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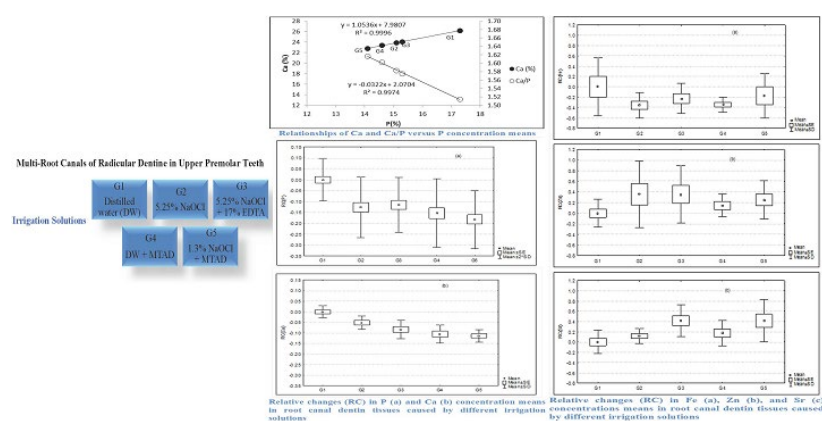
Effects of Different Irrigation Solutions on the Chemical Composition of Multi-Root Canals of Radicular Dentine in Upper Premolar Teeth

Morgana Quteifani Quteifan * [ID] [a] and Ali Khuder [ID] [b]

The objective of this research was to assess the effects of several irrigation solutions (ISOLs) on two major (P and Ca) and some minor (Fe, Zn, and Sr) elements of multi-root canals of radicular dentin in upper premolar teeth using X-ray fluorescence analysis (XRF). Powder of fifty radicular dentin specimens were prepared and divided into five groups based on treatments with different single and combined ISOLs. These groups were: G1-distilled water (control); G2- sodium hypochlorite (NaOCl, 5.25%); G3- sodium hypochlorite (NaOCl, 5.25%) and ethylendiaminetetraacetic acid (EDTA, 17%); G4- distilled water and a mixture of tetracycline, an acid, and a detergent (MTAD); and G5- sodium hypochlorite (NaOCl, 1.3%) and MTAD. The XRF results confirmed that the ISOL G5 (Ca/P=1.6254) was the most effective one. The ratio-to-ratio correlations revealed the common origins of Fe, Zn, and Sr in root canals of radicular dentin, which was highly interacted with the major elements of P and Ca each. These interactions were insignificantly affected by the used ISOLs. Finally, XRF is highly recommended as a safe, fast, nondestructive, and relatively not expensive technique to analyze the endodontic therapy of teeth.

Graphical abstract

Relationships of Ca and Ca/P versus P concentration means

Multi-Root Canals of Radicular Dentine in Upper Premolar Teeth

Irrigation Solutions

| G1 Distilled water (DW) | G2 5.25% NaOCl | G3 5.25% NaOCl + 17% EDTA |
| G4 DW + MTAD | G5 1.3% NaOCl + MTAD | |

Relative changes (RC) in P (a) and Ca (b) concentration means in root canal dentin tissues caused by different irrigation solutions

Relative changes (RC) in Fe (a), Zn (b), and Sr (c) concentrations means in root canal dentin tissues caused by different irrigation solutions

1. Introduction

Teeth are a composite of organic, inorganic, and water fractions in various amounts. The organic components may consist of reacted coagulated proteins, necrotic or viable pulp tissue, odontoblastic processes, and microorganisms [1, 2]. The inorganic phase consists of the unit cell $(Ca,X)10(P,C)6(O,OH)26$, in which Ca and P are the two main elements in teeth [3]. Any change in the Ca:P ratio may change the original proportion of the organic and inorganic components, which in turn changes different properties of the tooth such as microhardness, permeability, and solubility characteristics of dentin and may also adversely affect the sealing ability and adhesion of dental materials such as resin-

[a] Endodontic and Operative Dentistry Department, College of Dentistry, Damascus University, Damascus, Syrian Arab Republic. [b] Department of Chemistry, Atomic Energy Commission of Syria (AECS), P. O. Box 6091, Damascus, Syrian Arab Republic. *Corresponding author. E-mail: **morgana.quteifan@yahoo.com**

based cements and root canal sealers to dentin [4]. Changes in the Ca:P ratio may be caused by the chelating agents, which are used to improve chemomechanical debridement in the root canal treatment by removing the smear layer created during root canal instrumentation and composed of dentin structure and some nonspecific inorganic contaminants [1, 5].

In addition to the previous two major elements, teeth accumulate a variety of minor elements such Sr, Zn, and Fe, which can be ingested by humans via different routes, including mainly food and water sources [6-8]. Strontium and Zn can enrich in teeth by substituting in hydroxyapatite [9, 10]. A negative correlation between Ca and Sr in main parts of teeth, e.g. the enamel and cementum, was obtained, confirming that Ca can be substituted by Sr either as a whole or in fractions for the formation of hydroxyapatite [9]. Zinc is readily acquired by synthetic hydroxyapatite, competing with Ca for positions on the surface of the apatite crystal. Zinc pretreatment of hydroxyapatite produces a resistance to acid dissolution similar in magnitude to that produced by equivalent molar concentrations of fluoride [11]. Among the minor elements studied in this work, Fe is presented in teeth and its ions (Fe^{3+}) can induce the formation of apatite of high crystallinity and these ions can inhibit caries progression [12].

Major and minor elements in teeth were analyzed using methods from different techniques, i.e., atomic absorption spectroscopy (AAS) and inductively coupled plasma mass spectroscopy (ICP-MS) were becoming more routine, but they are considered destructive techniques [3,13]. Proton induced X-ray emission (PIXE) [9, 14] and X-ray fluorescence (XRF) [15, 16] were used for multi-elemental non-destructive analysis of teeth samples. XRF showed a very fast, simultaneous, reliable, quantitative, multi-elemental and non-destructive technique with a very good ability to determine major and minor elements in a wide range of concentrations.

One primary objective in the root canal treatments is to ensure chemomechanical removal of the microorganisms and organic/inorganic tissue residues from the infected root canals. Studies in the literature report that due to the structure of the root canals, mechanical instrumentation alone could not perform a satisfying cleaning in the canal. In ex vivo and clinical studies, some areas have remained unreached and infected, thus irrigation has played a key role in the achievement of root canal treatments [17, 18].

The aim of this work was to (i) assess the distributions of P and Ca as major elements and Fe, Zn, and Sr as minor elements in root canals of radicular dentine in upper premolar teeth using XRF technique; (ii) study the effect of several single and combined ISOLs on the chemical composition of root canal regimes; and (iii) study the mutual interactions between the previously mentioned major and minor elements in the control and the irrigation treatment groups.

2. Material and Methods

2.1 Teeth collection and preparation

This study was approved by the Research Ethics Committee at Damascus University. All patients signed an informed consent to use their teeth in this study. Fifty human teeth samples were collected from the patients who visited the dental clinics at the faculty of dentistry in Damascus University for orthodontic reasons. The teeth were extracted from the first upper molars of the left side of adults aged 20-35 year. The teeth were put in sodium hypochlorite NaOCl 5.25% for 2 hours [19]. Apical extrusion of debris was carried

out using reciprocation files and rotary instrumentation systems [20]. Then the specimens were stored in normal saline solution until used. The crowns of the teeth were removed at the cementoenamel junction using diamond disc (Brasseler, Savannah, GA, USA). Afterward, the digital X Rays of the specimens were performed to ensure that they are free from caries, fractures, and damages [20].

The teeth were randomly divided into the following five groups (n=10) according to protocol of ISOLs used. Group I-G1, the root canal dentin tissues were irrigated for 20 minutes with 5 mL of distilled water. Group II-G2, the root canal dentin tissues were irrigated for 20 minutes with 5 mL of 5.25% NaOCl (Clorox, KSA). Group III-G3, the root canal dentin tissues were irrigated for 19 minutes with 5 mL of NaOCl 5.25%, then for 1 minute with 5 mL of 17% EDTA (META BIOMED CO., LTD Republic of Korea). Group IV-G4, the root canal dentin tissues were irrigated for 15 minutes with 5 mL of distilled water, then for 5 minutes with 5 mL of BioPure MTAD as instructed by the company (Tulsa Dental Specialties Company, OK, USA). Finally, Group V-G5, the root canal dentin tissues were irrigated for 15 minutes with 5 mL of 1.3% NaOCl, then for 5 minutes with 5 mL of BioPure MTAD.

Dentin powders were obtained by Gates-Glidden burs after using the ISOLs. The powder of each canal dentin was kept in 5 cm^3 sterilized plastic vial for further XRF analysis.

2.2 XRF measurements

The measurements of dentin powders were performed using XRF instrument, which was assembled by the Syrian Atomic Energy Commission. The detector was PGT Si(Li) with an energy resolution of 160 eV at 5.9 keV and the preamplifier was a GAMMA-PGT system 4000 (Amp., ADC and MCA). The XRF instrument was equipped with a 2 kW Mo tube. Three secondary targets, i.e. Ti, Cu, and Mo, were used for X-ray excitations of (P), (Fe), and (Ca, Zn, and Sr) in teeth specimens with the operating conditions of (35 mA and 40 kV), (10 mA and 35 kV), and (20 mA and 35 kV), respectively. The live time was 2000 s for each of the X-ray excitation modes. The elements in the obtained spectra were determined using AXIL-QXAS software package, which was developed in the International Atomic Energy Agency (IAEA) [19].

The accuracy of XRF method was checked by the analysis of SRM 1486 - Bone Meal, NIST standard sample; while, the hydroxyapatite salt ($Ca_5(PO4)_3.OH$, GR, Merck, Darmstadt, Germany) was used for estimation of only P accuracy. To establish the reproducibility of the instrumental technique, five different standard samples with masses of 0.200 g each were put in polyethylene cups, having sample area of approximately 0.2826 cm^2. The cups were covered from one side with Mylar foils and sequentially exposed to XRF excitations using the previously mentioned operating conditions and modes. In a similar way as for the standard samples, the teeth powders were prepared and measured by XRF.

2.3 Statistics

The normal distributions of data were examined using Shapiro-Wilk test. The significant differences (p = 0.05) between the concentration means of the studied elements in treatment groups (G2-G5) and those in control group (G1) were analyzed with Tukey's honest significance test and Mann-Whitney U test. Pearson correlation analysis was used to investigate the mutual relations between each pair of the studied elements.

3. Results and Discussion

3.1 XRF measurements

The suitability of XRF method for the determination of the elements in dentin powders was checked by estimation of the accuracy (A), the precision (P), and the limits of detection (LOD). The A values were determined by the percentages of the relative differences between the obtained and the certified values of Ca, Fe, Zn, and Sr in five different subsamples (n = 5) of the bone meal standard reference material (SRM 1486 - Bone Meal, NIST), while the accuracy of P was determined by measurements of five different subsamples (n = 5) of the apatite reference sample. The results in **Table 1** showed that there were no significant differences between the obtained elemental concentrations and the certified values (*P*>0.05) according to Student's *t*-test for independent samples.

The precision of XRF measurements was evaluated in terms of the relative standard deviation (RSD = SD/Ci)×100, where SD is the standard deviation and Ci is the mean of ith element concentrations. The results showed that the precision was better than ± 8.3% for all determined elements.

The lower limits of detection (LOD) of the analyzed elements were estimated using the equation (1) [21]:

$$LOD = (3/S)\sqrt{(Ib/t)}, \qquad (1)$$

Where, S is the sensitivity (net peak counts per second per concentration); Ib is the intensity of the background of the respective peak (counts per second); and t is the counting time (s).

The results in **Table 1** showed that all studied elements were with concentrations much higher than the obtained LOD values.

Table 1. Characterization of element determination in the standard samples using XRF technique with different secondary targets

Element	Standard	Secondary target[a]	Obtained concentration	Certified values	(A%)[b]	(P%)[c]	(LOD)[d]	*p*[e]
P (%)	hydroxyapatite	Ti	18.1±0.4	18.5	-2.16	±2.21	0.484	0.085
Ca (%)	bone meal SRM	Mo	26.4±1.4	26.58	-0.677	±5.30	0.040	0.826
Fe (µg/g)	bone meal SRM	Cu	104±5	99	+5.05	±4.81	3.68	0.291
Zn (µg/g)	bone meal SRM	Mo	158±13	147	+7.48	±8.23	6.25	0.255
Sr (µg/g)	bone meal SRM	Mo	266±15	264	+0.758	±5.53	1.90	0.773

[a] The operating conditions were (35 mA and 40 kV) for P; (20 mA and 35 kV) for Ca, Zn, and Sr; and (10 mA and 35 kV) for Fe; collecting time was 2000 s for all used XRF targets each. [b] the percent accuracy, which is estimated by dividing the difference between the obtained and the certified values by the certified value and multiply the result by 100. [c] the percent precision, which is estimated by dividing the standard deviation by the obtained concentration and multiplying the result by 100. [d] the limits of detection. [e] the probability.

3.2 Root canal dentin analysis

Fifty fine and homogenized specimens of the collected root canal dentins were analyzed by the XRF technique with the previously mentioned operating conditions and modes. The XRF results of P, Ca, Fe, Zn, and Sr in the powders of the root canal dentins are shown in **Table 2**.

3.3 Element distribution

Several workers reported that X-ray fluorescence (XRF) is a useful technique for the analysis of major and minor elements in teeth samples [15,16,22]. In the present work, XRF showed the possibility to determine P and Ca as two major elements and Fe, Zn, and Sr as three minor elements in the root canal dentin powders. **Table 2** shows the XRF results of the means, the medians and the ranges of the studied elements in the root canal dentin specimens.

The results showed that P and Ca were the two main elements in the root canals dentin specimens of the control group with mean values of 17.3±0.9% and 26.2±0.8%, respectively. These two elements were normally distributed with relatively narrow ranges of P (16.0-18.6%) and Ca (25.0-27.1%), accepting the results obtained in literature [3,4,10], where P and Ca were distributed homogenously through the root dentin tissues. The results also showed that, the normal distributions of P and Ca were not influenced by treatments using the ISOLs. In addition, the normality was obtained for the minor elements in the control and the treatment groups, except two instances belonged to Zn in control (G1) and G2 treatment groups. Accordingly, Fe and Sr were normally distributed in the control group with concentration values of 69.0 (28-132) µg/g and 102 (48-154) µg/g, respectively, which were in good agreement with those reported in literature [3,14]. On the other hand, Zn concentrations in the control group were positively skewed with mean value of 273 (215-

434) µg/g. This could be explained as a result of high-protein diets, which characterized many individuals in the country; taking into account that, beef and lamb, as well as nuts, whole grains, and legumes are significant sources of zinc in the diet [23]. According to the previously mentioned reasons, diet and other individual factors could affect the Zn distributions in teeth specimens.

3.4 ISOLs effects

3.4.1 Major elements

A very well positive linear relationship was obtained between the concentration means of P and Ca in the treatment groups (**Fig. 1**). The coefficient of determination (R^2) was with a value of 0.9996. The results in **Fig. 1** also shows a very interesting correlation between the ratios of Ca/P and the concentration means of P with R^2 value of 0.9974. It was obviously that, the least value of Ca/P was for the control group (1.5145), accepting those obtained in literature [24]. Distilled water (DW) was used in the control group (G1) as an ISOL, which had only a mechanical flushing action to remove material from the root canal system [25]. According to this application, the instrumented specimens in G1 group were expected to contain significant amounts of P not only in the hydroxyapatite fraction form, but also in the organic and the remnant fraction forms. Depending on latter interpretation, higher P concentrations and thus a minimal ratio of Ca/P was expected to find in the control group compared to the other treatment groups. As a result, the different ratios of Ca/P means were to reflect the different effects of the single and the combined ISOLs on the chemical compositions of the organic and inorganic fractions of the root canal dentin tissues. Overall, Ca/P ratios were increased in the following order:
(Ca/P)G1=1.5145<(Ca/P)G3=1.5849<(Ca/P)G2=1.5958<(Ca/

P)G4=1.6100<(Ca/P)G5=1.6254

Furthermore, the relative changes in P and Ca means were estimated using equation (2):

$$RC(P)Gi=(PGi-PG1)/PG1 \text{ and } RC(Ca)Gi=(CaGi-CaG1)/CaG1 \quad (2)$$

Where RC(P) and RC(Ca) are the relative changes of P and Ca concentration means, respectively; Gi is the ith group; PG1 and CaG1 are the concentration means of P and Ca in control group (G1), respectively.

Table 2. Means, medians and ranges of concentrations of elements in powders of root canals of radicular dentin specimens, following treatment with different test solutions

Group[a]	Descriptive statistics	P (%)	Ca (%)	Fe (µg/g)	Zn (µg/g)	Sr (µg/g)
G1	Mean[b]	17.3±0.9	26.2±0.8	69.0±39.0	273±71	102±23
	Median	17.5	26.3	59.0	249	96.8
	Range	16.0-18.6	25.0-27.1	28-132	215-434	74.7-154
G2	Mean	15.1±0.1	24.8±0.8	44.3±16.3	370±172	114±15
	Median	15.3	24.8	44.5	306	112
	Range	13.0-17.1	23.8-26.3	24.0-77.0	222-714	92.1-138
G3	Mean	15.3±1.1	24.1±1.1	53.4±20.1	369±147	145±31
	Median	15.5	24.0	49.0	345	139
	Range	13.6-17.2	22.4-26.2	30.0-81.0	163-704	109-207
G4	Mean	14.6±1.4	23.4±1.1	45.3±10.3	312±58	120±25
	Median	14.5	23.4	45.0	311	128
	Range	12.3-17.3	21.8-25.3	24.0-62.0	207-393	69.4-150
G5	Mean	14.1±1.2	22.8±0.3	57.0±29.9	341±97	144±42
	Median	14.3	23.1	43.0	309	134
	Range	11.9-15.6	22.4-23.4	34.0-108	239-526	98.7-225

[a] G1, G2, G3, G4, and G5 are the treatment groups of distilled water (DW), 5.25% NaOCl, 5.25% NaOCl+17% EDTA, distilled water+MTAD, and 1.3% NaOCl+MTAD, respectively. Number of specimens in each group, n=10. [b] the concentration presented as mean ± standard deviation.
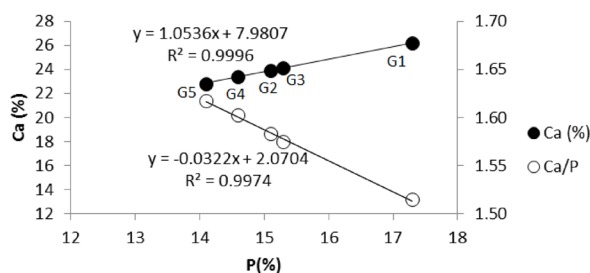

Fig. 1. Relationships of Ca and Ca/P versus P concentration. means. G1, G2, G3, G4, and G5 are the treatment groups with distilled water (DW), 5.25% NaOCl, 5.25% NaOCl+17% EDTA, DW+MTAD, and 1.3% NaOCl+MTAD ISOLs, respectively.

Fig. 2 shows the relative changes of P and Ca concentration means with the standard errors (SE) and the standard deviations (SD) in all studied treatment groups. The results shows significant negative changes (p<0.05) in P and Ca each in all treatment groups compared to the control. Thus, in the instance where the NaOCl ISOL in G2 treatment group was used, the significant RC(P) and RC(Ca) (p<0.05) with values of -0.127 and -0.052, respectively, were obtained; indicating significant losses in P and Ca concentrations, which could occur as a result of proteins losses from the organic components found in dentin canal system [4,26]. While, in the instance of MTAD ISOL use in G4 treatment group, the RC(P) and RC(Ca) values were of -0.153 and -0.106, respectively; indicating higher losses of P and Ca compared to those obtained in G2 group. This result confirmed the higher efficacy of MTAD ISOL in comparison to that of NaOCl. In the instance of G3 irrigation treatment group, the use of the combined ISOLs of 5.25% NaOCl+17% EDTA revealed RC(P) value of -0.115, confirming the negligible effect of 17% EDTA ISOL on P distribution when it was used after NaOCl. This could be explained by the fact that, dentin has a dense collagen network covered by hydroxyapatite; thus, when NaOCl used before EDTA, the hydroxyapatite coating appeared to protect

the collagen fibers, avoiding the direct NaOCl action [27]. Finally, the use of the combined ISOLs of 1.3% NaOCl+MTAD (G5 irrigation treatment group) revealed the greatest negative values of RC(P) (-0.182) and RC(Ca) (-0.114) compared to those of other instances in this work.
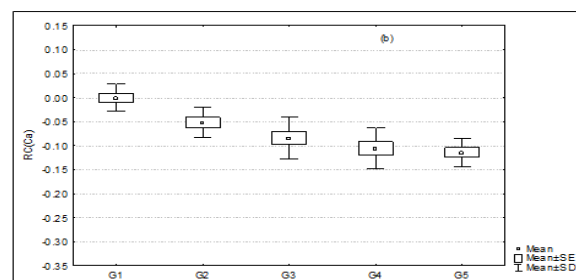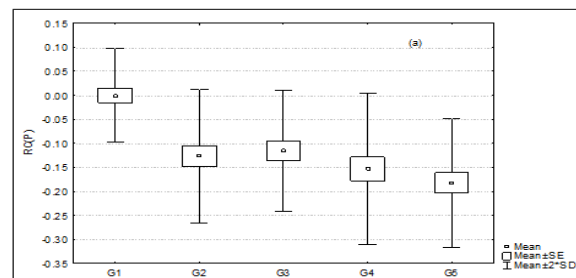

Fig. 2. Relative changes (RC) in P (a) and Ca (b) concentration means in root canal dentin tissues caused by different ISOLs. G1 is the control group; G2, G3, G4, and G5 are the treatment groups; SE and SD are the standard error and standard deviation, respectively.

3.4.2 Minor elements

Fig. 3a shows insignificant negative changes in concentration means of Fe in all treatment groups compared to that of the control. The relative changes in Fe means were in the following order:

RC(Fe)G2>RC(Fe)G4>RC(Fe)G3>RC(Fe)G5>RC(Fe)G1. According to the previous results, the single ISOLs of NaOCl and MTAD have more efficiencies on removing Fe from the dentin tissues than the combined ISOLs of NaOCl+EDTA and NaOCl+MTAD. Thus, the efficacies of EDTA as a chelating agent to sequester metal ions such as Fe^{3+} [28] or MTAD as a demineralizing agent [29] were reduced when they were used after the ISOL of NaOCl. Finally, the results indicated that the ratios of Fe/Ca and Fe/P for the treatment groups (G2-G5) were insignificantly changed compared to those of the control group: $(2.63\pm1.44).10^{-4}$ and $(3.96\pm2.10).10^{4}$, respectively.

Fig. 3b shows insignificant positive changes in concentration means of Zn in all treatment groups compared to the control. This could be attributed to Zn^{2+} ion which has the ability to substitute Ca^{2+} in the apatite lattice [30]. The changes in Zn means were in the following order: RC(Zn)G2≈RC(Zn)G3>RC(Zn)G5>RC(Zn)G4>RC(Zn)G1, indicating the higher substitution of Zn^{2+} for Ca^{2+} in treatment groups with NaOCl ISOL. In addition, the ratio of Zn/Ca was calculated. This was with a value of $(10.4\pm2.5).10^{-4}$ for the control group. Insignificant increases in Zn/Ca ratios were obtained for all treatment groups, except G3, which was estimated with a value of $(15.4\pm6.1).10^{-4}$. The ratio of Zn/P was also calculated. This was with a value of $(15.8\pm4.1).10^{-4}$ for the control group. The significant increase in Zn/P ratio was obtained only for G5 treatment group with a value of $(24.3\pm7.2).10^{-4}$.

It is well known that, Sr is one of the component elements of teeth and the Sr^{2+} ion would strongly replace Ca^{2+} in the apatite lattice [3,31]. The results in **Fig. 3c** showed positive changes in Sr concentration means in all irrigation treatment groups, confirming the specific substitution ability of Sr^{2+} ion for Ca^{2+} in the apatite lattice. The significant changes in Sr means were obtained by using the ISOLs in G3 and G5 treatment groups. Overall, the changes in Sr means were in the following order: RC(Sr)G3≈RC(Sr)G5>RC(Sr)G4>RC(Sr)G2>RC(Sr)G1, indicating the higher effect of the combined ISOLs on Sr compared to the effect of the single ISOLs.

Furthermore, the ratio of Sr/Ca was calculated with a value of $(4.95\pm3.40).10^{-4}$ for the control group, accepting those obtained in literature [3]. The previous ratio was insignificantly changed in the irrigation treatment groups. The ratio of Sr/P was also calculated for the control group. This was with a value of $(7.42\pm4.83).10^{-4}$. The significant differences were obtained for Sr/P ratios of G3, $(11.0\pm4.7).10^{-4}$, and G5, $(10.3\pm3.3).10^{-4}$, compared to that ratio of the control group.

3.4.3 Correlation study

The data on element-to-element correlations in the control and the treatment groups were studied. The results showed only one positive strong correlation in the control group: P-Ca (r = 0.65); while, there were two correlations in G2 treatment group: one strong positive correlation (r = 0.69) between P-Zn and one very strong negative correlation (r = -0.76) between Ca-Fe. Whereas, there were no strong correlations between the studied elements in the other treatment groups, i.e. G3, G4, and G5.

The data on ratio-to-ratio correlations in all treatment groups were also studied. The results in **Table 3** showed surprisingly many strong and very strong correlations in the studied groups. There were four very strong positive correlations and a similar number of the very strong negative correlations in the control group (G1): Fe/Ca-Fe/P (r = 1.00), Zn/Ca-Zn/P (r = 0.99), Sr/Ca-Sr/P (r = 1.00), Zn/Fe-Sr/Fe (r =

0.75), Fe/Ca-Zn/Fe (r = -0.87), Sr/Ca-Zn/Sr (r = - 0.73), Fe/P-Zn/Fe (r = -0.87), and Sr/P-Zn/Sr (r = -0.73); further indications showed two strong positive correlations: Zn/Ca-Zn/Sr (r = 0.67) and Zn/P-Zn/Sr (r = 0.68).
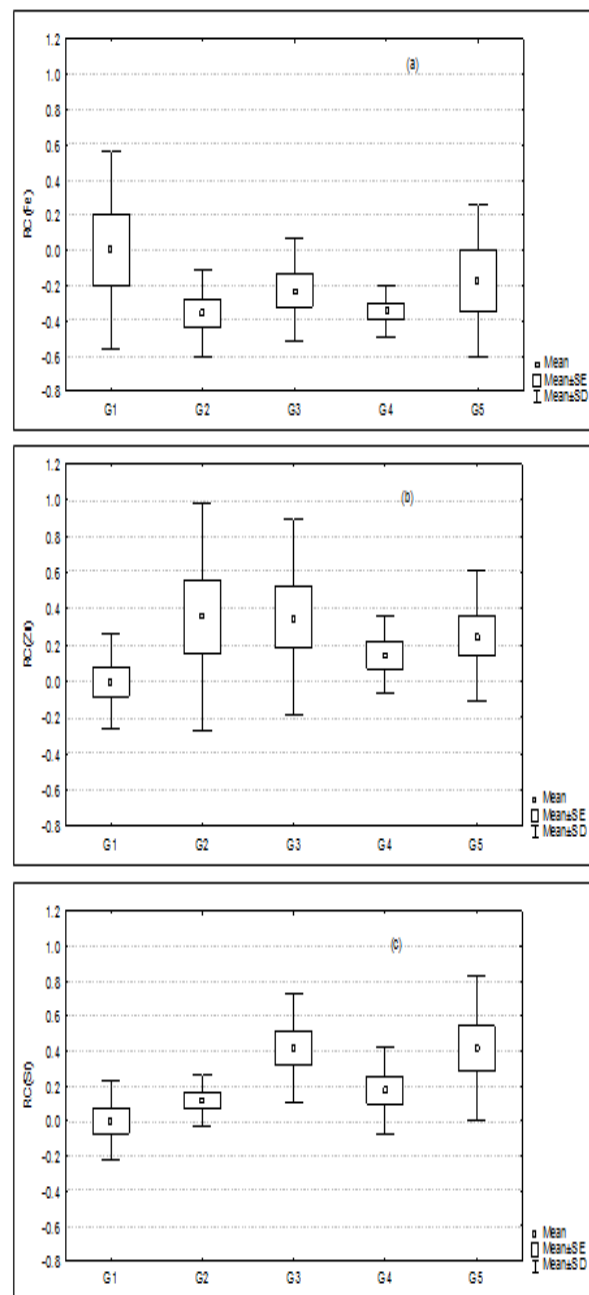

Fig. 3. Relative changes (RC) in Fe (a), Zn (b), and Sr (c) concentrations means in root canal dentin tissues caused by different ISOLs. G1 is the control group; G2, G3, G4, and G5 are the treatment groups; SE and SD are the standard error and standard deviation, respectively.

In G2 treatment group, seven very strong positive correlations were obtained: Fe/Ca-Sr/Ca (r = 0.78), Fe/Ca-Fe/P (r = 0.93), Zn/Ca-Zn/P (r = 0.95), Zn/Ca-Zn/Sr (r = 0.91), Zn/P-Zn/Fe (r = 0.70), Zn/P-Zn/Sr (r = 0.97), and Zn/Fe-Zn/Sr (r = 0.72); while, there were four very strong negative correlations: Ca/P-Fe/Ca (r = -0.77), Ca/P-Zn/Ca (r = -0.77), Ca/P-Sr/Ca (r = -0.78), and Fe/P-Sr/Fe (r = -0.91). In G2 treatment group there was only one strong positive correlation: Zn/Fe-Sr/Fe (r = 0.68).

Table 3. Pearson correlation coefficient matrix of element ratios in treatment and control groups.

Group[a]	Ratio	Ca/P	Fe/Ca	Zn/Ca	Sr/Ca	Fe/P	Zn/P	Sr/P	Zn/Fe	Sr/Fe	Zn/Sr
G1	Ca/P	1.00									
	Fe/Ca	-0.35	1.00								
	Zn/Ca	0.31	-0.03	1.00							
	Sr/Ca	-0.40	0.50	-0.09	1.00						
	Fe/P	-0.30	**1.00**	-0.02	0.45	1.00					
	Zn/P	0.43	-0.08	**0.99**	-0.14	-0.07	1.00				
	Sr/P	-0.35	0.48	-0.07	**1.00**	0.43	-0.12	1.00			
	Zn/Fe	0.49	**-0.87**	0.29	-0.25	**-0.87**	0.35	-0.21	1.00		
	Sr/Fe	0.19	-0.53	-0.05	0.35	-0.56	-0.02	0.39	**0.75**	1.00	
	Zn/Sr	0.34	-0.18	**0.67**	-0.73	-0.13	0.68	-0.73	0.11	-0.54	1.00
G2	Ca/P	1.00									
	Fe/Ca	**-0.77**	1.00								
	Zn/Ca	**-0.77**	0.59	1.00							
	Sr/Ca	**-0.78**	**0.78**	0.68	1.00						
	Fe/P	-0.51	**0.93**	0.31	0.55	1.00					
	Zn/P	-0.58	0.35	**0.95**	0.53	0.08	1.00				
	Sr/P	0.10	0.21	-0.02	0.51	0.13	0.03	1.00			
	Zn/Fe	-0.06	-0.41	0.47	-0.01	-0.63	**0.70**	-0.03	1.00		
	Sr/Fe	0.32	-0.77	-0.12	-0.23	**-0.91**	0.07	0.22	**0.68**	1.00	
	Zn/Sr	-0.57	0.28	**0.91**	0.36	0.03	**0.97**	-0.23	**0.72**	0.05	1.00
G3	Ca/P	1.00									
	Fe/Ca	-0.18	1.00								
	Zn/Ca	-0.21	**0.82**	1.00							
	Sr/Ca	**0.85**	-0.36	-0.46	1.00						
	Fe/P	-0.17	**1.00**	**0.82**	-0.35	1.00					
	Zn/P	-0.02	**0.77**	**0.98**	-0.33	**0.78**	1.00				
	Sr/P	**0.90**	-0.31	-0.45	**0.99**	-0.30	-0.31	1.00			
	Zn/Fe	0.35	**-0.63**	-0.25	0.26	-0.63	-0.14	0.24	1.00		
	Sr/Fe	**0.79**	-0.49	-0.63	**0.90**	-0.48	-0.51	**0.92**	0.44	1.00	
	Zn/Sr	-0.40	**0.92**	**0.93**	-0.63	**0.91**	**0.86**	-0.60	-0.46	**-0.73**	1.00
G4	Ca/P	1.00									
	Fe/Ca	0.26	1.00								
	Zn/Ca	0.14	0.12	1.00							
	Sr/Ca	0.08	-0.31	0.02	1.00						
	Fe/P	0.57	**0.94**	0.16	-0.22	1.00					
	Zn/P	0.67	0.30	**0.83**	0.07	0.53	1.00				
	Sr/P	0.57	-0.12	0.09	**0.86**	0.12	0.44	1.00			
	Zn/Fe	-0.35	**-0.85**	0.38	0.40	**-0.76**	0.09	0.10	1.00		
	Sr/Fe	-0.29	**-0.84**	-0.06	**0.70**	**-0.78**	-0.23	0.42	**0.84**	1.00	
	Zn/Sr	-0.08	0.28	0.49	**-0.85**	0.15	0.32	**-0.70**	-0.08	-0.58	1.00
G5											
	Ca/P	1.00									
	Fe/Ca	-0.35	1.00								
	Zn/Ca	0.11	-0.11	1.00							
	Sr/Ca	-0.05	-0.19	-0.26	1.00						
	Fe/P	-0.30	**1.00**	-0.11	-0.15	1.00					
	Zn/P	0.39	-0.19	**0.95**	-0.20	-0.16	1.00				
	Sr/P	0.20	-0.22	-0.23	**0.95**	-0.17	-0.08	1.00			
	Zn/Fe	0.14	**-0.67**	0.46	-0.15	**-0.70**	0.43	-0.13	1.00		
	Sr/Fe	0.18	**-0.71**	0.03	0.24	**-0.74**	0.05	0.24	**0.80**	1.00	
	Zn/Sr	0.19	0.05	**0.80**	-0.76	0.04	**0.77**	**-0.69**	0.37	-0.15	1.00

[a] G1, G2, G3, G4, and G5 are the treatment groups of distilled water (DW), 5.25% NaOCl, 5.25% NaOCl+17% EDTA, distilled water+MTAD, and 1.3% NaOCl+MTAD, respectively. Number of specimens in each group, n=10.

Higher numbers of the very strong positive and negative correlations were obtained in G3 treatment group. Thus, there were sixteen very strong positive and one very strong negative correlations: Ca/P-Sr/Ca (r = 0.85), Ca/P-Sr/P (r = 0.90), Ca/P-Sr/Fe (r = 0.79), Fe/Ca-Zn/Ca (r = 0.82), Fe/Ca-Fe/P (r = 1.00), Fe/Ca-Zn/P (r = 0.77), Fe/Ca-Zn/Sr (r = 0.92), Zn/Ca-Fe/P (r = 0.82), Zn/Ca-Zn/P (r = 0.98), Zn/Ca-Zn/Sr (r = 0.93), Sr/Ca-Sr/P (r = 0.99), Sr/Ca-Sr/Fe (r = 0.90), Fe/P-Zn/P (r = 0.78), Fe/P-Zn/Sr (r = 0.91), Zn/P-Zn/Sr (r = 0.86), Sr/P-Sr/Fe (r = 0.92), and Sr/Fe-Zn/Sr (r = -0.73). Whereas, there were only two strong negative correlations in G3 treatment group: Fe/Ca-Zn/Fe (r = -0.63) and Sr/Ca-Zn/Sr (r = -0.63).

In G4 treatment group, there were five very strong positive and six very strong negative correlations: Fe/Ca-Fe/P (r = 0.94), Zn/Ca-Zn/P (r = 0.83), Sr/Ca-Sr/P (r = 0.86), Sr/Ca-Sr/Fe (r = 0.70), Zn/Fe-Sr/Fe (r = 0.84), Fe/Ca-Zn/Fe (r = -0.85), Fe/Ca-Sr/Fe (r = -0.84), Sr/Ca-Zn/Sr (r = -0.85), Fe/P-Zn/Fe (r = -0.76), Fe/P-Sr/Fe (r = -0.78), and Sr/P-Zn/Sr (r=-0.70). Strong positive and strong negative correlations were not obtained in G4 treatment group.

In G5 treatment group, there were six very strong positive and four very strong negative correlations: Fe/Ca-Fe/P (r = 1.00), Zn/Ca-Zn/P (r = 0.95), Zn/Ca-Zn/Sr (r = 0.80), Sr/Ca-Sr/P (r = 0.95), Zn/P-Zn/Sr (r = 0.77), Zn/Fe-Sr/Fe (r = 0.88), Fe/Ca-Sr/Fe (r = -0.71), Sr/Ca-Zn/Sr (r = -0.76), Fe/P-Zn/Fe (r = -0.70), and Fe/P-Sr/Fe (r = -0.74). Only two strong negative

correlations were obtained in G5 treatment group: Fe/Ca-Zn/Fe (r = -0.67) and Sr/P-Zn/Sr (r = -0.69).

It could be concluded hereby that, the correlation between pairs of the studied elements (element-to-element) in canals of radicular dentin specimens was observed only for a limited number of instances, whereas the higher numbers of the relationships were observed by correlating the ratios of the studied elements by each other (ratio-to-ratio).

The results showed that several important ratio-to-ratio correlations in the control group such (Sr/Ca-Sr/P), (Zn/Fe-Sr/Fe), (Fe/Ca-Zn/Fe), (Sr/Ca-Zn/Sr), (Fe/P-Zn/Fe), (Sr/P-Zn/Sr), (Zn/Ca-Zn/Sr), and (Zn/P-Zn/Sr) were not found in the treatment groups: (G2), (G3), (G2 and G5), (G2 and G4), (G2 and G3), (G2 and G3), (G4), and (G4), respectively.

The results showed very strong correlations for Fe/Ca-Fe/P and Zn/Ca-Zn/P each, regardless to the ISOL used in treatment process; this well agrees with those reported in literature, where minor elements such Fe and Zn have the ability to incorporate into the hydroxyapatite lattice of the dental tissues [32,33]. The results also showed that Sr could incorporate in the hydroxyapatite lattice, but NaOCl solution was likely to affect the distribution of Sr in the root canal dentin. Strong correlations between the studied minor elements were obtained in all instances, in exception of the combined ISOLs in G3 treatment group, reflecting the possible effect of EDTA solution on the minor elements distribution in root canal system.

4. Conclusions

In the present work, XRF technique was well suited for the determination of major elements (P and Ca) and minor elements (Fe, Zn, and Sr) in powders of root canal dentins with errors less than 8.3%. The previously mentioned elements, except Zn in G2 and G3 treatment groups, were normally distributed.

The XRF results showed greater decreases in P means of the treatment groups than those of Ca. The ratios of Ca/P were decreased as follows: $Ca/P_{G5(1.3\% NaOCl+MTAD)} > Ca/P_{G4(MTAD)} > Ca/P_{G2(5.25\% NaOCl)} > Ca/P_{G3(5.25\% NaOCl+17\% EDTA)} > Ca/P_{G1(Distilled water)}$. Accordingly, the highest direct action on the root canal dentin was attributed to the combined ISOL of 1.3% NaOCl+MTAD. On the other hand, the ISOLs used in this work showed different effects on the distribution of the minor elements (Fe, Zn, and Sr) in root canal dentin. Accordingly, higher removal of Fe from dentin tissues was investigated by using the single ISOLs of NaOCl or MTAD, while Zn and Sr had, apparently, the ability to replace Ca^{2+} in the apatite lattice regardless of the type of the used ISOLs.

Study on ratio-to-ratio correlations showed significant mutual interactions between minor-major elements in all studied treatment groups. Moreover, several minor-to-minor element correlations were significantly affected by the type of the ISOLs. This indicated the re-distribution ability of the minor elements during the exposure of the root canal dentin tissues to the action of the ISOL regimes.

Acknowledgments

Author Contributions

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