopic examination showed that the collagen structure of the analysed samples is intact after laser application associated with 15% EDTA, a fact that suggests

the hypothesis of a minimum invasive treatment. The samples that have been treated for 20, respectively 40 s, show the complete removal of SL with open dentinal tubules and absence of thermal phenomena [15].

Other authors reported the presence of smear plug in apical third when a 7-watt diode was used with a 20s cycle [16].

Likewise, Faria MIA et al., in their study performed with Diode laser 980 nm, 1.5 W and 3W irradiation, showed the absence of SL on the one hand and the presence of partially obliterated tubules on the other hand.

The choice for the two power settings (1.5 and 3W), as well as the 100 Hz frequency was based on the results presented by Alfredo et al. who proved that these parameters resulted in a temperature increase of approximately 10° C, a value that is not above the threshold beyond which thermal damage may appear at the periapical thermal layer level [17, 18].

## Conclusions

SL removal was more effectively achieved when teeth benefited from irrigation associated with the laser diode.

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