

Research Article



Mineral content analysis of root canal dentin using laser-induced breakdown spectroscopy

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Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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ABSTRACT

Objectives: This study aimed to introduce the use of laser-induced breakdown spectroscopy (LIBS) for evaluation of the mineral content of root canal dentin, and to assess whether a correlation exists between LIBS and scanning electron microscopy/energy dispersive spectroscopy (SEM/EDS) methods by comparing the effects of irrigation solutions on the mineral content change of root canal dentin.

Materials and Methods: Forty teeth with a single root canal were decoronated and longitudinally sectioned to expose the canals. The root halves were divided into 4 groups ($n = 10$) according to the solution applied: group NaOCl, 5.25% sodium hypochlorite (NaOCl) for 1 hour; group EDTA, 17% ethylenediaminetetraacetic acid (EDTA) for 2 minutes; group NaOCl+EDTA, 5.25% NaOCl for 1 hour and 17% EDTA for 2 minutes; a control group. Each root half belonging to the same root was evaluated for mineral content with either LIBS or SEM/EDS methods. The data were analyzed statistically.

Results: In groups NaOCl and NaOCl+EDTA, the calcium (Ca)/phosphorus (P) ratio decreased while the sodium (Na) level increased compared with the other groups ($p < 0.05$). The magnesium (Mg) level changes were not significant among the groups. A significant positive correlation was found between the results of LIBS and SEM/EDS analyses ($r = 0.84$, $p < 0.001$).

Conclusions: Treatment with NaOCl for 1 hour altered the mineral content of dentin, while EDTA application for 2 minutes had no effect on the elemental composition. The LIBS method proved to be reliable while providing data for the elemental composition of root canal dentin.



Keywords: Dentin; Endodontics; Microscopy; Spectrum analysis

INTRODUCTION

Root canal irrigation is an essential part of endodontic treatment that provides the removal of tissue remnants and eradication of microorganisms during instrumentation [1]. After mechanical instrumentation, the dentin surface is covered with a smear layer, which is composed of both organic and inorganic materials. To remove the smear layer completely, the sequential use of sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) solutions has been recommended as an effective irrigation regimen. As a proteolytic

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agent, NaOCl denatures organic components of the smear layer, while EDTA demineralizes the inorganic components via calcium (Ca) chelation [2].

The use of root canal irrigants may alter the chemical composition of dentin [3,4], by the removal of Ca and phosphorus (P) present in hydroxyapatite crystals, which are the major inorganic components of dentin [5,6]. The alterations in the Ca/P ratios may change the permeability, microhardness, and solubility characteristics of dentin and may also adversely affect the adhesion of filling materials to dentin [7-9]. Moreover, the change in dentin composition may cause endodontically treated teeth to be more prone to fracture [10].

The mineral content of root canal dentin has been evaluated using various methodologies including X-ray diffraction analysis [11], inductively coupled plasma-atomic emission spectrometry [4], wavelength dispersive X-ray fluorescence spectrometry [12], or scanning electron microscopy/energy dispersive spectroscopy (SEM/EDS) analysis [3,13]. SEM/EDS analysis is a well-established method, which is based on the energy emitted in the form of X-ray photons when the electrons from external sources hit the atoms in a material, thus generating characteristic X-rays of that element [13,14]. This method allows fast and quantitative microanalysis estimating the amounts of mineral in a given tooth sample in a non-destructive manner [13,14].

Laser-induced breakdown spectroscopy (LIBS) is another technique for elemental analysis, in which a small amount of sample is ablated by high-energy laser pulse [15-17]. Due to the high temperature of the plasma, sample breakdowns into atoms, which emit light of characteristic wavelengths. The emission spectrum is collected with fiber optics and directed into the spectrometer. Thus, the spectral signals are constituted and multi-elemental analysis is performed [18]. In this technique, no or minimum sample preparation is necessary and there is no limitation in sample size and shape [15]. LIBS has the unique property that allows depth profiling by mapping and performing layer-by-layer analysis, thus providing spatial distribution profiles of the targets [15]. LIBS can provide rapid and *in situ* multi-elemental analysis [18]. Moreover, it is easy to use and relatively cost effective [18].

The use of the LIBS technique in dentistry is limited to anthropology studies [15,19] and the studies that analyzed elemental distribution in carious teeth [16,17]. The LIBS technique can also provide valuable information in the field of endodontics. However, no study has evaluated the efficacy of LIBS on the elemental analysis of root canal dentin. Therefore, the purpose of this study was to introduce a new method for assessing mineral content of root canal dentin *ex vivo*, and to assess whether a correlation exists between the LIBS and SEM/EDS analyses by comparing the effects of irrigation solutions on the mineral content change of root canal dentin.

MATERIALS AND METHODS

Specimen preparation

Following ethics committee approval, 40 single rooted freshly extracted human teeth with a single root canal were obtained for this study. The soft tissues covering the root surfaces were removed with gauze and a fine brush. The teeth were decoronated and pulp tissues were removed with barbed broaches. Then, the teeth were longitudinally sectioned to expose the root canals. The root halves were placed in an ultrasonic bath containing distilled water for

10 minutes to remove any remaining soft tissue. Thereafter, the root canal area of each root half was brushed with a microbrush (SDI, Bayswater, Victoria, Australia) for 2 minutes to create smear layer on each surface. The specimens were divided into 4 groups by allocating each root half belonging to the same root to the same group ($n = 10$). Twenty root halves were served as the control group and did not receive any further treatment. The remaining root halves were then immersed in the solutions as follows:

Group NaOCl: 10 mL of 5.25% NaOCl for 1 hour.

Group EDTA: 5 mL of 17% EDTA for 2 minutes.

Group NaOCl+EDTA: 10 mL of 5.25% NaOCl for 1 hour, then rinsed with 5 mL of distilled water, and immersed in 5 mL of 17% EDTA for 2 minutes.

Final irrigation was performed with 5 mL of distilled water in each group. Each root half belonging to the same root was divided into 2 subgroups according to the analysis method to be applied.

SEM/EDS analysis

Following sputter coating with gold in Ion Sputter Coater (Bal-Tec SCD 005, Leica Microsystems, Nussloch, Germany), the specimens were mounted on SEM (JSM-6400, JEOL Ltd., Tokyo, Japan) equipped with EDS (Oxford Inca X-Sight, Abingdon, Oxfordshire, UK). For the analysis, the system was operated at 20 kV, with a constant working distance. The spectra of atomic composition of the specimens' surface were obtained by spot SEM/EDS analysis from three spots located at 2, 5, and 8 mm from the root apex to represent apical, middle, and coronal thirds of the root canal, respectively. The levels of Ca, P, magnesium (Mg), and sodium (Na) elements in the root dentin surface of each specimen were measured. The EDS software automatically evaluated relative contribution of each of the detected elements within the region of interest to a total of 100%. Three different measurements were done for each root third, and the mean value was calculated to represent the respective root third. The value of each root third for each specimen was recorded and the resulting mean value was accepted as representative of root canal area.

LIBS analysis

The LIBS experiments were conducted using an Nd:YAG laser (Litron Nano SG, Litron Lasers, Warwickshire, England) which emits a laser pulse at 1,064 nm with an output energy of 150 mJ and 5 channel Aurora LIBS spectrometer (Applied Spectra, Fremont, CA, USA) which records the spectrum between 186 and 888 nm. **Figure 1** shows the experimental setup of the LIBS system. The laser was operated in Q-switched mode at a repetition rate of 8 Hz and 40 mJ/pulse while the spectrometer was operated at 650 ns gate delay and 1.05 ms integration time. The samples were scanned with the laser at 2, 5, and 8 mm from the root apex to represent apical, middle, and coronal thirds of the root canal, respectively, and 4 shots were obtained from each root third in order to examine the elemental composition of the samples. The first shot was used to clean the sample surface. The mean value of 3 shots was calculated for each root third, as described in SEM evaluation. Then, the value of each root third for each specimen was recorded and the resulting mean value was accepted as representative of root canal area.

Since LIBS spectra contain large number of data with high dimensionality, only characteristic emission lines of Ca, P, Mg, and Na elements were taken into consideration. To this end, the peak intensity of Ca II at 393.4 nm, P I at 213.5 nm, Mg I at 279.5 nm, and Na I at 589.5

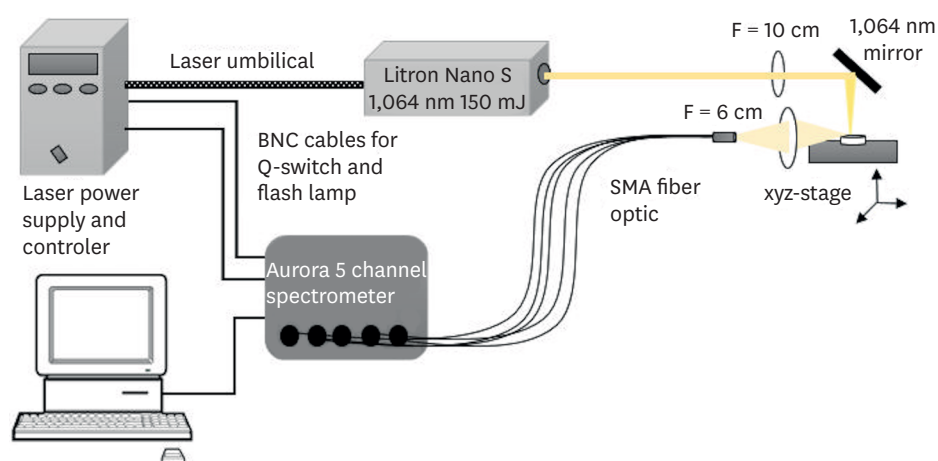


Figure 1. Schematic diagram of experimental setup for the laser-induced breakdown spectroscopy (LIBS) system. BNC, Bayonet Neill–Concelman; SMA, shape memory alloy.

nm were confirmed with the help of National Institute of Standards and Technology (NIST)-atomic spectra database [20] and utilized for the analysis.

To compare the LIBS results with those obtained with the SEM/EDS analysis, the data of the experimental groups were proportioned to the control group for each analysis. By this way, the results of the analyses were compared in terms of the change in the elemental distribution of root canal dentin after the application of the irrigants.

Normalization of analyses data

Atomic % concentration values of the elements were obtained with the SEM/EDS analysis, while intensity (atomic unit) values of the elements were obtained with the LIBS analysis. Because the measurement unit of each analysis was different, normalization of data was performed to test the correlation statistically between the analyses. Therefore, the values obtained from both analyses were normalized between 0 and 1 according to following formula:

$$X' = \frac{X - X_{min}}{X_{max} - X_{min}}$$

Statistical analysis

Data were statistically analyzed for normal distribution using the Shapiro-Wilk test. The mineral content change between the groups for the SEM/EDS analysis was analyzed with one-way analysis of variance and Bonferroni correction for pairwise comparisons ($p = 0.05$). Following the normalization of data, the correlation between the SEM/EDS and LIBS methods was statistically analyzed using Pearson's correlation coefficient. The level of significance was set at $p = 0.05$. All statistical analyses were performed using SPSS software (SPSS 22 for Windows, SPSS Inc., Chicago, IL, USA).

RESULTS

SEM/EDS analysis

The results of the SEM/EDS analysis regarding the mineral content of the teeth in each experimental group are presented in **Table 1**. In groups NaOCl and NaOCl+EDTA, the Ca/P level decreased, while the Na level increased, compared with the other groups ($p < 0.05$).

Table 1. Mineral content values of the groups (atomic concentration, %)

Group	Ca	P	Mg	Na	Ca/P
Control	58.390 ± 1.265	36.491 ± 0.829	2.647 ± 0.346	2.472 ± 0.474	1.603 ± 0.074
NaOCl	55.264 ± 0.578*	37.647 ± 0.401*	2.541 ± 0.205	4.549 ± 0.789*	1.468 ± 0.022*
EDTA	57.821 ± 1.246	36.427 ± 0.948	2.643 ± 0.357	3.109 ± 0.644	1.593 ± 0.073
NaOCl+EDTA	55.601 ± 1.411*	36.547 ± 0.533	2.797 ± 0.398	5.053 ± 0.882*	1.523 ± 0.060*

The data were shown as mean ± standard deviation.

Ca, calcium; P, phosphorus; Mg, magnesium; Na, sodium; group NaOCl, 5.25% sodium hypochlorite (NaOCl) for 1 hour; group EDTA, 17% ethylenediaminetetraacetic acid (EDTA) for 2 minutes; group NaOCl+EDTA, 5.25% NaOCl for 1 hour and 17% EDTA for 2 minutes.

*The difference between the experimental group and the control group is significant ($p < 0.05$).

There was no significant difference between the elemental composition of group EDTA and the control group. The Mg level changes were not significant among the groups ($p > 0.05$).

LIBS analysis

The wavelengths of the identified elements in the LIBS spectra of root canal dentin are shown in **Table 2**. The LIBS spectra of the groups presented a collection of characteristic emission lines of Ca II, P I, Mg I, and Na I (**Figure 2**). According to this spectrum, the experimental groups showed similar spectral intensity of Ca II compared with the control group. The spectral intensity of P I was higher in group NaOCl than the control group, while groups EDTA and NaOCl+EDTA presented a similar peak intensity compared with the control

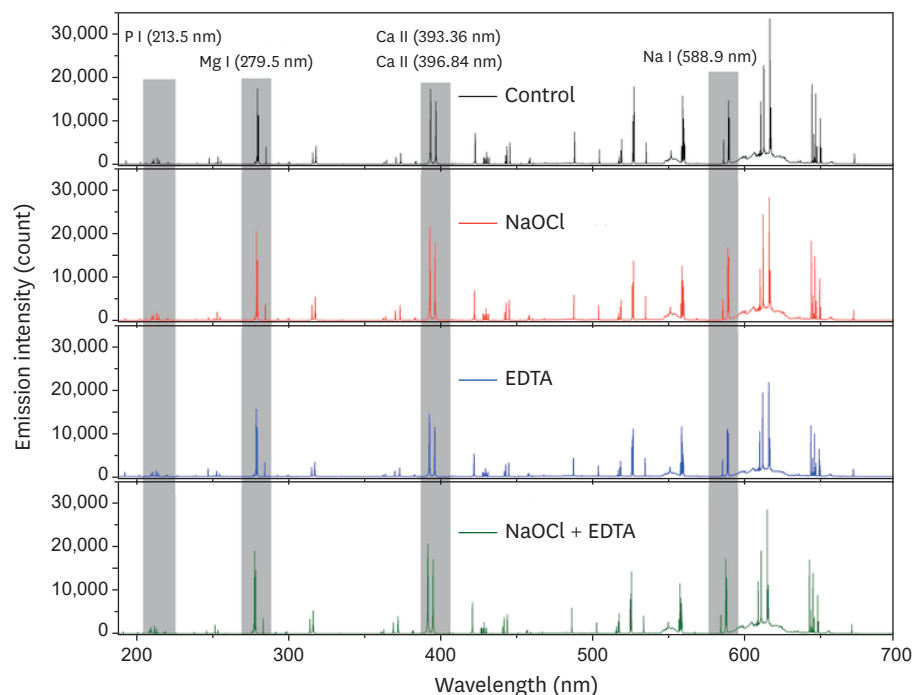


Figure 2. Laser-induced breakdown spectroscopy (LIBS) spectra for the groups. Ca, calcium; P, phosphorus; Mg, magnesium; Na, sodium; NaOCl, sodium hypochlorite; EDTA, ethylenediaminetetraacetic acid.

Table 2. Identified elements in the laser-induced breakdown spectroscopy (LIBS) spectra of root canal dentin and their observed wavelength

Identified element	Observed wavelength (nm)
Calcium (Ca)	210.3, 211.2, 315.8, 317.9, 370.6, 373.6, 393.3, 396.8, 422.6, 428.3, 430.2, 442.5, 443.5, 445.5, 458.5
Phosphorus (P)	213.5, 214.9, 215.3, 253.5, 255.3
Magnesium (Mg)	277.9, 279.5, 279.7, 280.2, 285.2, 383.2, 383.8
Sodium (Na)	588.9, 589.5

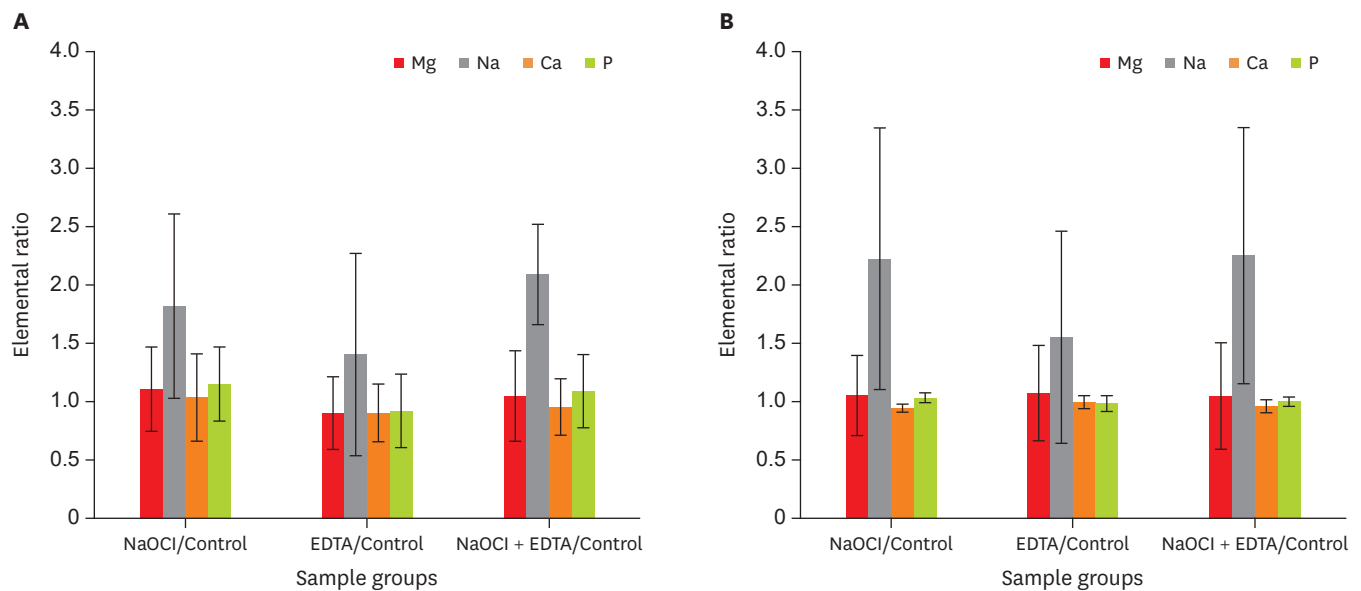


Figure 3. The change in the elemental distribution of the experimental groups compared with the control group. (A) Laser-induced breakdown spectroscopy (LIBS) analysis, (B) scanning electron microscopy/energy dispersive spectroscopy (SEM/EDS) analysis.

Ca, calcium; P, phosphorus; Mg, magnesium; Na, sodium; NaOCl, sodium hypochlorite; EDTA, ethylenediaminetetraacetic acid.

group. The spectral intensity of Na I was higher in groups NaOCl and NaOCl+EDTA than the control group, while all groups exhibited similar spectral intensity of Mg I. The change in the elemental distribution of the experimental groups compared with the control group is shown in **Figure 3**, for each analysis. Accordingly, the LIBS and SEM/EDS results of the experimental groups exhibited similar elemental distribution pattern with respect to the control group.

Following the normalization of experimental data sets, a significant positive correlation was found between the results of the LIBS and SEM/EDS analyses ($r = 0.84$, $p < 0.001$).

DISCUSSION

In the present study, the mineral content change of root canal dentin after applying different irrigation solutions was evaluated using the LIBS method. Previously, this method was found to be useful in the detection and monitoring of a wide range of elements in human teeth [21]. On the other hand, there is no available data regarding the application of LIBS on the mineral content analysis of root canal dentin. Since the present study is the first to use LIBS in endodontics, one root half was analyzed with LIBS, while the other root half of the same tooth was examined with SEM/EDS, in order to validate the LIBS analysis results. The SEM/EDS analysis was chosen as a standard technique for comparison, because it provides spatial distribution of elements with little or no sample preparation [13], similar to the LIBS analysis [15]. The present findings indicate that the LIBS results exhibit significant correlation with those obtained from the SEM/EDS analysis. Similarly, previous studies also indicated a strong correlation of LIBS analysis in comparison to other analytical techniques applied in determination of elements in herbs, plants, or fruits [22-24].

Mineral contents of dentin vary depending on the anatomical location of dentin tissue samples [25]. Therefore, separate measurements from each root third were performed for both analyses

to determine the mean contents of the root canal surface and not of one single point. To obtain high signal quality and optimize the signal to noise ratio, the parameters of the LIBS analysis including laser repetition rate, energy, and gate delay were determined before starting the experiments. Each line of the spectral peaks was corrected with NIST-atomic spectra database. Although the LIBS analysis provided valuable information on the elemental distribution of root canal dentin, the absence of calibration data set with known elemental concentration was a limitation of this particular work. The LIBS analysis is unable to produce quantitative results without calibration and it is relatively hard to obtain a sample group to construct a calibration model, especially in samples like teeth. Therefore, it was impossible to obtain quantitative results for the elemental concentration of the root canal dentin in terms of weight, volume, or mass. However, to overcome this limitation, a normalization procedure was applied to the LIBS and SEM/EDS data sets and thus the results that obtained by the analyses could be compared. This procedure confirmed the validity of the LIBS technique. Furthermore, the change in the elemental distribution of the experimental groups with respect to the control group presented similar patterns in both analyses, indicating a positive correlation between them. Of note, there may be an alternative way to overcome the above limitation, which is the use of calibration free LIBS (CF-LIBS) technique. In this approach, quantitative results can be obtained without a calibration data set by measuring the plasma temperature and electron density [26]. However, the CF-LIBS technique is relatively new and the ability of this technique to quantify the elemental concentration of the root canal dentin can be assessed in future studies.

As a common procedure, the root canal system is flooded with 1%–6% NaOCl during the entire period of root canal treatment to enhance its tissue dissolution and antimicrobial effects, while 17% EDTA solution is used as a final rinse for several minutes to remove smear layer from the canal walls [10,27]. Thus, 10 mL of 5.25% NaOCl and 5 mL of 17% EDTA were used as irrigants in the current study and the application durations were 1 hour for the NaOCl solution and 2 minutes for the EDTA solution to simulate the clinical situation. The specimens were immersed in the solutions in the current study, thus all surfaces were exposed to the irrigants which does not reflect the exact clinical situation. However, this method allowed standardization in the treatment time and volume of the solutions.

Earlier studies indicated that root canal irrigants cause alterations in the chemical and structural composition of dentin, similar to the present findings [27-29]. Dentin is composed of approximately 22% organic material, consisting of type I collagen mostly [27]. Since NaOCl is a non-specific proteolytic antimicrobial agent, the exposure of dentin to NaOCl could lead to undesired changes in dentin structure. In several studies, the change in mechanical properties of dentin after exposure to different concentrations of NaOCl was tested, and NaOCl irrigation was associated with a reduction of elastic modulus and flexural strength of dentin [27-29]. It is worth to mention that the negative effect of NaOCl on the mechanical properties of dentin was generally explained with its ability to dissolve the organic component, whilst the inorganic component of dentin was accepted to remain intact [29]. On the other hand, different studies have shown that NaOCl treatment might decrease [4,12] or increase the inorganic content of dentin [3,30]. In the present study, 1 hour exposure of root canal dentin to 5.25% NaOCl resulted in significant decrease in the Ca/P ratio. It can be assumed that different results in the literature are caused by the concentration- and time-dependent effects of NaOCl on root canal dentin [10].

Based on the present results, the use of EDTA alone did not significantly change the Ca and P levels compared with the control group. Contrarily, some studies have found a decrease in

the inorganic content of dentin after the application of EDTA alone [4,13,31]. The decalcifying efficacy of EDTA depends on the application time and the pH of the solution [2,32]. Reportedly, the greatest demineralizing efficiency of EDTA is achieved between pH 5 and 6 [32]. In the present study, an EDTA solution which had a neutral pH was used and left in contact with the specimens for 2 minutes. In line with the present results, there were studies that indicated a stable Ca/P ratio after the application of EDTA alone [3,12,33]. It was suggested that the organic part of dentin plays a critical role during the decalcification process of EDTA [34]. When EDTA is used as the only irrigation solution, the accumulated organic component on dentin surface may limit the penetration of EDTA to the inorganic part, preventing its demineralizing ability [35].

Trace amounts of Mg and Na are usually present in the apatite crystals [3,13]. Although little is known about the role of these minerals, the change in the levels of Mg and Na was also analyzed in the present study. The Mg level was not affected after the treatment with the irrigants, as in the previously reported study [4]. On the other hand, a significant increase in the Na level was detected in groups which NaOCl was used. NaOCl exhibits its mechanism of action by dissociating into ions and this may explain the increase in the Na level on dentin surface after the treatment with the solution for 1 hour.

CONCLUSIONS

Within the limitations of the present study, it can be concluded that the treatment with 5.25% NaOCl for 1 hour altered the mineral content of root canal dentin, while 17% EDTA application for 2 minutes had no effect on the elemental composition. The LIBS analysis presented a significant positive correlation with the SEM/EDS analysis, and proved to be a reliable method while providing data for the elemental composition of root canal dentin. The LIBS method can be used to analyze the effect of root canal irrigants on the mineral content change of the deeper layers of root canal dentin in future studies.

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