IEJ Iranian Endodontic Journal

The Impact of Different Irrigation Regimens on the Chemical Structure and Cleanliness of Root Canal Dentin

Amira Elgawish [a], Hossam Tawfik [b], Abeer El Gendy [c], Roy George [a], Mahmoud M Bakr [a*]

a School of Medicine and Dentistry, Griffith University, Queensland, Australia; *b* Department of Endodontics, Faculty of Oral and Dental Medicine, Misr International University, Cairo, Egypt; *c* Department of Endodontics, Faculty of Dentistry, Ain Shams University, Cairo, Egypt

Article Type: **Original Article**

Received: 10 May 2023
Revised: 12 Jul 2023
Accepted: 07 Aug 2023
Doi: *10.22037/iej.v18i4.38004*

****Corresponding author***: Mahmoud Bakr, Director of Clinical Education, School of Medicine and Dentistry, Griffith University, Queensland, Australia.

E-mail: m.bakr@griffith.edu.au

Introduction: This *in vitro* study aimed to evaluate the effect of different irrigation regimens on the chemical composition and cleanliness of root canal dentin. **Materials and Methods:** Forty-eight extracted single-rooted permanent human teeth were collected. Root canals were instrumented using step-back technique up to master apical file size 60. Samples were divided into 3 groups (n=16) based on the type of the irrigant used. The irrigation solutions were 5.25% sodium hypochlorite, 2% chlorhexidine gluconate, and saline solution as a control. Root canal cleanliness was assessed using stereomicroscope and scanning electron microscope. Scanning electron microscope energy dispersive X-ray was used for the inorganic analysis. Fourier transform infrared spectrometer was used for the organic analysis. One-way analysis of variance (ANOVA) and multiple comparison post hoc test were used for comparison between the three groups. **Results:** The highest mean percentage of remaining debris was in saline group followed by chlorhexidine gluconate group. Sodium hypochlorite group showed the lowest mean value of remaining debris. Furthermore, our results showed that canal irrigation with sodium hypochlorite affected the chemical structure of root canal dentin more than chlorhexidine gluconate. **Conclusions:** Based on the results, 5.25% sodium hypochlorite emerges as the preferred irrigant for root canal treatment. This research sheds light on the significance of irrigation regimens in endodontics and emphasizes the need for careful consideration of irrigant selection in clinical practice.

Keywords: Chlorhexidine; Dentin; Infrared Spectrometer; Irrigation; Sodium Hypochlorite

Introduction

The most common irrigant used in root canal treatment is sodium hypochlorite (NaOCl) in a concentration ranging from 0.5% to 6% [1-3]. Sodium hypochlorite solutions are known for their bactericidal activity and capacity to dissolve organic matter and necrotic tissues [4, 5]. Chlorhexidine gluconate (CHX) is a broad-spectrum antimicrobial agent that is also used as an irrigant as it has an antibacterial efficacy comparable to that of NaOCl [6-8]; however, the inability of CHX to dissolve organic matter is perceived as a drawback [9]. Several studies have investigated the effect of root canal irrigants *e.g.*, NaOCl, EDTA, water, CHX and hydrogen peroxide on the cleanliness, histological structure, dissolution of necrotic tissues and removal of smear layer and have achieved varying results [10-22].

Dentin consists of several identifiable structures: tubules with cell processes and fluid, highly mineralized peritubular dentin, and intertubular dentin consisting mainly of collagen and deposited apatite. The structural organization and microstructural variations reflect formative influences, such as tooth size and shape, and alterations caused by age, insult and disease [23]. In the literature, the effects of root canal irrigants on the chemical nature of root canal dentin was addressed by several studies [24, 25]. The studies investigated the removal of organic material [26, 27], microhardness, roughness and mechanical properties of dentin [28-33]. In addition to the above, mineral content (calcium) removal [34-38], overall changes in tissue weight [39], cervical dentin permeability [40], molecular composition and morphology of smear layer were also studied [41]. Furthermore, human dentin, the mineralized bio-composite cellular tissue which makes up the bulk of the tooth, is formed of type-I mineralized collagen fibers and nanocrystalline hydroxyapatite crystals [42]. The mineral content of dentin is mainly composed of calcium-phosphate-carbonate mineral with the inclusion of lower concentrations of sodium, magnesium, chloride, potassium and a large number of trace elements, the most significant of which is fluorine [43]. Some chemical agents have been proven to induce alterations in the chemical structure of human dentin and change the calcium/phosphate ratio of the dentin surface [43-45]. This, in turn, changes the permeability and solubility characteristics of dentin which may inadvertently affect the adhesion of dental materials to dentin tissues [45-50]. Therefore, this study aimed to evaluate the effect of different irrigation regimens on canal cleanliness and changes in the chemical structure of human root dentin.

Materials and Methods

The project was granted an exemption from the Ethical Committee, Faculty of Dentistry, Ain Shams University, Cairo, Egypt (FDASU-RecEM072103).

The irrigants used in this study were 2% CHX solution (Sigma, St. Louis, Mo, USA), 5.25% NaOCl solution (Egyptian Co. for household, products under license of Clorox Co., Egypt) and Isotonic saline (Egypt Otsuka pharmaceutical Co., 10th of Ramadan city, Egypt) [51]. 5.25% sodium hypochlorite was used in the presented study to evaluate the ability of high concentration irrigant to dissolve organic debris and necrotic tissue.

Appropriate sample size to find the efficacy of different irrigation solutions on the cleanliness and chemical composition of dentin was calculated by taking large effect size (d=0.6), alpha error (α)=0.05, and power of study as 80% in the sample size estimation formula for determination of the mean difference among continuous parameters between the three groups provided the total minimum sample size of 45. With allocation ratio being kept as 1:1, in each group, a minimum of 15 samples per group was deemed an appropriate sample size. The above sample size estimation is conducted by using G power (v3.1.9.2).

Tabe 1: Treatment groups

Groups	Subgroup A, Root canal cleanliness	Subgroup B, Chemical structure of dentine	
		Organic	Inorganic
Group I: 5.25% NaOCl	*n*=16	*n*=8	*n*=8
Group II: 2% chlorhexidine gluconate	*n*=16	*n*=8	*n*=8
Group III: (control group) saline	*n*=16	*n*=8	*n*=8

Teeth selection and preparation

Forty-eight freshly extracted permanent human teeth of maxillary anterior teeth with mature roots were selected and inspected under stereomicroscope (25× magnification). Teeth with root cracks were excluded.

Adherent soft tissue was mechanically removed using ultrasonic scaler. Buccolingual and mesiodistal radiographic views were taken to ensure that the canals were not calcified and to rule out additional canals or complex anatomies. All crowns of the samples were decapitated using safe-sided diamond disc mounted on a high-speed contra-angle handpiece, under water coolant. Teeth were randomly allotted to one of the three treatment groups (Table 1).

Root canal instrumentation

Working length was determined by introducing size 10 K-file to the apical foramen and then withdrawn by 1 mm. All root canals were prepared using Flex-O files (Mani, Tochigi, Japan) using the step-back technique. All canals were assessed to determine the initial apical size for all teeth. As the majority of the teeth had an initial apical size corresponding to a size 25 file it was decided to standardize the canal preparation with a master apical file of size 40 and stepped back to size 60 till the apical third before the teeth were tapered coronally using progressively larger files to the coronal third. Apical patency was preserved by using K-file size 15.

To assess chemical changes as a result of the use of NaOCl and CHX, it was decided to irrigate the canals with the specific irrigant associated with each treatment group for the entire canal preparation. Two mL of the irrigant was delivered into canal using a 27-gauge needle for over 10 sec between each file.

Methods of evaluation

Each sample was sectioned longitudinally into two halves by drilling two longitudinal grooves along the whole length of the root using tapered diamond disc with a high-speed contra-angle handpiece, under water coolant [52].

The teeth were then split by placing a #15 surgical blade (Henry Schein, Port Washington, NY, USA) in the groove and striking the blade with a small mallet.

Each section was then randomly subdivided for evaluation of root canal cleanliness and chemical structure (organic and inorganic) of root canal dentin (Table 1).

(A) Evaluation of canal cleanliness: (Subgroup A)

1-Stereomicroscopic examination:

Samples were evaluated under stereomicroscope (Olympus, Tokyo, Japan). Both halves of the split roots were dried with compressed air and lines were superimposed over the canal thirds (apical, middle and cervical). Images of the split roots were taken using a digital camera (Nikon N90S DCS 420 digital camera; Nikon Corp., Tokyo, Japan) at a 1:1 setting. The images were transferred to a computer with Adobe Photoshop (Photoshop software, version 5.0, Adobe Systems Inc., San Jose, CA, USA) and enlarged to 12 times the original size. The debris in each canal was traced and the total number of pixels occupied by the debris was reported using the histogram function in the software program.

The outline of the canal was then traced and the same feature of the software reported the total pixels occupied by the canal. Percentage of debris was calculated by dividing the pixels of debris by the total pixels representing the entire area of the canal. Percentage of debris was calculated for each third separately and for the total debris in each canal.

2- Scanning electron microscopic examination:

Selected samples were examined under the scanning electron microscope. Specimens were first dehydrated in water and ethanol mixture with increasing content of ethanol (70%, 80%, 96%, and 100% for 24 h each), followed by mixtures of ethanol and acetone (increasing acetone content: 80%, 96%, and 100% for 24 h each). Then they were dried for 24 h in a desiccator under vacuum with glass filter pump. Finally, the specimens were mounted on a single stub, and sputter coated with gold using scanning electron microscope coating unit E5100 (Polaron Equipment, Whatford, UK). Dentin surface was observed with scanning electron microscope (Joel JXA 840 A, electron probe microanalyzer, Japan) at 15 kV with 1,000× magnification.

(B) *Evaluation of chemical changes: (Subgroup B)*

1-Inorganic analysis:

One half of each split root of subgroup B (groups I, II, III & IV) was used for the inorganic analysis using scanning electron microscope energy dispersive X-ray (INCA-SIGHT, Oxford, England).

Specimens were critical point dried and a spatter of gold was applied to give a good image and quality; then the specimens were pasted on the specimen holder of the device. Three points (spectrum) in the prepared root canal dentin were examined and representative micrographs were taken. Changes in the inorganic content of dentin were evaluated by recording the alterations in levels of calcium, magnesium and phosphorus.

The levels of calcium, phosphorus, magnesium and carbon in dentin were measured to a minimum detectable level of 300 ppm for each specimen, over an area of 200 μm^2 that was irradiated at a voltage of 30 kV for 50 sec.

2-Organic analysis:

A section of each split tooth of subgroup B (groups I, II, III) was used for evaluation of the organic content of root dentin.

For the purpose of fourier transform infrared spectrometer (FTIR) analysis by Jasco FTIR-6100 spectrometer (Jasco Inc., Mary's Court Easton, MD, USA), a diamond disc (Komet #991 HP-220 Double Sided HP; Brasseler GmbH, Lemgo, Germany) was passed over the prepared root canal dentin surface to a depth of 1 mm and the dentin dust was collected.

The FTIR radiation source emits infrared light that passes through the sample. Part of the infrared light is absorbed through the sample and the rest is transmitted. The transmitted part for each sample is passed through a photocell (detector) to change its light intensity into electro-motive force. Electro-motive force of each sample through the recording system draws an infrared chart which is a plot of frequency versus intensity. The changes in infrared spectroscopy can be detected through the changes in intensity of some bands and by shifting in the band position which indicates the apparent changes in the behavior of the material.

The fourier transform infrared-attenuated total reflectance spectra was measured in the frequency region 400 to 4000 cm^{-1} as a wave number, *via* JASCO FTIR using the reflection unit; and then the collected spectra in a reflectance percentage were transformed into the absorbance percentage units in Y-axis by the JASCO software (Software of FTIR device for molecular analysis; Jasco Inc., Easton, MD, USA).

Statistical analysis

Data was presented as mean and standard deviation (SD) values. The normality of the data has been assessed using the Shapiro-Wilk test and the data was found normally distributed. One-way Analysis of Variance (ANOVA) was used for comparison between the three groups. Multiple comparison post-hoc test was used for pair-wise comparison between the means when ANOVA test is significant. The significance level was set at $P \le 0.05$.

Results

Root canal cleanliness

Assessment of stereomicroscopic images of sectioned samples irrigated with NaOCl showed that the mean value of debris was significantly lower than samples irrigated with saline, mean difference [27.8; 95%CI (1.6,54.0); P=0.04] (Table 2).

According to location

Coronal third:

Samples irrigated with NaOCl showed significantly lower mean value of debris than samples irrigated with CHX [mean difference 24.8; 95%CI (7.0, 42.6) *P*=0.006] (Table 3).

Middle third:

Samples irrigated with NaOCl showed significantly lower mean value of debris than samples irrigated with CHX [21.1; 95%CI (0.6, 41.5)].

Samples irrigated with NaOCl showed significantly lower mean value of debris than samples irrigated with saline [21.5; 95%CI (1.0, 41.9)] (Table 3).

Apical third:

Samples irrigated with NaOCl showed significantly lower mean value of debris than samples irrigated with saline [48.0; 95%CI (33.8, 63.2)].

Samples irrigated with CHX group showed significantly lower mean value of debris than samples irrigated with saline [58.0; 95%CI (43.8, 72.2)] (Table 3).

According to irrigant

Sodium hypochlorite group showed no statistically significant differences between the segments when NaOCl was used (Table 4) (Figure 1). Chlorhexidine gluconate group showed significantly higher mean percentage of debris at the middle segment followed by the coronal third (Table 4). The saline group showed a significantly higher percentage of debris at the apical third when compared to other sections of the root.

B- Changes in dentin chemical structure:

I-Inorganic content:

The evaluation of the inorganic content of root canal dentin following different irrigation regimens showed no significant difference in the calcium, phosphorus, magnesium and carbon content when compared to saline group (Table 5).

II-Organic Analysis:

FTIR spectra of root canal treated dentin measured in the frequency region 4000-400 cm^{-1} showed the following reflection bands:

1- Phosphate $(PO_4)^{-3}$ appears in frequencies 562, 633 & 685 cm^{-1}, and as asymmetric stretching (υ_2) in frequency 1018 cm^{-1} and as symmetric stretching (υ_1) in 965 cm^{-1}.
2- Carbonate $(CO3)^{-2}$ (υ_2) appears in 882 cm^{-1} and as $(CO3)^{-2}$ (υ_3) in a broad band from 1418-1451 cm^{-1}.
3- (-CO-NH_2-vibrations) in amide I protein appears in frequency in 1648 cm^{-1}.
   (-CO-NH-vibrations) in amide II protein appears in frequency 1543 cm^{-1}.

Table 2. Comparison between the mean percentages of debris of different groups [95% (CI)]

5.25% NaOCl gp I	2% CHX gp II	Saline gp III	*P*-value	gp I vs gp II	gp I vs gp III	gp II vs gp III
Mean (SD)	Mean (SD)	Mean (SD)		Mean difference	Mean difference	Mean difference
25.7 (5.3)	37.5 (16.7)	53.5 (31.5)	0.04*	11.8 (-14.4, 38.0)	**27.8 (1.6, 54.0)**	16.0 (-10.2, 42.2)

*CI; Confidence interval. * Significant at P≤0.05. Values in **bold** indicate significant results.*

Table 3. Mean (SD) of remaining percentage of debris at different root levels (thirds) within each group. [95% (CI)]

Group Level	5.25% NaOCl gp I	2% CHX gp II	Saline gp III	*P*-value	gp I vs gp II	gp I vs gp III	gp II vs gp III
Coronal	21.4 (4.2)	46.2 (22.3)	35.7 (6.8)	0.006*	24.8 (7.0, 42.6)	14.3 (-3.5, 32.1)	-10.5 (-28.3,7.3)
Middle	28.1 (5.2)	49.2 (31.8)	49.6 (25.9)	0.03*	21.1 (0.6 to 41.5)	21.5 (1.0, 41.9)	0.4 (20.1 to 20.9)
Apical	27.2 (23.0)	17.2 (15.8)	75.2 (27.9)	<0.001*	-10.0 (-24.2, 4.2)	48.0 (33.8, 63.2)	58.0 (43.8, 72.2)

*CI; Confidence interval, * Significant at P≤0.05. Values in **bold** indicate significant results*

Table 4. Mean (SD) of percentage of debris at different thirds for different irrigating regimes

Group Level	5.25% NaOCl gp I	2% CHX gp II	Saline gp III
Coronal	21.4 (4.2)	46.2 (22.3)	35.7 (6.8)
Middle	28.1 (5.2)	49.2 (31.8)	49.62 (5.9)
Apical	27.2 (23.0)	17.2 (15.8)	75.22 (7.9)
***P*-value**	0.60	0.03*	0.04*

** Significant at P≤0.05.*

Table 5. Mean (SD) of the effect of different irrigating solutions on mineral content of root canal dentin

	5.25%NaOCl gp I	2%CHX gp II	Saline gp IV	*P*-value
Calcium	24.2 (6.2)	20.3 (8)	21.3 (8.1)	0.50
Phosphorus	12.1 (2.5)	9.4 (3)	11.3 (3.4)	0.62
Magnesium	0.5 (0.2)	0.7 (0.2)	0.5 (0.2)	0.92
Carbon	18.8 (9.7)	20 (6.1)	18.9 (8)	0.94

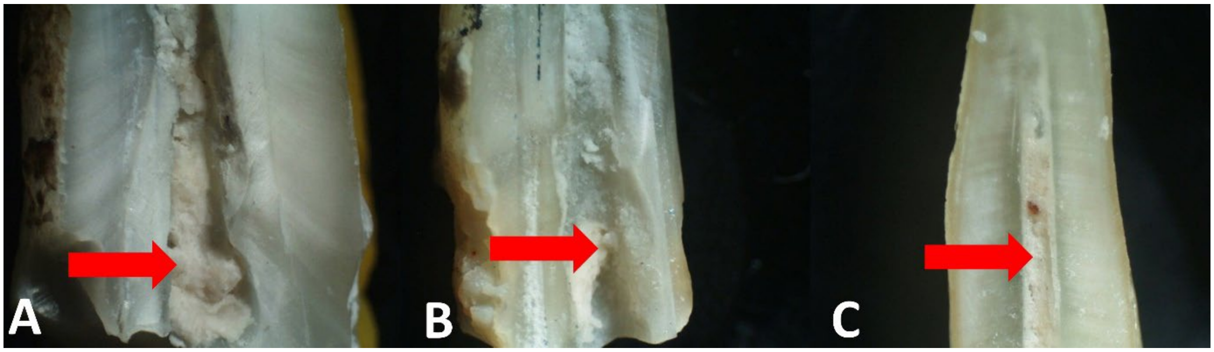

Figure 2. *A, B* and *C)* representing coronal, middle and apical thirds of the root, respectively, of samples irrigated with saline showing that the canal system is not clean and full of debris in root thirds (red arrows point to debris)

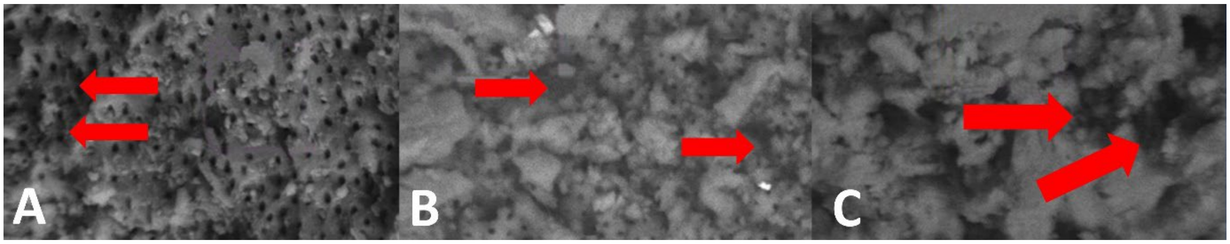

Figure 3. *A*) SEM image of specimens irrigated with NaOCl showing significantly lower debris value with partially opened dentinal tubules (red arrows); *B*) SEM image of specimens irrigated with CHX showing slightly more debris (red arrows) when compared to the NaOCl group; *C*) SEM image showing that the root canal system of the control specimen was not properly cleansed (red arrows representing debris)

The fourier transform infrared-attenuated total reflectance spectroscopic spectra of human dentin is shown in (Figure 5), representing the root canal irrigation with the three regimens of treatments as NaOCl, CHX, and saline solution to point out the effect of each one on the dentin composition. Root canal irrigation with the tested irrigants induced changes in the FTIR spectra of root canal dentin. These changes involved both the frequency and the intensity of bands.

Changes in frequency:

The vibrational bands of dentin under the effect of the tested irrigants is presented in Table 6. None of the tested irrigants showed changes in the frequency of the organic or inorganic components except for the samples irrigated with NaOCl, which showed total disappearance of the amide II.

Table 6. A summary of the changes in frequency of organic and inorganic components of dentin with different irrigants

Group	PO_4	CO_3	Amide I	Amide II
NaOCl	1032	1400	1600	disappeared
CHX	1032	1400	1600	1550
Saline	1032	1400	1600	1550

Changes in intensity

The tested irrigation regimens induced changes in the intensity of different bands as reflected by the normalized absorbance percent (Figures 5A, 5B, 5C). When compared to the control group, the NaOCl absorbance peak for carbonate and phosphate showed an increase. The absorbance peak for carbonate and phosphate was marginally lower than that of the control group (Figure 5). The changes in the intensity of the organic components showed that the absorbance peak of amide I remained unchanged, while the absorbance peak for amide II is not visible when compared to the control group. Chlorhexidine gluconate showed a similar absorbance peak for both amide I and II when compared to the control group (Figure 5).

Discussion

Root canal cleanliness was investigated using stereomicroscopy and scanning electron microscopy for obtaining a complete overview of the canal surface and regions. The evaluation of the organic and inorganic changes was done by splitting each sample into two longitudinal halves, where one half was tested for the organic changes, and the second half was tested for the inorganic changes. This method was employed in an attempt to minimize dental structure variability.

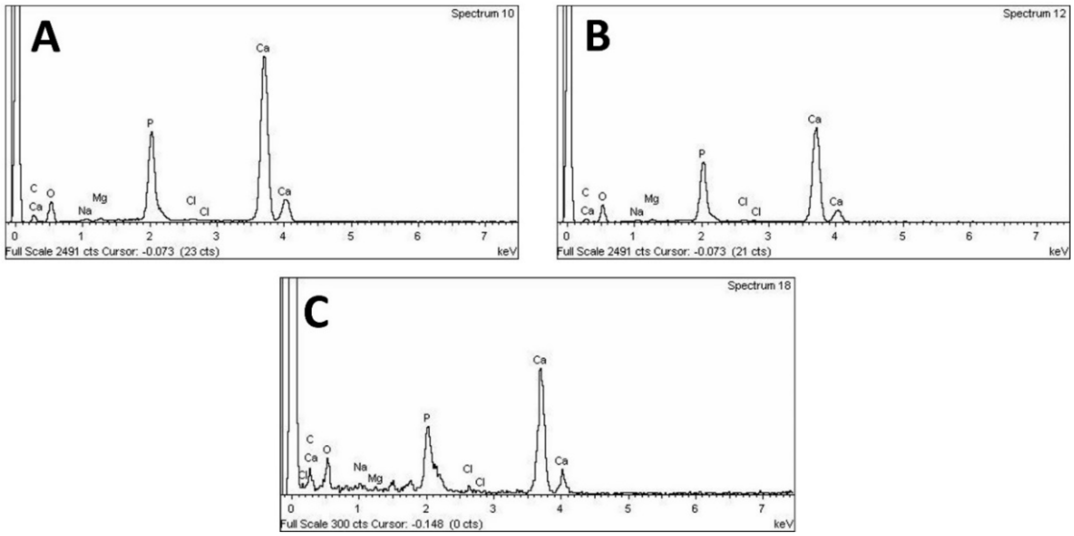

Figure 4. *A*) EDX analysis for samples irrigated with 5.25% a NaOCl showing an increase in the calcium and phosphorus levels; *B*) EDX analysis for samples irrigated with 2% CHX showing a decrease in the calcium and phosphorus levels; *C*) EDX analysis for samples irrigated with saline (control group)

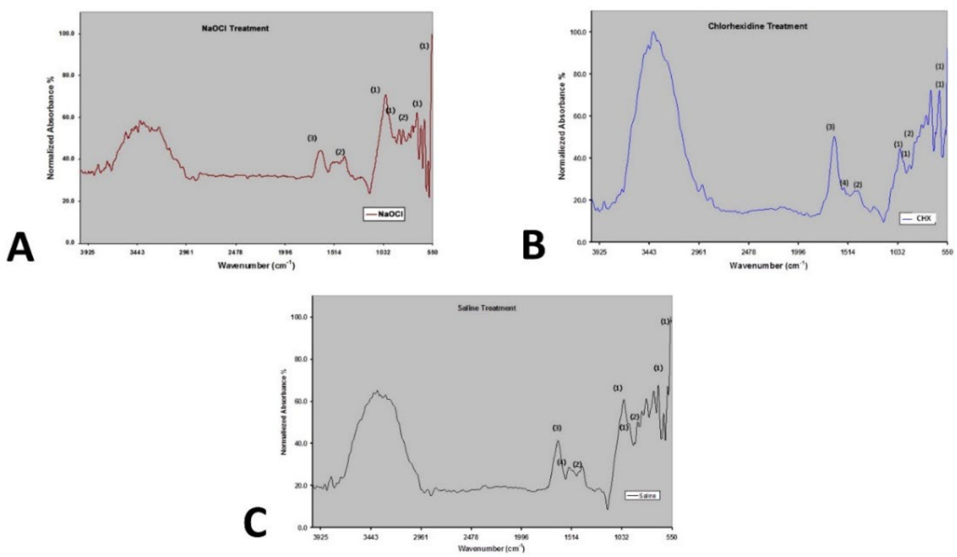

Figure 5: *A*) ATR FTIR Spectra of dentin irrigated with 5.25% NaOCI showing total disappearance of amide II; *B*) The amide II group remained unchanged with 2% CHX; *C*) Control group

To evaluate the changes in the inorganic structure of root canal dentin, the scanning electron microscope energy dispersive X-ray system was used. Scanning electron microscope energy dispersive X-ray has a significant technological advance in instrumentation and is ideally suited for detailing surface morphology and identifying surface composition. Infrared spectroscopy was used in this study for measurement of changes in dentin organic content. This method was selected to overcome the problems encountered with the X-ray diffraction method. The latter method requires total grinding of the sample, thus combining the affected superficial layers of dentin with the deeper unaffected layers [53].

Results demonstrated that the saline group showed the highest mean debris value. This was followed by CHX group. Sodium hypochlorite group showed the lowest mean debris value. Sodium hypochlorite is known to fragment long peptide chains and chlorinate protein terminal group; the resulting N-chloramines are broken down into other species [54-56]. Sodium hypochlorite acts as organic and fat solvent degrading fatty acids [26, 57]. Cleaning efficiency of NaOCl is better than CHX [19]. However, because of the outstanding substantive property of CHX, it remains as an irrigant used for endodontic treatment [58].

Regarding the segments, in the NaOCl group, cleanliness of the apical third was less than the coronal third. This may be due to small diameter of root canals; it is often difficult for the irrigant to reach the apex of the tooth. There is a little chance of tissue contact for the chemical action of the irrigant to be effective; however, the coronal third acts as a reservoir for the irrigant, thus increasing its cleaning and flushing action. This is in agreement with Abbott *et al.* [10] and Yamashita *et al.* [19], who also found that cleanliness of the apical third was less than the coronal third.

In the CHX group, cleanliness of the apical third was better than the coronal third. This could be supported by the experimental findings of Giardino *et al.* [59], who measured surface tension of different irrigant solutions and found out that Cetrexidine (0.2% cetrimide + CHX in aqueous solution) had low surface tension. However, this does not explain why the cleanliness of CHX group in the apical third was better than the coronal third, hence this observation needs to be further explored.

The results of the presented investigation showed that root canal irrigation with NaOCl affected the chemical structure of root canal dentin more than CHX. This was clearly demonstrated in this study where irrigation with NaOCl resulted in total disappearance of amide II group. This is attributed to the fact that NaOCl fragments the peptide chains into amine groups which further decomposes to other biproducts including inter- and intramolecular crosslinks *via* Schiff base formation [56, 60]. This was in agreement with other studies reporting high weight loss due to dissolution of organic components, especially the amide groups [24, 25, 41]. Amide II remained unchanged with CHX. This is supported by studies reporting that CHX had no effect on roughness and hardness of the root canal dentin [30].

Furthermore, NaOCl was capable of changing carbonate and phosphate groups. This is in agreement with Tsuda *et al.* [25] and Dogan and Calt [34]. On the contrary, Sakae *et al.* [26] reported a decrease in carbonates following NaOCl irrigation. In addition to the above, the CHX group resulted in an increase in amide I group, and a decrease in phosphate and carbonate group. This may be due to the substantivity of CHX; however, further studies are needed to verify this.

Being a proteolytic agent of alkaline nature, NaOCl is not expected to affect the mineral content of dentin. However, in the presented study, irrigation with NaOCl increased calcium and phosphorus. This may be due to the suggestion that dissolution of organic ions results in an increase in the mineralized layer. This is in agreement with Baumgartner *et al.* [61, 62] who suggested that irrigation with NaOCl may expose the inorganic material that prevents further dissolution of dentin or it may dissolve the organic components and leave a smear layer of mineralized tissue. In our study, irrigation with NaOCl did not alter the magnesium levels. This is in agreement with Dogan and Qalt [34] who suggested that magnesium in dentin may be bound to phosphate, carbonate, or the organic matrix.

In this study, there was a trend that indicated a decrease in calcium and phosphorus levels with CHX irrigation; however, this was not statistically significant. This is in agreement with Sayin *et al.* [38] who suggested that because CHX is a cationic compound, it has the ability to bind anionic molecules, such as the phosphate present in the structure of hydroxyapatite. Phosphate exists in the calcium carbonate complexes in dentin. Chlorhexidine gluconate can bind phosphate, which in turn can lead to the release of small amounts of Ca2+ ion from the root canal dentin. Chlorhexidine gluconate might show a possible indirect effect on the removal of Ca2+ ion by binding the phosphate. Further studies should be carried out to clarify the exact nature of the interaction between hydroxyapatite crystals and CHX.

Optimizing the concentration of irrigant solutions is crucial for enhancing the tissue-dissolving properties of irrigants [21-47, 51-63]. The 5.25% concentration of NaOCl used in this study was consistent with previous studies [13, 15] and proved to have the most superior properties in terms on antibacterial (bactericidal) effect, and dissolution of organic debris and necrotic tissues [62, 64, 65]. Despite higher concentrations of NaOCl being more effective as irrigants, this should be considered as a double-edged sword due to reports about higher concentrations of NaOCl being toxic to the periapical tissues [66-68], as well as negatively influencing the mechanical properties of radicular dentin through decreasing hardness and increasing roughness [30]. Therefore, caution should be always practiced in a clinical setting and clinical judgment should be made to decide the best NaOCl concentration to be used in individual cases based on a risk *versus* benefit analysis.

Furthermore, with the rapid advancement of irrigation systems, the irrigation delivery mode is equally important to the irrigant concentration, and should be considered carefully in treatment planning and clinical decision making [69-72]

Conclusions

1- Cleanliness of root canal dentin was affected by the type of irrigant where NaOCl produced the best effect.
2- The tested irrigation regimens did not affect the inorganic content of root canal dentin.
3- Sodium hypochlorite adversely affected the organic content of root canal dentin.

Therefore, based on the results of the presented study, 5.25% NaOCl is the irrigant of choice.

Conflict of Interest: 'None declared'.

References

1. Leonardo MR, Tanomaru Filho M, Silva LA, Nelson Filho P, Bonifacio KC, Ito IY. In vivo antimicrobial activity of 2% chlorhexidine used as a root canal irrigating solution. J Endod. 1999;25(3):167-71.
2. Kuruvilla JR, Kamath MP. Antimicrobial activity of 2.5% sodium hypochlorite and 0.2% chlorhexidine gluconate separately and combined, as endodontic irrigants. J Endod. 1998;24(7):472-6.
3. Ohara P, Torabinejad M, Kettering JD. Antibacterial effects of various endodontic medicaments on selected anaerobic bacteria. J Endod. 1993;19(10):498-500.
4. Inaba D, Ruben J, Takagi O, Arends J. Effect of sodium hypochlorite treatment on remineralization of human root dentine in vitro. Caries Res. 1996;30(3):218-24.
5. Zehnder M, Kosicki D, Luder H, Sener B, Waltimo T. Tissue-dissolving capacity and antibacterial effect of buffered and unbuffered hypochlorite solutions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2002;94(6):756-62.
6. Vieira Rde S, da Silva IA, Jr. Bond strength to primary tooth dentin following disinfection with a chlorhexidine solution: an in vitro study. Pediatr Dent. 2003;25(1):49-52.
7. Siqueira JF, Jr., Batista MM, Fraga RC, de Uzeda M. Antibacterial effects of endodontic irrigants on black-pigmented gram-negative anaerobes and facultative bacteria. J Endod. 1998;24(6):414-6.
8. Heling I, Chandler NP. Antimicrobial effect of irrigant combinations within dentinal tubules. Int Endod J. 1998;31(1):8-14.
9. Jeansonne MJ, White RR. A comparison of 2.0% chlorhexidine gluconate and 5.25% sodium hypochlorite as antimicrobial endodontic irrigants. J Endod. 1994;20(6):276-8.
10. Abbott PV, Heijkoop PS, Cardaci SC, Hume WR, Heithersay GS. An SEM study of the effects of different irrigation sequences and ultrasonics. Int Endod J. 1991;24(6):308-16.
11. Walker TL, del Rio CE. Histological evaluation of ultrasonic debridement comparing sodium hypochlorite and water. J Endod. 1991;17(2):66-71.
12. Franchi M, Eppinger F, Filippini GF, Montanari G. NaOCl and EDTA irrigating solutions for endodontics: SEM findings. Bull Group Int Rech Sci Stomatol Odontol. 1992;35(3-4):93-7.
13. Berutti E, Marini R. A scanning electron microscopic evaluation of the debridement capability of sodium hypochlorite at different temperatures. J Endod. 1996;22(9):467-70.
14. Turkun M, Cengiz T. The effects of sodium hypochlorite and calcium hydroxide on tissue dissolution and root canal cleanliness. Int Endod J. 1997;30(5):335-42.
15. Takeda FH, Harashima T, Kimura Y, Matsumoto K. A comparative study of the removal of smear layer by three endodontic irrigants and two types of laser. Int Endod J. 1999;32(1):32-9.
16. Peters OA, Barbakow F. Effects of irrigation on debris and smear layer on canal walls prepared by two rotary techniques: a scanning electron microscopic study. J Endod. 2000;26(1):6-10.
17. Guerisoli DM, Marchesan MA, Walmsley AD, Lumley PJ, Pecora JD. Evaluation of smear layer removal by EDTAC and sodium hypochlorite with ultrasonic agitation. Int Endod J. 2002;35(5):418-21.
18. Torabinejad M, Cho Y, Khademi AA, Bakland LK, Shabahang S. The effect of various concentrations of sodium hypochlorite on the ability of MTAD to remove the smear layer. J Endod. 2003;29(4):233-9.
19. Yamashita JC, Tanomaru Filho M, Leonardo MR, Rossi MA, Silva LA. Scanning electron microscopic study of the cleaning ability of chlorhexidine as a root-canal irrigant. Int Endod J. 2003;36(6):391-4.
20. Irala LE, Grazziotin-Soares R, Salles AA, Munari AZ, Pereira JS. Dissolution of bovine pulp tissue in solutions consisting of varying NaOCl concentrations and combined with EDTA. Braz Oral Res. 2010;24(3):271-6.
21. Stojicic S, Zivkovic S, Qian W, Zhang H, Haapasalo M. Tissue dissolution by sodium hypochlorite: effect of concentration, temperature, agitation, and surfactant. J Endod. 2010;36(9):1558-62.
22. Yamazaki AK, Moura-Netto C, Salgado RJ, Kleine BM, Prokopowitsch I. Ex vivo analysis of root canal cleaning using Endo-PTC associated to NaOCl and different irrigant solutions. Braz Oral Res. 2010;24(1):15-20.
23. Marshall GW, Jr. Dentin: microstructure and characterization. Quintessence Int. 1993;24(9):606-17.
24. Nadalin MR, Perez DE, Vansan LP, Paschoala C, Souza-Neto MD, Saquy PC. Effectiveness of different final irrigation protocols in removing debris in flattened root canals. Braz Dent J. 2009;20(3):211-4.
25. Tsuda H, Ruben J, Arends J. Raman spectra of human dentin mineral. Eur J Oral Sci. 1996;104(2 (Pt 1)):123-31.
26. Sakae T, Mishima H, Kozawa Y. Changes in bovine dentin mineral with sodium hypochlorite treatment. J Dent Res. 1988;67(9):1229-34.
27. Zhang K, Tay FR, Kim YK, Mitchell JK, Kim JR, Carrilho M, Pashley DH, Ling JQ. The effect of initial irrigation with two different sodium hypochlorite concentrations on the erosion of instrumented radicular dentin. Dent Mater. 2010;26(6):514-23.
28. Saleh AA, Ettman WM. Effect of endodontic irrigation solutions on microhardness of root canal dentine. J Dent. 1999;27(1):43-6.
29. Sim TP, Knowles JC, Ng YL, Shelton J, Gulabivala K. Effect of sodium hypochlorite on mechanical properties of dentine and tooth surface strain. Int Endod J. 2001;34(2):120-32.
30. Ari H, Erdemir A, Belli S. Evaluation of the effect of endodontic irrigation solutions on the microhardness and the roughness of root canal dentin. J Endod. 2004;30(11):792-5.
31. Slutzky-Goldberg I, Maree M, Liberman R, Heling I. Effect of sodium hypochlorite on dentin microhardness. J Endod. 2004;30(12):880-2.
32. Oliveira LD, Carvalho CA, Nunes W, Valera MC, Camargo CH, Jorge AO. Effects of chlorhexidine and sodium hypochlorite on the microhardness of root canal dentin. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007;104(4):e125-8.
33. Patil CR, Uppin V. Effect of endodontic irrigating solutions on the microhardness and roughness of root canal dentin: an in vitro study. Indian J Dent Res. 2011;22(1):22-7.
34. Dogan H, Qalt S. Effects of chelating agents and sodium hypochlorite on mineral content of root dentin. J Endod. 2001;27(9):578-80.
35. Ari H, Erdemir A. Effects of endodontic irrigation solutions on mineral content of root canal dentin using ICP-AES technique. J Endod. 2005;31(3):187-9.
36. Gonzalez-Lopez S, Camejo-Aguilar D, Sanchez-Sanchez P, Bolanos-Carmona V. Effect of CHX on the decalcifying effect of 10% citric acid, 20% citric acid, or 17% EDTA. J Endod. 2006;32(8):781-4.
37. Sayin TC, Serper A, Cehreli ZC, Kalayci S. Calcium loss from root canal dentin following EDTA, EGTA, EDTAC, and tetracycline-HCl treatment with or without subsequent NaOCl irrigation. J Endod. 2007;33(5):581-4.
38. Sayin TC, Cehreli ZC, Deniz D, Akcay A, Tuncel B, Dagli F, Gozukara H, Kalayci S. Time-dependent decalcifying effects of endodontic

irrigants with antibacterial properties. J Endod. 2009;35(2):280-3.
39. Beltz RE, Torabinejad M, Pouresmail M. Quantitative analysis of the solubilizing action of MTAD, sodium hypochlorite, and EDTA on bovine pulp and dentin. J Endod. 2003;29(5):334-7.
40. Surapipongpuntr P, Duangcharee W, Kwangsamai S, Ekka A. Effect of root canal irrigants on cervical dentine permeability to hydrogen peroxide. Int Endod J. 2008;41(10):821-7.
41. Mountouris G, Silikas N, Eliades G. Effect of sodium hypochlorite treatment on the molecular composition and morphology of human coronal dentin. J Adhes Dent. 2004;6(3):175-82.
42. Nalla RK, Balooch M, Ager JW, 3rd, Kruzic JJ, Kinney JH, Ritchie RO. Effects of polar solvents on the fracture resistance of dentin: role of water hydration. Acta Biomater. 2005;1(1):31-43.
43. Hennequin M, Douillard Y. Effects of citric acid treatment on the Ca, P and Mg contents of human dental roots. J Clin Periodontol. 1995;22(7):550-7.
44. Hennequin M, Pajot J, Avignant D. Effects of different pH values of citric acid solutions on the calcium and phosphorus contents of human root dentin. J Endod. 1994;20(11):551-4.
45. Rotstein I, Dankner E, Goldman A, Heling I, Stabholz A, Zalkind M. Histochemical analysis of dental hard tissues following bleaching. J Endod. 1996;22(1):23-5.
46. Van Meerbeek B, Lambrechts P, Inokoshi S, Braem M, Vanherle G. Factors affecting adhesion to mineralized tissues. Oper Dent. 1992;Suppl 5:111-24.
47. Ruse ND, Smith DC. Adhesion to bovine dentin--surface characterization. J Dent Res. 1991;70(6):1002-8.
48. Afkhami F, Sadegh M, Sooratgar A, Montazeri E. Effect of Smear Clear and Conventional Root Canal Irrigants on Push-Out Bond Strength of Resilon/Epiphany System. Iran Endod J. 2018;13(4):481-5.
49. Razmi H, Bolhari B, Karamzadeh Dashti N, Fazlyab M. The Effect of Canal Dryness on Bond Strength of Bioceramic and Epoxy-resin Sealers after Irrigation with Sodium Hypochlorite or Chlorhexidine. Iran Endod J. 2016;11(2):129-33.
50. Baxter S, Nickoll M, Konietschke F, Rödig T. Efficacy of Different Irrigation Techniques in Removing Ledermix Paste from Simulated Root Canal Irregularities. Iran Endod J. 2019;14(1):28-34.
51. Mokhtari F, Anvar E, Mirshahpanah M, Hemati H, Danesh Kazemi A. The Probable Effect of Irrigation Solution and Time on Bond Strength to Coronal Dentin: An In Vitro Evaluation. Iran Endod J. 2017;12(4):439-42.
52. Karade P, Chopade R, Patil S, Hoshing U, Rao M, Rane N, Chopade A, Kulkarni A. Efficiency of Different Endodontic Irrigation and Activation Systems in Removal of the Smear Layer: A Scanning Electron Microscopy Study. Iran Endod J. 2017;12(4):414-8.
53. Gurbuz T, Ozdemir Y, Kara N, Zehir C, Kurudirek M. Evaluation of root canal dentin after Nd:YAG laser irradiation and treatment with five different irrigation solutions: a preliminary study. J Endod. 2008;34(3):318-21.
54. Davies JM, Horwitz DA, Davies KJ. Potential roles of hypochlorous acid and N-chloroamines in collagen breakdown by phagocytic cells in synovitis. Free Radic Biol Med. 1993;15(6):637-43.
55. Stoward PJ. A histochemical study of the apparent deamination of proteins by sodium hypochlorite. Histochemistry. 1975;45(3):213-26.
56. Hawkins CL, Davies MJ. Hypochlorite-induced damage to proteins: formation of nitrogen-centred radicals from lysine residues and their role in protein fragmentation. Biochem J. 1998;332 (Pt 3)(Pt 3):617-25.
57. Estrela C, Estrela CR, Barbin EL, Spano JC, Marchesan MA, Pecora JD. Mechanism of action of sodium hypochlorite. Braz Dent J. 2002;13(2):113-7.
58. Carrilho MR, Carvalho RM, Sousa EN, Nicolau J, Breschi L, Mazzoni A, Tjaderhane L, Tay FR, Agee K, Pashley DH. Substantivity of chlorhexidine to human dentin. Dent Mater. 2010;26(8):779-85.
59. Giardino L, Ambu E, Becce C, Rimondini L, Morra M. Surface tension comparison of four common root canal irrigants and two new irrigants containing antibiotic. J Endod. 2006;32(11):1091-3.
60. Daumer KM, Khan AU, Steinbeck MJ. Chlorination of pyridinium compounds. Possible role of hypochlorite, N-chloramines, and chlorine in the oxidation of pyridinoline cross-links of articular cartilage collagen type II during acute inflammation. J Biol Chem. 2000;275(44):34681-92.
61. Baumgartner JC, Cuenin PR. Efficacy of several concentrations of sodium hypochlorite for root canal irrigation. J Endod. 1992;18(12):605-12.
62. Baumgartner JC, Mader CL. A scanning electron microscopic evaluation of four root canal irrigation regimens. J Endod. 1987;13(4):147-57.
63. Lima Nogueira BM, da Costa Pereira TI, Pedrinha VF, de Almeida Rodrigues P. Effects of Different Irrigation Solutions and Protocols on Mineral Content and Ultrastructure of Root Canal Dentine. Iran Endod J. 2018;13(2):209-15.
64. Hand RE, Smith ML, Harrison JW. Analysis of the effect of dilution on the necrotic tissue dissolution property of sodium hypochlorite. J Endod. 1978;4(2):60-4.
65. Yamada RS, Armas A, Goldman M, Lin PS. A scanning electron microscopic comparison of a high volume final flush with several irrigating solutions: Part 3. J Endod. 1983;9(4):137-42.
66. Mehra P, Clancy C, Wu J. Formation of a facial hematoma during endodontic therapy. J Am Dent Assoc. 2000;131(1):67-71.
67. Gernhardt CR, Eppendorf K, Kozlowski A, Brandt M. Toxicity of concentrated sodium hypochlorite used as an endodontic irrigant. Int Endod J. 2004;37(4):272-80.
68. Barnhart BD, Chuang A, Lucca JJ, Roberts S, Liewehr F, Joyce AP. An in vitro evaluation of the cytotoxicity of various endodontic irrigants on human gingival fibroblasts. J Endod. 2005;31(8):613-5.
69. Widjiastuti I, Rudyanto D, Yuanita T, Bramantoro T, Aries Widodo W. Cleaning Efficacy of Root Canal Irrigation with Positive and Negative Pressure System. Iran Endod J. 2018;13(3):398-402.
70. Shalan LA, Al-Huwaizi HF. Cleaning Efficiency of Root Canal after Irrigation with New Irrigation Technique: A Scanning Electron Microscopic Study. Iran Endod J. 2018;13(1):102-7.
71. Ghoddusi J, Moushekhian S, Arian E, Ghiasi J, Forghani M. The Effectiveness of Sonic-Activated Irrigation in Reducing Intratubular Enterococcus faecalis. Iran Endod J. 2019;14(1):63-7.
72. de Vasconcelos Neves G, Dos Santos KSA, de Souza Sales Rocha EAL, de Moura RQ, Morais Barros DG, Gominho LF, de Castro Gomes DQ. Antibacterial Effect of Photodynamic Therapy on Root Canal Disinfection Combined with Different Irrigation Protocols. Iran Endod J. 2020;15(2):90-5.

Please cite this paper as: Elgawish A, Tawfik H, El Gendy A, Roy George , Bakr MM. The Impact of Different Irrigation Regimens on the Chemical Structure and Cleanliness of root canal Dentin. Iran Endod J. 2023;18(4): 224-32. *Doi: 10.22037/iej.v18i4.38004.*