


# Effects of different lasers on organic/inorganic ratio of radicular dentin

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**Abstract** The aim of this study was to evaluate the chemical stability of endodontic-treated root dentin after different laser irradiations through Raman spectroscopy. Fifty maxillary canines were selected and prepared with K3 system. Roots were randomly distributed into five groups ( $n = 10$ ) according to the surface treatment: GI (water), GII (NaOCl+EDTA), GIII (NaOCl+EDTA+980 nm Diode laser), GIV (NaOCl+EDTA+1064 nm Nd: YAG laser) and GV (NaOCl+EDTA+2780 nm Er,Cr: YSGG laser). Lasers were applied for 20 s. Samples were bisected, and the organic and inorganic content of dentin was analyzed by Raman spectroscopy. Data were submitted to ANOVA and Tukey tests ( $p < 0.05$ ). None of the surface treatments alter the inorganic content (cts) ( $p = 0.183$ ). Roots irradiated with Er,Cr: YSGG laser had a reduced collagen content (GV-290.7±41.7) compared with the water-treated roots (GI-328.3±63.5) and those treated with NaOCl+EDTA (GII-333.9±55.8). Roots irradiated with Er,Cr: YSGG laser also showed a higher inorganic/organic ratio (GV-9.5±1.1) than roots treated with water (GI-7.7±1.5), NaOCl+EDTA (GII-8.0±1.4) and diode laser (GIII-8.2±1.6). Both organic and inorganic contents increased from cervical to apical thirds in all groups. None of the surface treatments were able to promote changes in the inorganic

content of the root dentin; treatment with NaOCl+EDTA combined with Er,Cr: YSGG altered collagen.

**Keywords** Lasers · Raman spectroscopy · Root canal treatment

## Introduction

Technological advances have made possible new approaches to endodontic therapy with reduced operative time and greater safety and efficacy of treatment [1]. Laser technology has become an important tool when combined with endodontic treatment, providing the ability to operate in the root canal system. Different lasers have been used in endodontics such as erbium, neodymium, and diode lasers [2–8].

The 2780-nm Er,Cr: YSGG laser beam is highly absorbed by the components of dental tissues because of its affinity for water and hydroxyapatite [9]. This interaction is called ablation, a photothermal effect caused by the laser [10]. It promotes microbial reduction [5], removes the smear layer debris when applied to root canal walls [5, 9, 11], and increases dentin permeability [12].

In contrast, the 980-nm diode laser and 1064-nm Nd: YAG laser have a short wavelength and low absorption by hydroxyapatite and water [13], thus, high penetrating power and high absorption coefficient in the dark [14], with a consequent reduction in permeability [2, 3, 14, 15], besides removing the smear layer [16]. The 980-nm diode laser promotes exposure of dentinal tubules as well as surface modification, reducing microleakage [7, 17–19]. This laser also promotes microbial reduction in areas difficult to access, because it has a greater penetration capacity compared to other lasers [8, 19].

Alterations in morphology, permeability, hardness, and solubility of root dentin promoted by chemical solutions and high

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power lasers may be caused by changes in dentin microstructure [2, 20–23]. Different methods have been used to characterize the chemical composition of hard tissues, including Fourier transform infrared spectroscopy (FTIR) and Raman spectroscopy [20, 24, 25]. The key advantage of Raman spectroscopy is that it requires little to no sample preparation while the FTIR method has constraints on sample thickness, uniformity, and dilution to avoid saturation.

The effect of 980-nm diode laser irradiation on dentin microstructure is still unknown. Previous reports have evaluated Er:Cr:YSGG and Nd: YAG laser irradiation effects by different methods of microstructure analysis [11, 25–27]. However, the former studies do not evaluate the changes in the intensity of the spectra after laser irradiation used to endodontic therapy, which is usually performed by mechanical debridement in the presence of irrigation solutions in combination with chelating and auxiliary agents used with instrumentation.

Although several investigations have evaluated the effects of the lasers under study on mechanical properties and morphology of radicular dentin [2, 3, 6, 7, 12], there is still a lack of information regarding the effects of these laser irradiation treatments on radicular dentin components. Studies of chemical changes in the radicular dentin are necessary to determine clinical and adhesive procedures after the endodontic therapy. The purpose of this *ex vivo* study was to evaluate the chemical changes in root dentin of teeth treated with high power lasers combined with 1 % sodium hypochlorite (NaOCl) and 17 % ethylenediaminetetraacetic acid (EDTA) through the use of Raman spectroscopy.

## Materials and methods

The experimental protocol was approved by the ethical committee on human research of School of Dentistry of Ribeirão Preto, University of São Paulo (#24449713.6.0000.5419), and by the Institutional Research Boards, Case Western Reserve University, exemption file #EM-13-17. Maxillary canines, stored in 0.1 % thymol solution at 4 °C, were washed with running water for 24 h to remove thymol residues. The teeth were examined macroscopically and radiographed in a buccopalatal orientation in order to select canines with fully formed roots and a single canal without calcifications or curvatures. Roots were sectioned with a diamond saw, standardizing the root length at 17 mm.

Initially, 40 samples were irrigated and flushed with 2 ml of 1 % NaOCl. Exploration of the canal was performed with a hand file # 10 type K. The coronal third of the canals was preflared at low speed with LA Axxes drills

(SybronEndo, Orange, CA, USA), following the sequence: 35/06, 45/06. A manual file # 10 K-type stainless steel (Dentsply Maillefer, Ballaigues, Switzerland) was introduced into each canal until the tip was visible at the apical foramen. The working length was established by subtracting 1 mm from this length. Roots that had a diameter of 250 µm at the working length were selected.

Mechanical preparation of root canals was performed with K3® rotary instruments (SybronEndo, Orange, CA, USA) according to the following sequence: 25/.02, 25/.04, 30/.02, 30/.04, 35/.02, 40/.02, 45/.02, and finally 50/.02. The root canals were irrigated with 2 mL of 1 % NaOCl at each change of file and finally prepared with 5 mL of 17 % EDTA for 5 min. The canals then were irrigated with 2 mL of 1 % NaOCl and finally with 10 ml of distilled water. Ten other samples ( $n=10$ ) underwent the same process of mechanical preparation; however, distilled deionized water was used as the irrigation solution at all times.

The specimens were randomly distributed into five groups according to surface treatment: GI=irrigation with distilled water; GII=irrigation with 1 % NaOCl and 17 % EDTA; GIII=irrigation with 1 % NaOCl, 17 % EDTA, followed by irradiation with 980 nm diode laser (Sirona Dental Bensheim HE, Germany); GIV=irrigation with 1 % NaOCl, 17 % EDTA followed by irradiation with 1064 nm Nd: YAG laser (Deka, Florence, Tuscany, Italy); GV=irrigation with 1 % NaOCl, 17 % EDTA followed by irradiation with 2780 nm Er,Cr: YSGG laser (Biolase Technology, San Clemente, CA, USA).

The laser-treated specimens were irradiated for 20 s beginning with the tip at the working length, and performing a helical motion along the same to the cervical third, ultimately returning to the apex. GIII specimens ( $n=10$ ) were irradiated with a 980-nm diode laser through a flexible optical fiber of 200-µm diameter, 1.5 W of power and continuous mode. In GIV ( $n=10$ ), the specimens were irradiated with an Nd: YAG laser, wavelength of 1064 nm, using a quartz optical fiber having 250-µm diameter, with 1.5-W power and a repetition rate of 25 Hz. Before irradiation, the root canals of the specimens of the GIII and GIV were flooded with distilled deionized water, keeping them moist. GV ( $n=10$ ) were irradiated with the Er,Cr: YSGG laser, wavelength of 2780 nm, with a tip diameter of 275-µm diameter, and a length of 25 mm, 25-Hz frequency, power 1.5 W, and percentage of 10 % air and 10 % water of the cooling system equipment.

The samples were then bisected at low speed with the aid of diamond disc (KG Sorensen, Barueri, SP, Brazil), allowing analysis of chemical changes generated in root dentin after surface treatments.

## Analysis of the organic and inorganic composition by Raman micro spectroscopy

The contents of organic and inorganic phases of the dentin surface of the root canal were characterized by a Raman microscope (Horiba Jobin Yvon, Edison, NJ, USA). The intensity of  $\text{PO}_4^{3-}$  and  $\text{CH}_2$  peaks in the Raman spectrum are proportional to the amount of inorganic and organic, respectively, scattering Raman signals very strongly (Fig. 1). The organic phase of dentin is predominantly composed of collagen. Further analysis of the ratio between inorganic and organic compounds also was performed.

The light generated by the laser source had a wavelength of 785 nm. The resulting excitation point was about 10  $\mu\text{m}$  in diameter, and the laser penetration was about 100  $\mu\text{m}$ . Initially, the system was calibrated using the known peak of a Si wafer at  $520.7\text{ cm}^{-1}$ . Three measurements were performed in each root third of each hemisection, for a total of six spectra per root third for each specimen (Fig. 1). In order to statistically analyze the values of collagen, mineral, and mineral/collagen ratio, the average of these six measurements on each root third was utilized.

Measurements were obtained using 1200 lines/mm grating, which provided a wave number resolution of 1.25 pixels/ $\text{cm}^{-1}$ . Each spectrum was the average of 20 consecutive spectra, each collected for 4 s.

### Statistical analysis

The data obtained were examined for normal distribution (Shapiro-Wilk test,  $P > 0.05$ ) and homogeneity of variance (Levene test,  $P > 0.05$ ). Data of inorganic content, organic content, and inorganic/organic ratio were analyzed by one-way ANOVA in a split-plot arrangement with the plot represented by surface treatments and the subplot represented by

the root thirds. Tukey's test was used for multiple comparisons between groups and the level of probability was 95 % for all analyses.

## Results

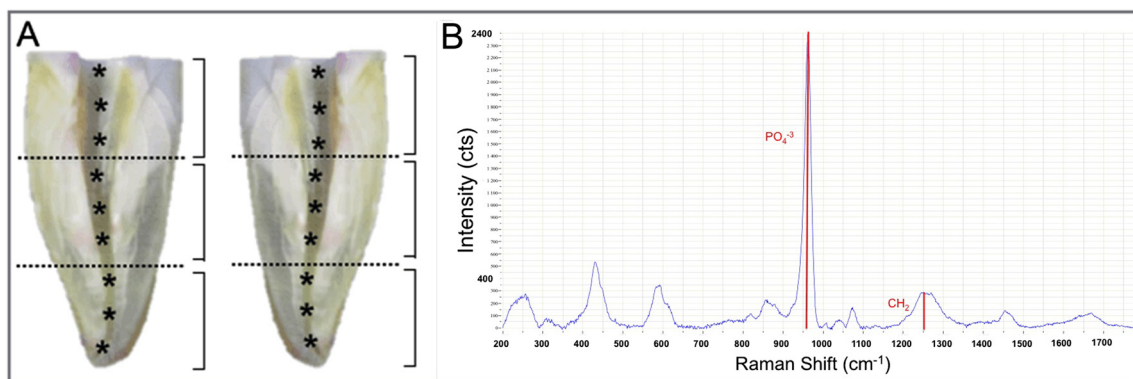
### Raman spectroscopy

A one-way ANOVA in a split-plot arrangement showed that inorganic content was not affected by surface treatments ( $P = 0.183$ ) and interaction between the surface treatments and root thirds ( $P = 0.256$ ). However, the inorganic content was significantly affected by the subplot root thirds ( $P < 0.001$ ).

Furthermore, the organic content was affected by surface treatments ( $P = 0.006$ ) and root thirds ( $P < 0.001$ ). However, the interaction between the surface treatments and root thirds did not affect the organic content ( $P = 0.207$ ). The Er,Cr:YSGG promoted the greatest change in collagen content compared to the groups treated with water and the group treated with 1 % NaOCl + 17 % EDTA ( $P < 0.05$ ). The groups treated with diode laser and Nd:YAG laser showed intermediate values ( $P > 0.05$ ) (Table 1).

Similarly, the inorganic/organic ratio was affected by surface treatments ( $P < 0.001$ ) and root thirds ( $P < 0.001$ ). However, the interaction between the surface treatments and root thirds did not affect the inorganic/organic ratio ( $P = 0.346$ ). The Er,Cr:YSGG laser irradiation resulted in higher values compared to the groups treated with water or the 1 % NaOCl + 17 % EDTA group or the diode laser-irradiated group ( $P < 0.05$ ) with intermediate values only for the Nd:YAG laser-treated group (Table 2).

Regarding root thirds, the statistic analysis showed that in the cervical third, mineral and collagen intensity peaks were lower than in the middle third, which in turn were lower than



**Fig. 1** a Photographs of hemisected root thirds on which *points of observation* are indicated by asterisks b Root dentin Raman spectrum— $\text{PO}_4^{3-}$  peak related to the mineral content and  $\text{CH}_2$  peak related to organic phase, which is rich in collagen

**Table 1** Means  $\pm$  standard deviations of organic peak intensity for each surface treatment condition

Surface treatment	Organic content
Water	328.3 $\pm$ 63.5 A
NaOCl + EDTA	333.9 $\pm$ 55.8 A
NaOCl + EDTA + 980 nm diode	303.8 $\pm$ 51.8 AB
NaOCl + EDTA + Nd: YAG	319.9 $\pm$ 30.4 AB
NaOCl + EDTA + Er,Cr: YSGG	290.7 $\pm$ 41.7 B

*Different letters* indicate significant differences between the experimental groups—Tukey test ( $p < 0.05$ )

in the apical third ( $P < 0.05$ ). Thus, the inorganic/organic ratio was lower for the cervical third compared to the middle and apical thirds ( $P < 0.05$ ), which had no difference between them ( $P > 0.05$ ) (Table 3).

## Discussion

This study determined the chemical changes in root canal dentin treated with diode, Nd: YAG, and Er,Cr: YSGG lasers. The effects of irradiation by different lasers have been studied in order to prove its effectiveness and reliability in endodontics, but the specific changes in organic and inorganic composition were not considered [2, 4, 6, 28].

The results obtained in our study by Raman spectroscopy showed that treatment with Er,Cr: YSGG laser significantly reduced the intensity of collagen peaks. The difference found between the lasers probably is due to the greater affinity of the Er,Cr: YSGG laser for water compared to the other systems, causing vaporization of water and other organic components such as hydrated collagen [11]. Conversely, the Nd: YAG laser and diode laser have short wavelengths, 1064 and 980 nm, respectively, and thus have a low absorption coefficient by hydroxyapatite and water [13]; treating the surface with these systems showed intermediate values.

However, the mineral/collagen ratio designed to measure the ratio of organic and inorganic components showed that the

**Table 2** Means  $\pm$  standard deviations of inorganic/organic ratio for each surface treatment

Surface treatment	Inorganic/organic ratio
Water	7.7 $\pm$ 1.4 A
NaOCl + EDTA	8.0 $\pm$ 1.3 A
NaOCl + EDTA + 980 nm diode	8.2 $\pm$ 1.6 A
NaOCl + EDTA + Nd: YAG	8.8 $\pm$ 1.4 AB
NaOCl + EDTA + Er,Cr: YSGG	9.5 $\pm$ 1.1 B

*Different letters* indicate significant differences between the experimental groups—Tukey test ( $p < 0.05$ )

980-nm diode laser was unable to elicit significant changes, matching the water-treated group and the group treated with 1 % NaOCl + 17 % EDTA. Comparative studies of the morphological changes using laser irradiation and different irrigating solutions on dentin showed that the morphological changes generated by the 980-nm diode laser are quite similar to those obtained by NaOCl combined with EDTA, and results in less intense changes compared to other lasers [3, 17]. It is noteworthy that studies on the chemical effects generated in the dentin surface of root canals irradiated with 980-nm diode laser have not been reported, but there were no significant chemical changes in the present study. The analysis of the mineral/collagen ratio confirms the significant reduction of organic compounds in roots irradiated with Er,Cr: YSGG laser when compared to other groups.

Our study showed that lasers did not change the inorganic compounds of the root dentin. This was probably due to the presence of water in the root canal at the time of irradiation, reducing heat generation due to excessive energy absorption, thus preserving the inorganic components [11]. The temperature increase of the dentin during irradiation by high-power lasers promotes vaporization of water, decomposition of proteins, and apatite crystals may melt and recrystallize rapidly, causing defects in the structure changing the Ca/P ratio [29]. Gurbuz et al. (2008) [25] showed that the mineral content of the root dentin was not affected after irradiation by Nd: YAG laser (1.5 W). Topcuoglu and Koseoglu (2015) [26] studied by atomic emission spectrometry the effect of Er: YAG (1 W) and Nd: YAG (1 W) lasers combined with 1 % NaOCl and determined that these lasers do not affect the mineral content. Dilber et al. (2013) [30] showed that irradiation of the dentin with the Er: YAG, Nd: YAG, and KTP lasers did not alter the concentration of Ca, K, Mg, Na, P, or mineral Ca/P ratio of the dentin surface.

Altundasar et al. (2006) [11] and Secilmis et al. (2008) [27], using Er,Cr: YSGG laser at 3 and 1 W, respectively, found alterations in chemical elements Ca, P, Mg, and Na, and no alteration on Ca/P ratio of dentin after irradiation, suggesting that the changes are confined to the molecular level. The differences in the evaluation method, the power of the lasers used, and the concentration and action time of the solutions make it difficult to compare these studies with ours.

The outcomes of our study indicated that irrigation with 1 % NaOCl followed by 17 % EDTA did not change the composition of the root dentin. However, there was a variation in the proportion of organic and inorganic compounds from the root thirds in all groups, including the control (water-treated group). The mineral/collagen ratio is lower in the cervical third compared to other portions, with a lower degree of mineralization. This was probably caused by the higher number of tubules in the cervical third [31]. The difference in the chemical characteristic between root thirds interferes directly with restorative procedures. According to Neelakantan et al. (2012)

**Table 3** Means  $\pm$  standard deviations of the inorganic content (cts), organic content (cts) and mineral/collagen ratio in the different root thirds considering all experimental groups

Radicular Third	Inorganic content	Organic content	Inorganic/organic ratio
Cervical	2262.0 $\pm$ 395.6 A	289.1 $\pm$ 28.5 A	7.7 $\pm$ 1.1 A
Middle	2681.3 $\pm$ 691.6 B	308.9 $\pm$ 41.0 B	8.6 $\pm$ 1.6 B
Apical	3047.3 $\pm$ 677.3 C	347.9 $\pm$ 62.1 C	8.8 $\pm$ 1.5 B

*Different letters (capital letters, comparison among columns) indicate statistical difference ( $P < 0.05$ )*

[32], the adhesion of epoxy resin-based sealers is smaller in the apical region.

In order to properly determine the desired surface treatment for each clinical situation, it is important to understand the chemical changes induced by different surface treatments used in endodontic therapy. Further studies should be conducted to assess the real advantages of using associations of high-power laser systems with irrigating solutions in order to establish the optimal protocol for each case.

## Conclusions

Radicular dentin treated with irrigating solutions combined with Er,Cr: YSGG laser undergoes chemical changes, with reduced amount of collagen, thus increasing the mineral/collagen ratio: surface treatment can directly interfere in dentin permeability and with the quality of bond strength of the root canal filling materials. Clinical studies should be carried out to determine whether the alterations revealed by this study have practical consequences, especially in regard to the longevity of endodontic sealers.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no competing interests.

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