Root Canal Dentin Erosion Following Different Irrigation Protocols

by

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The following individuals certify that they have read, and recommend to the Faculty of Graduate and Postdoctoral Studies for acceptance, a thesis entitled:

Root Canal Dentin Erosion Following Different Irrigation Protocols

submitted by Michael Magnusson in partial fulfillment of the requirements for

the degree of Master of Science

in Craniofacial Science

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Abstract

Objectives: It has been previously shown that root dentin erosion increases if sodium hypochlorite (NaOCl) is used again following final ethylenediaminetetraacetic acid (EDTA). The purpose of this study is to quantitatively examine the erosion of root dentin when alternating these irrigants during instrumentation.

Methods: Eight extracted teeth were instrumented to size #40/04, sequentially from #15/04, using Vortex Blue rotary files (Dentsply Tulsa Dental, Tulsa, OK). Teeth were then randomly divided into four groups based on irrigation protocol as follows: Group 1: negative controls, irrigated with saline throughout the instrumentation process and as a final rinse. Group 2: 6% NaOCl was used between files at a flow rate of 5mL/min for 15 seconds. Final irrigation was 2 minutes 6% NaOCl followed by 2 minutes 17% EDTA. Group 3: Same protocol as group 2, plus an additional rinse with 6% NaOCl (2 minutes, 5mL/min) after EDTA. Group 4: Alternating irrigation using NaOCl and EDTA, each for 15 seconds between files during instrumentation. A final rinse was carried out alternating NaOCl, EDTA and NaOCl for 15 seconds each. All teeth were then sectioned into 1mm disks and the dentin element content was analyzed by continuous line scanning up to 300 µm from the canal lumen using energy-dispersive X-ray spectroscopy.

Results: Statistically significant differences in atomic percentages of carbon, oxygen and calcium were observed between groups. Group 4 had significantly lower atomic percentages of calcium and significantly higher atomic percentages of carbon than both group 1 and group 2. Significantly lower calcium was also observed in group 3 compared to group 1 while higher levels of carbon and oxygen were observed in group 3 compared to group 2. A trend toward lower levels of calcium and phosphorus was seen in group 4 compared to group 3, however differences were not statistically significant.

Conclusion: Alternating NaOCl and EDTA throughout instrumentation resulted in dentin composition with the lowest percentages of Ca and P, indicating higher potential for root dentin erosion.

Lay Summary

Different chemical solutions are used during the cleaning of root canals. Two of the most common solutions used are NaOCl and EDTA. When used in sequence, NaOCl first, EDTA second, these two chemicals enable disinfection of the root canal system, as well as removal of both hard and soft tissue debris. However, a reversed sequence used during the cleaning process can lead to erosion of dentin, resulting in weakened mechanical properties of the tooth root. This may affect long term outcomes of root canal treatment.

It is a common practice by many clinicians to alternate NaOCl and EDTA during the instrumentation process of root canal treatment instead of using only NaOCl followed by EDTA as a final rinse. The goal of this study is to see what changes in root dentin result from alternating these chemicals during root canal treatment using a method of structural analysis.

Preface

This thesis is the principal work Michael Magnusson, as part of the requirements of a Master of Science in Craniofacial Science with a Diploma in Endodontics. Study design was developed by the supervisor Dr. Markus Haapasalo, Dr. Zhejun Wang and Dr. Michael Magnusson. Collection and preparation of samples, data analysis, and thesis writing were completed by Michael Magnusson. Imaging and data reporting was carried out by Dr. Zhejun Wang. Dr. Markus Haapasalo further contributed by editing the thesis. The relative contribution to this research project by Michael Magnusson was 80%.

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List of Symbols

%	Percent
µ	Micro
±	Plus minus
℃	Degrees Celsius

List of Abbreviations

C	Carbon
Ca	Calcium
EDS	Energy-dispersive X-ray spectroscopy
EDTA	Ethylenediaminetetraacetic acid
GPa	Gigapascals
kg/mm^2	Kilogram per millimetre squared
kV	Kilovolts
min	Minutes
mL	Millilitres
MPa	Megapascals
NaOCl	Sodium hypochlorite
nm	Nanometers
NiTi	Nickel Titanium
O	Oxygen
P	Phosphorus
pH	Power of hydrogen

SEM	Scanning electron microscopy
WL	Working length

Acknowledgements

I am very grateful to have had the opportunity to complete my research with my supervisor Dr. Markus Haapasalo. I have greatly appreciated and benefited from his wisdom, kindness and humour throughout the process. I would also like to thank my committee members Dr. Ya Shen and Dr. Zhejun Wang, who have been generous with their time and knowledge, and offered their support without hesitation. My sincere thanks to our program director Dr. Jeff Coil, as well as our clinical instructors for their invaluable mentorship. They lead by example, demonstrating the value of research in their clinical excellence. I'd also like to express my appreciation for the hard work and support of our clinical staff Shauna, Francisco and Lois who continually go above and beyond to help us achieve our best efforts. Finally, a special thanks for the friendship and camaraderie of my past and present co-residents whom I have had the pleasure of sharing this experience with.

Dedication

This work is dedicated to my parents, Stewart and Susan Magnusson, who have always encouraged me to pursue my curiosity, and to my nephews, Jack and Simon Magnusson, whom I encourage to follow theirs.

Chapter 1: Review of the Literature

1.1 Introduction

The goal of endodontic treatment is to prevent or eliminate apical periodontitis while preserving the strength of the tooth (Ørstavik & Pitt Ford, 2008; Haapasalo et al, 2010). Apical periodontitis occurs as a result of microbial contamination of the root canal system (Kakehashi et al, 1965; Sundqvist, 1976). Therefore, in order to provide effective endodontic treatment, it is essential to eradicate or entomb pathogenic bacteria (Sundqvist & Figdor, 1998). This is achieved by root canal instrumentation, irrigation and obturation of the canal, as well as coronally restoring the tooth.

Mechanical cleaning alone cannot address all areas of the root canal system as significant portions of the root canal wall remain untouched during instrumentation with rotary files (Peters et al, 2001). Irrigation solutions with tissue dissolving ability and antimicrobial activity are utilized to enhance disinfection and address bacteria that remain after mechanical canal preparation (Byström et al, 1981). Furthermore, the use of an irrigating solution during instrumentation helps to increase cutting efficiency, flush debris and dissipate heat (Basrani & Haapasalo, 2012). Irrigation solutions are relied upon to dissolve both inorganic and organic tissues. Effective cleaning of the canal is facilitated by dissolution of vital and necrotic pulp tissue, biofilm, and dentin debris created during instrumentation. Ideally, the dissolution of these tissues is achieved while leaving the healthy canal wall dentin intact.

In order to achieve endodontic disinfection, it is important to instrument the canals to an extent in which irrigation solutions can be distributed effectively (Abou-Rass & Piccinino, 1982). Syringe needle irrigation remains one of the most common methods of distributing solutions within the root canal. However, fluid delivered by syringe and needle does not extend far beyond the needle tip (Chow, 1983; Park et al, 2013). Because of this, it is important that sufficient instrumentation is completed to allow for the tip of the needle to reach the apical portion of the root so that maximal disinfection of this area can be achieved (Siqueira et al, 2002). On the other hand, it is important not to over-enlarge the canal in order to preserve the strength of the tooth. It has been suggested that maximal dentin preservation during treatment can help to reduce the incidence of vertical root fracture (Trope & Ray, 1992). This has led to the concept of conservative endodontic treatment, which relies less on the mechanical shaping of root canals and more on the action of irrigation solutions. Further to conservative mechanical shaping, the effects of irrigation sequences capable of altering the structure of root dentin should be considered in the goal of preserving maximal root strength.

1.2 Dentin properties

The structural and mechanical properties of dentin help to provide tooth strength (Table 1). Dentin is a porous hard tissue that consists of a tubular network embedded within collagen and hydroxyapatite (Tjäderhane et al, 2009). Making up the bulk of tooth structure, intact dentin functions as a barrier to chemical and mechanical stresses on the tooth. By weight, dentin composition is approximately 70% mineral, 20% organic and 10% water. These numbers represent an average, as dentin is not uniform throughout its thickness. Dentin adjacent to the pulp consists of greater tubule density as well as greater tubule cross-sectional area compared to peripheral dentin. This is a result of a constant number of tubules extending between the smaller circumference of the pulp lumen to the larger circumference at the cemento-dentino junction. Furthermore, it is a result of greater amounts of highly mineralized peritubular dentin towards the periphery. These structural differences throughout the thickness of dentin translate to differences in mechanical properties (Craig et al, 1959; Pashley et al, 1985). Therefore, reported values for mechanical properties present as wide ranges. The microhardness of bulk dentin ranges from 40-70 kg/mm² while modulus of elasticity ranges from approximately 10-19 GPa (Table 1). Compared to enamel, dentin is both softer and more elastic. This creates a stress breaking function for the overlying enamel and allows dentin to be cut efficiently with stainless steel and NiTi endodontic files. Moreover, the variation of properties throughout the width of dentin provides effective distribution of mechanical forces through the tooth.

Table 1: Dentin properties (Pashley et al, 2012)

Compressive strength (MPa)	**217-300**
Young modulus of elasticity (GPa)	**10-19**
Shear strength (MPa)	**45-132**
Tensile strength (MPa)	**31-106**
Microhardness (kg/mm²)	**40-70**

1.3 Smear Layer

The smear layer as it relates to endodontic treatment was first described by the scanning electron microscope (SEM) study on instrumented root canal walls by McComb and Smith (1975). They noticed that standard endodontic instrumentation resulted in a canal wall that was smeared and packed with debris made up of dentin, remnants of odontoblastic processes, pulp tissue and bacteria (McComb & Smith, 1975). The smear layer is amorphous, irregular and granular with a thickness of 1-2 μm on instrumented areas of the canal (Goldman et al, 1981;

Mader et al, 1984). It has also been shown that smear plugs can extend 40 μm into dentinal tubules (Mader et al, 1984). Although there is debate on the need to remove the smear layer prior to obturation, it is recommended in order to enhance cleaning of the root canal system and enable an effective seal. With regards to cleaning, smear layer removal facilitates greater bacterial removal within the root canal system (Yoshida et al, 1995). First, and simply, removal of the smear layer results in the removal of its microbial component. Furthermore, it removes a substrate which may allow bacteria to penetrate deeper within the dentinal tubules (Violich & Chandler, 2010). The smear layer acts as a barrier between disinfecting solutions and bacteria within the dentinal tubules, therefore decreasing bacterial killing. Upon smear removal the dentinal tubules are exposed, allowing for enhanced irrigant effectiveness (Orstavik & Haapasalo, 1990; Wang et al, 2013, 2018).

The barrier effect of the smear layer also prevents filling materials from maximal adaptation to the root canal wall (White et al, 1987). Sealer penetration into dentinal tubules can occur beyond 60 μm when smear is removed, in comparison to no penetration with smear present (Okşan et al, 1993). The irregular structure and high content of water in the smear layer also provides a potential leakage pathway between the filling material and canal wall. Thus, smear removal is thought to increase the sealing ability of obturation material, which may translate to improved success rates (Pitt-Ford & Roberts, 1990).

1.4 Properties of irrigating solutions

The ideal endodontic irrigant serves many functions. Irrigation solutions facilitate instrumentation by providing lubrication, dispersing heat from friction and flushing debris. In addition, the ideal irrigant works to dissolve organic and/or inorganic matter, such as the smear layer and soft tissue remnants of the necrotic pulp, and effectively kill bacteria, yeasts and viruses. It is important for this to be done in a cost-effective manner, without adverse effects to the surrounding tissues or strength of the tooth. Currently, there is no single solution that achieves all these goals and as such, irrigant solutions used in combination is common in clinical practice.

1.4.1 Sodium hypochlorite

The first use of sodium hypochlorite (NaOCl) in endodontics is attributed to Coolidge (1919). Its beneficial properties make it a common irrigant used throughout endodontic cleaning and shaping. These properties include soft tissue dissolution of necrotic and vital tissue, and effective antibacterial action. Bystrom and Sundqvist (1983) showed that even at concentrations as low as 0.5%, NaOCl is able to reduce root canal bacteria to undetectable levels, when culturing was used to detect residual microbes after irrigation.

The tissue dissolving and antibacterial mechanisms by which NaOCl acts have been summarized by Estrella et al (2002). Saponification is the conversion of fatty acids to soap and alcohol. Neutralization involves the conversion of amino acids to water and salt. Chlorine dissolved in water and in contact with organic matter forms hypochlorous acid, a weak acid and oxidizer. Both hypochlorous acid and hypochlorite facilitate amino acid degradation and hydrolysis. Chloramination results when chlorine combines with protein amino groups. The chloramines that are formed are strong oxidants that irreversibly inhibit bacterial enzymes by oxidation of their sulfhydryl groups, ultimately impeding cell metabolism. Finally, the pH of sodium chloride is greater than 11. This has beneficial antibacterial effects by interfering with the bacterial cytoplasmic membrane, altering cell metabolism, and phospholipid degradation (Estrela et al, 2002).

Clinical factors can influence the effectiveness of NaOCl. Sufficient volume of solution and contact time are required to adequately clean the system (Siquiera et al, 2000). Chlorine is consumed rapidly in reaction and therefore it is important to replenish the fluid often (Moorer & Wesselink, 1982). Effectiveness can also be increased using agitation devices and by raising the temperature of the solution (Moorer & Wesselink, 1982; Cunningham & Joseph, 1980). Furthermore, higher concentrations of solution allow for faster tissue dissolution and disinfection (Hand, Smith, & Harrison, 1978). It has also been shown that higher concentrations of NaOCl are favoured in the killing of bacteria within a biofilm (Clegg et al, 2006; Stojicic et al, 2012). For this reason, NaOCl is often used at a concentration of 6% during root canal treatment. However, NaOCl toxicity is directly proportional to its concentration (Spangberg et al, 1973). Inadvertent extrusion from the root canal system to the periradicular area can result in a sodium hypochlorite accident, which is characterized by severe pain, swelling, ecchymosis and potential paresthesia. Other undesirable aspects of NaOCl include its bad taste, corrosive activity and lack of substantivity. Furthermore, NaOCl cannot remove the inorganic component of the smear layer, thereby leaving the smear layer seemingly unaffected if it is used as the sole irrigant (Goldman et al, 1981).

1.4.2 Ethylenediaminetetraacetic acid

Ethylenediaminetetraacedic acid (EDTA) is used in endodontic treatment to facilitate removal of the smear layer caused by instrumentation. As a chelating agent, EDTA dissolves dentin hydroxyapatite, including the inorganic tissue (smear layer and dentin debris) that remains attached to the root canal wall after instrumentation and irrigation with sodium hypochlorite (Yamada et al, 1983). The reaction is self-limiting and ceases after all molecules of solution have bound calcium (Hülsmann et al, 2003). When used alone, EDTA is unable to remove the entirety of the smear layer and leaves behind a fibrous soft tissue layer on the root canal wall (Baumgartner & Mader, 1987). However, when used following sodium hypochlorite, it has been shown that a final 1-minute rinse of 1mL of 17% EDTA can achieve smear layer removal in areas accessible to fluid exchange (Çalt & Serper, 2002; Saito et al, 2008). Therefore, it is important to ensure adequate time, volume and circulation of the EDTA solution within the root canal system.

In endodontic applications, EDTA is thought to have indirect antibacterial activity. Yoshida et al (1995) found that final irrigation with EDTA resulted in significantly less detection of bacteria at the subsequent appointment when compared to saline. This effect was thought to occur because of the removal of bacteria associated with the smear layer and not because of a direct antimicrobial action (Yoshida et al, 1995).

1.5 Effects of irrigants on dentin

Both NaOCl and EDTA have been shown to alter the physical properties of dentin. NaOCl exerts changes to the organic structure of dentin that result in adverse effects on its mechanical properties (Marending et al, 2007). Time dependent decreases in flexural strength and elastic modulus have been shown to occur with increasing concentrations of NaOCl (Sim et al, 2001). However, the smear layer can have a barrier effect, preventing NaOCl from reaching patent dentinal tubules (McComb & Smith, 1975). Therefore, when sodium hypochlorite is used as the sole irrigant during instrumentation, its effect on the structural properties of canal wall dentin is limited.

EDTA has also been shown to alter dentinal structure (Nygaard-Østby, 1957; Hülsmann et al, 2003). As a chelator, EDTA can demineralize exposed dentin, leaving behind its underlying organic component (Baumgartner & Mader, 1987). The result of such demineralization is a reduction of dentin microhardness particularly in the most superficial layers in relation to the root canal lumen (Cruz-Filho et al, 2011). Furthermore, the reduction of microhardness varies among canal region and appears to a lesser degree in the apical portion of the root due to higher amounts of sclerotic dentin (Sayin et al, 2007; Lottanti et al, 2009). However, the demineralizing effect of EDTA when used as a final rinse for 2 minutes does not manifest changes in the flexural strength of root dentin (Zhang et al, 2010).

1.6 Irrigant combinations

As neither NaOCl nor EDTA fulfil the requirements of endodontic irrigation when used alone, combination regimens have been suggested. It has been suggested that the alternating use of NaOCl and EDTA may facilitate more efficient reduction of bacterial loads (Basrani & Haapasalo, 2012). Additionally, in order to remove bacteria from the dentinal tubules, it has been shown that antimicrobial solutions are more effective after smear layer removal (Orstavik & Haapasalo, 1990; Wang et al, 2013, 2018). Yamada et al (1983) found that they were able to achieve a canal surface free of superficial debris with patent dentinal tubules when a final rinse was carried out with 17% EDTA followed by 5.25% NaOCl. Furthermore, use of EDTA after NaOCl results in a greater antimicrobial effect than when NaOCl is used alone (Byström & Sundqvist, 1985). However, use of NaOCl after smear layer removal with EDTA results in structural changes to root canal dentin. SEM images show eroded dentin surfaces along the canal wall when NaOCl is used after EDTA (Baumgartner & Mader, 1987; Qian et al, 2011). It has also been shown that NaOCl can penetrate dentinal tubules up to 300 µm (Zou et al, 2010). Wang et al (2016) showed that in samples using a final rinse of NaOCl after EDTA, alterations in dentin atomic percentages were constant up to that depth. These alterations included a decrease in calcium and phosphorous levels compared to samples in which a final rinse was completed with EDTA alone (Wang et al, 2016).

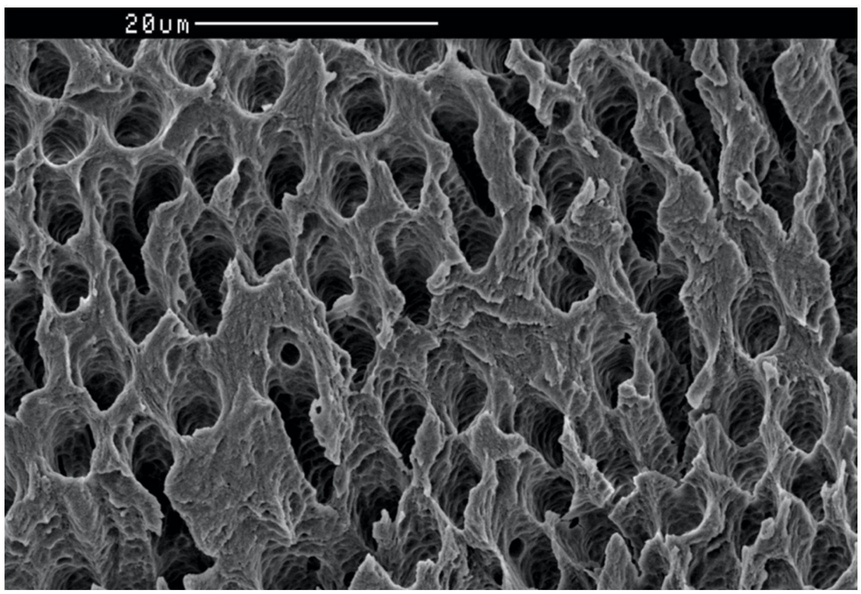

Figure 1: Erosion of root canal dentin after irrigation sequence NaOCl – EDTA - NaOCl. Image courtesy of Qian, Shen, Haapasalo.

1.7 Impact of dentin erosion on treatment outcome

Negative clinical outcomes resulting from the alteration of dentin mechanics due to irrigating solutions have yet to be proven. Vertical root fracture is regarded as a catastrophic failure of the tooth. Endodontic treatment and restorative procedures have been implicated as the primary causes of vertical root fracture. Morfis (1990) found that the incidence of vertical root fracture was higher when teeth were obturated by lateral condensation and when a prefabricated or cast post was present. Fuss et al (2001) examined 154 vertically fractured roots and found that 95 (61.7%) had posts placed, of which, 64 (67.3%) were screw posts. Rotary instrumentation of the root canal can also result in microcracks in root dentin which may propagate to vertical root fractures (Bier et al, 2009; Yoldas et al, 2012). Vertical root fracture is comparatively rare in untreated teeth (Tamse, 1988). Studies have indicated the importance of remaining dentinal thickness on fracture resistance of the roots (Lertchirakarn et al, 2003). This has led to regimens aimed at conserving the maximal amount of dentin while still achieving effective cleaning of the canal system. It is logical to assume that negative alterations to the mechanical properties of dentin may also affect long term outcomes. Therefore, structural alterations of dentin should be considered when recommending irrigation protocols.

1.8 Energy-dispersive X-ray spectroscopy

Energy-dispersive X-ray spectroscopy (EDS) can be used to analyze element composition within a sample. Focused X-ray energy on an element can excite inner shell electrons to move to an outer shell. The electron hole that is left is then filled by an outer shell electron of higher energy. This produces a characteristic X-ray from the unique anatomic structure of each element in the sample which can be measured by an energy dispersive spectrometer. Using this technology, qualitative and quantitative measurements of inorganic (Ca and P) as well as organic (C and O) elements of a dentin sample can be obtained (Newbury et al, 2017).

Chapter 2: Aims and Hypothesis

2.1 Rationale

Irrigation is a crucial component of endodontic success. Emphasis is focused on the ability of irrigation sequences to achieve a root canal system free of bacteria and their by-products, as well as elimination of hard and soft tissue debris. However, less attention is directed toward the structural changes in dentin when different sequences of NaOCl and EDTA are used. Previous research using SEM has illustrated dentin surface changes when NaOCl and EDTA are used in an alternating manner (Baumgartner & Mader, 1987; Qian et al, 2011). There are no reports about the effect on dentin properties and the atomic proportions of Ca, P, O and C in dentin by the alternating use of the irrigating solutions.

2.2 Aim

To analyze structural changes of dentin up to 300 μm from the root canal lumen by measuring atomic percentages of key elements in dentin when NaOCl and ETDA are used in alternating sequence during instrumentation, in comparison to saline irrigation, NaOCl irrigation with final EDTA rinse, and NaOCl irrigation with NaOCl as a final rinse after EDTA.

2.3 Null Hypothesis

The irrigation sequences compared in this study do not differ in their capacity to cause changes in the chemical composition of dentin up to 300 µm from the root canal lumen.

Chapter 3: Materials and Methods

3.1 Tooth selection and preparation

Eight single rooted extracted anterior human teeth without previous endodontic treatment were obtained. The teeth were accessed with a surgical length high speed round bur using water spray. Teeth were confirmed to have one root canal under operating microscope visualization. A #10 K-file (Dentsply Maillefer, Ballaigues. Switzerland) was inserted into the canal until the tip was just visible at the apical foramen. A rubber stopper on the file was secured at a stable reference point and the distance from the instrument tip to the stopper was measured. The working length (WL) of each canal was determined by subtracting 1mm from the measured length. Roots were covered with orthodontic wax in order to prevent leakage of the irrigant from the apex. All teeth were instrumented sequentially with Vortex Blue (Dentsply Tulsa Dental, Tulsa, OK) rotary files starting with size 15/04 and working up to a final size of 40/04 totaling six files in each tooth.

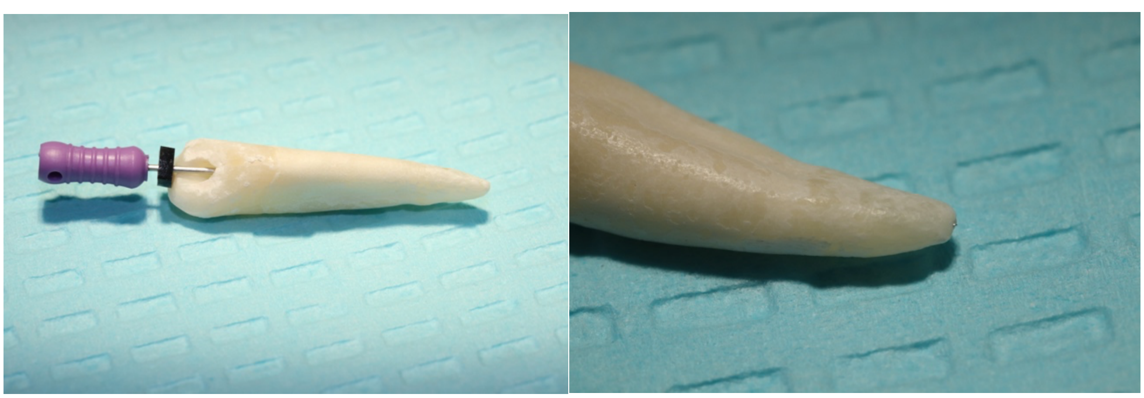

Figure 2: Working length determination

3.2 Irrigation protocols

All irrigation procedures were carried out using a 30-G side-vented closed-ended needle (ProRinse; Dentsply Tulsa Dental). The needle tip was inserted as far apically as it could reach before binding. In order to facilitate even distribution of irrigant within the canal, the tip was rotated and moved using an apical-coronal pumping action with an amplitude of 10mm throughout the irrigation process. The flow rate for all irrigation protocols was 5mL/min. The teeth were divided into 4 groups based on irrigation protocol as follows:

1. Group 1: two teeth irrigated with saline throughout canal instrumentation were used as negative controls.
2. Group 2: two teeth were irrigated with 6% NaOCl for 15 seconds before each rotary file was used. After shaping was complete, the final rinse protocol consisted of 6% NaOCl for 2 minutes, followed by 17% EDTA for 2 minutes. Total active irrigation was 3 minutes 30 seconds for NaOCl and 2 minutes for EDTA. Total working time was approximately 7 minutes.
3. Group 3: two teeth were irrigated using the same protocol as group 2, with the addition of 6% NaOCl for 2 minutes as a final rinse after the 2 minutes of EDTA. Total active irrigation was 5 minutes 30 seconds for NaOCl and 2 minutes of EDTA. Total working time was approximately 9 minutes.
4. Group 4: two teeth were irrigated with 6% NaOCl for 15 seconds, followed by 17% EDTA for 15 seconds before each of the 6 rotary files were used. After instrumentation was complete, the canals were irrigated again with 6% NaOCl for 15 seconds, 17% EDTA for 15 seconds and 6% NaOCl for 15 seconds. Total active irrigation was 2

minutes for NaOCl and 1 minute 45 seconds for EDTA. Total working time was approximately 6 minutes.

The teeth were stored in water at 4°C until sectioning of the roots was carried out.

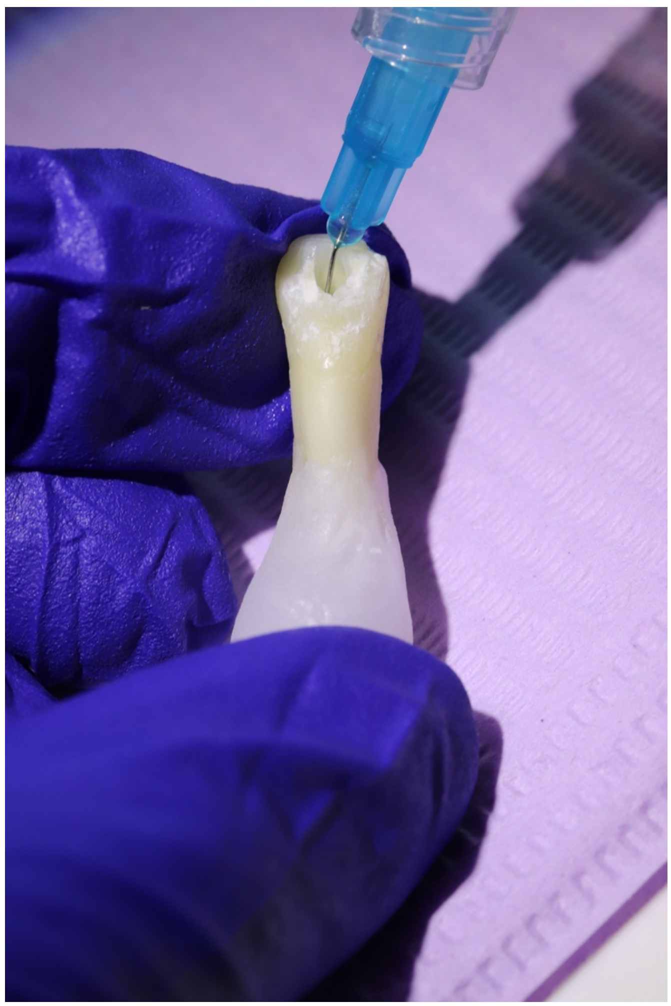

Figure 3: Experiment set up for irrigation

3.3 Tooth sectioning

Prior to sectioning, a groove was prepared longitudinally along the buccal root surface for orientation. Three dentin discs were horizontally sectioned from each root corresponding to the apical, middle, and coronal thirds. The root discs were sectioned to a thickness of 1 mm using a 0.6 mm precision diamond saw (Isomet 5000; Buehler Ltd, Lake Bluff, IL) at 2700 rpm under water coolant. Each disc was transferred to a well filled with water, labelled to represent the irrigation regimen it underwent as well as the part of the root it was sectioned from. The root discs were stored up to 72 hours at 4°C until preparation for EDS was carried out.

Figure 4: Preparation of buccal groove for orientation

Figure 5: Prepared buccal groove

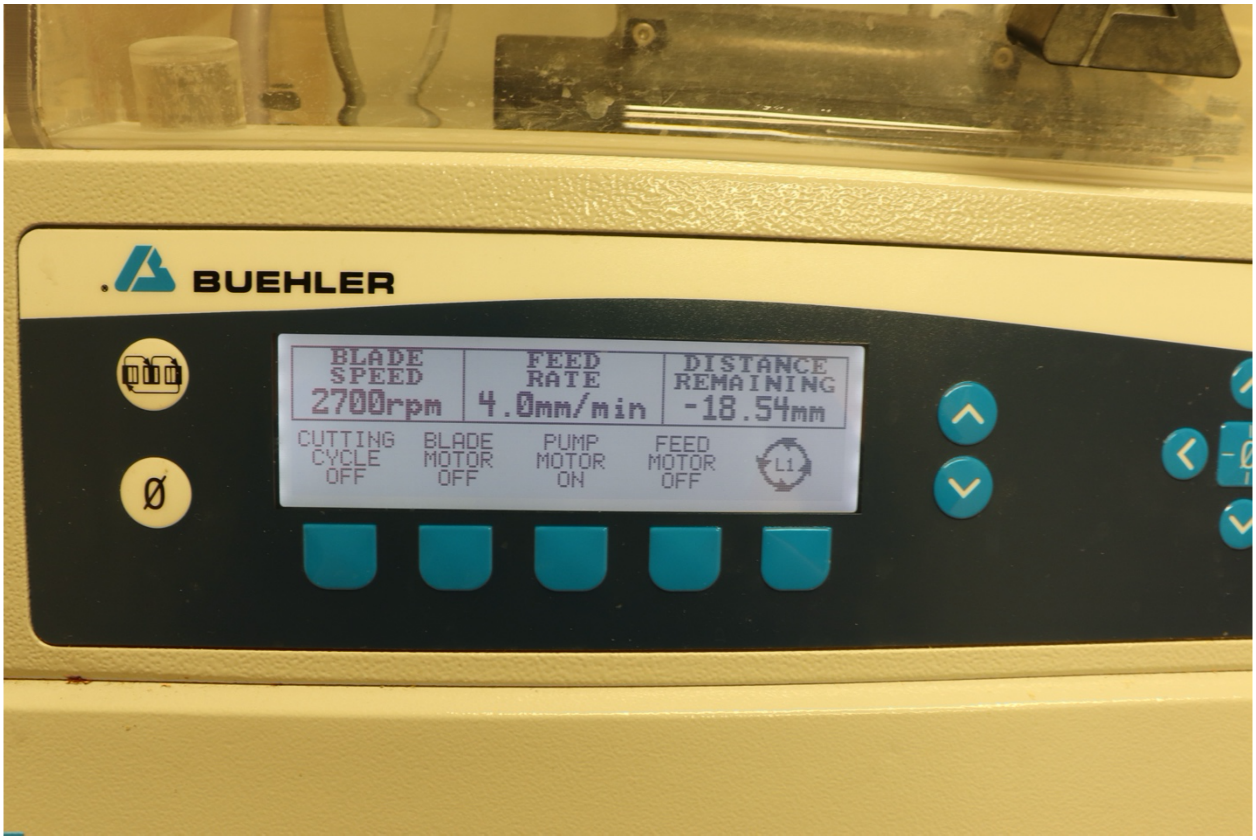

Figure 6: Settings for cutting of the dentin discs

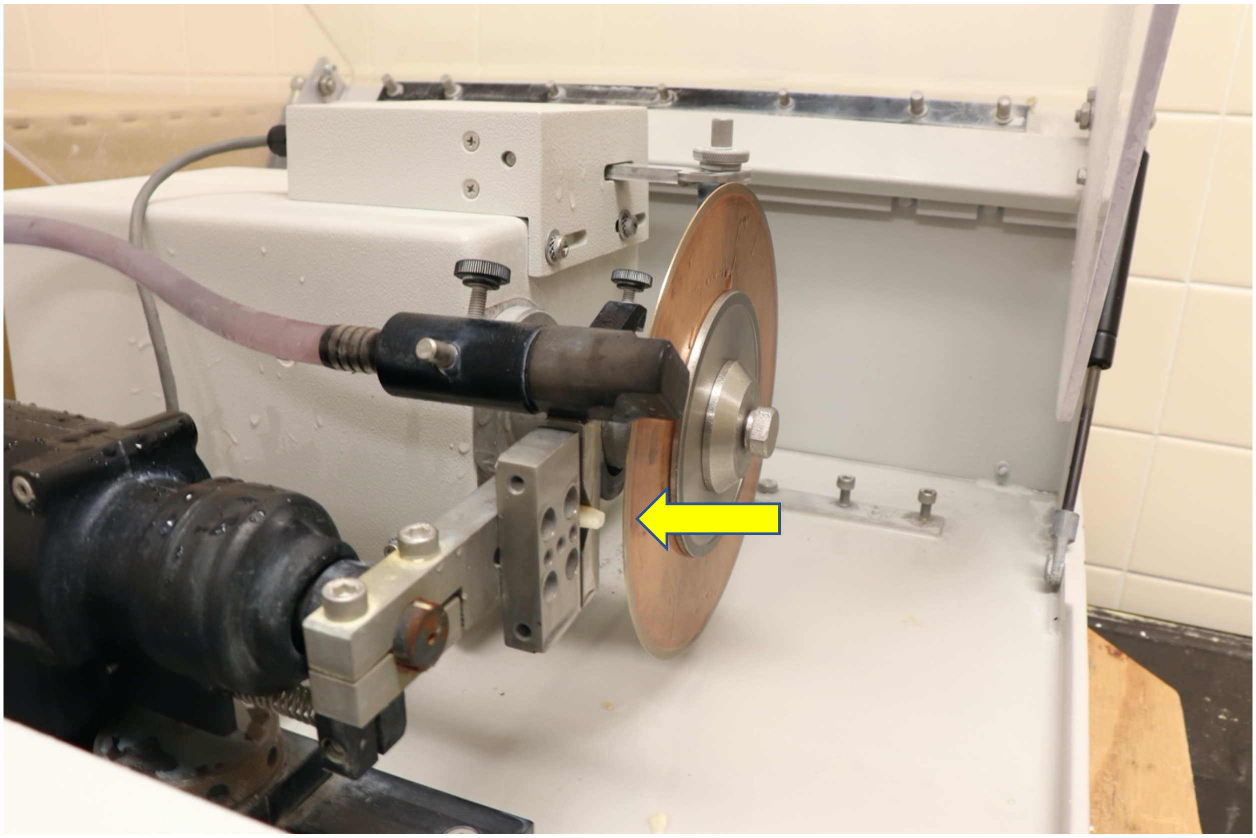

Figure 7: Mounted extracted tooth after middle dentin disc was cut.
Arrow shows the specimen. Water cooling was used during cutting.

Figure 8: Dentin disc with prepared buccal groove

3.4 EDS analysis

EDS analysis was carried out as per Wang et al (2016). Serial dehydration of the dentin blocks was carried out using increasing concentrations of ethanol (50%, 70%, 80% and 100%) for 10 minutes at each concentration. Each block was then immersed in 2mL of 100% pure ethanol for at least 1 hour to complete the dehydration process. Once dehydrated, the discs were sputter coated with iridium (4 nm thickness) in a Leica EM MED020 Coating System (Leica Microsystems Inc, Tokyo, Japan) for EDS analysis.

The line scanning function was used to determine the atomic percentage levels of carbon, oxygen, phosphorus and calcium in dentin up to 300 µm from the canal lumen. Measurements were taken by EDS at a voltage of 10 kV (Helios Nanolab 650; FEI, Eindhoven, Netherlands) every 5 µm, totaling 61 measurements for each line. Each disc was line scanned at four areas corresponding to the mesial, buccal, distal and lingual surfaces. For each element, the mean value of the atomic percentage in the 300 µm line scan was calculated and the mean of the 4 lines was used for each disc.

3.5 Statistics

Statistical analysis was performed using SPSS 27 for windows (IBM Corp, Armonk, NY). Normality of data was tested using the Kolmogorov-Smirnov test. Because the data was not normally distributed, atomic percentages were evaluated using the independent samples Kruskal-Wallis test.

Chapter 4: Results

Mid-root dentin disks were used for EDS analysis. Significant differences were seen between irrigation protocols with respect to atomic percentages of carbon, oxygen and calcium in the dentin samples. Atomic percentages of the samples are presented in Table 2.

The relative proportion of atomic carbon was significantly higher in the alternating group (Group 4) compared to the control group (Group 1) as well as the EDTA final rinse group (Group 2). The relative proportion of carbon was also significantly higher for the NaOCl final rinse group (Group 3) compared to the EDTA final rinse group. No other significant differences were observed with respect to carbon

Dentin samples where EDTA was the final rinse (Group 2) had significantly higher relative proportions of oxygen than both the control and NaOCl final rinse groups. These were the only statistically significant differences between groups with respect to relative proportions of oxygen.

The alternating irrigation protocol resulted in the lowest atomic percentage of calcium compared to the other protocols. These percentages were statistically significant in comparison to Group 2 (EDTA final rinse), and also highly significant in comparison to Group 1 (control). A statistically significant lower atomic percentage of calcium was also observed in the NaOCl final rinse group compared to Group 1 (control). No other significant differences with respect to relative proportions of calcium were observed.

There were no significant differences regarding atomic percentage of phosphorus between groups. However, there was a trend towards lower relative proportions of phosphorus in the alternating group and NaOCl final rinse group compared to group 2 (EDTA final rinse) and group 1 (control).

Table 2: Mean relative atomic percentages of four elements in root dentin after instrumentation and four different irrigation protocols

Mean relative atomic percentage and standard deviation				
Irrigation Protocol	Carbon	Oxygen	Phosphorus	Calcium
Control[1]	23.2 ± 1.8^{ab}	37.2 ± 3.1^{a}	16.9 ± 2.2^{a}	22.7 ± 1.4^{a}
EDTA[2]	21.7 ± 1.7^{a}	41.3 ± 1.4^{b}	15.6 ± 0.7^{a}	21.4 ± 1.1^{ab}
NaOCl[3]	24.9 ± 2.6^{bc}	39.3 ± 1.1^{a}	15.3 ± 1.2^{a}	20.5 ± 0.9^{bc}
Alter[4]	27.0 ± 2.4^{c}	39.2 ± 2.0^{ab}	14.6 ± 0.9^{a}	19.2 ± 0.6^{c}

Different superscript letters within each vertical column indicate statistically significant difference ($p < .05$)
[1]Group 1, Instrumentation: Saline; Final rinse: Saline
[2]Group 2, Instrumentation: NaOCl; Final rinse: NaOCl then EDTA (2 minutes each)
[3]Group 3, Instrumentation: NaOCl; Final rinse: NaOCl, EDTA then NaOCl (2 minutes each)
[4]Group 4, Instrumentation: NaOCl and EDTA; Final rinse: NaOCl, EDTA then NaOCl (15 seconds each)
Kruskal-Wallis test

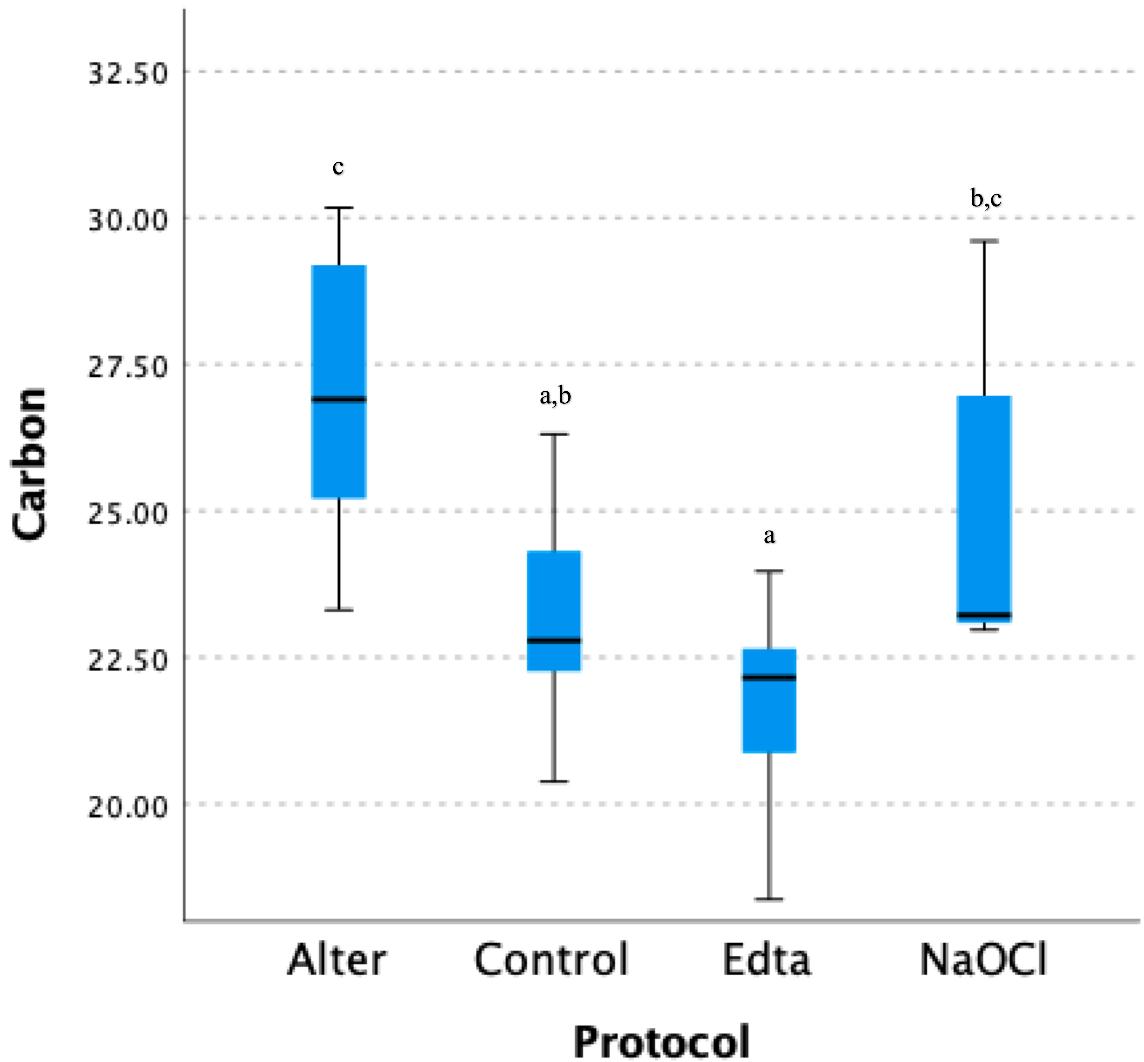

Figure 9: Relative atomic percentage of carbon resulting from different irrigation protocols. Protocols: Alter = (Group 4) Alternating use of NaOCl and EDTA (15 seconds each) before each file, final rinse NaOCl, EDTA, NaOCl (15 seconds each); NaOCl = (Group 3) NaOCl (15 seconds) before each file, final rinse NaOCl, EDTA, NaOCl (2 minutes each). Control = (Group 1) Saline irrigation (negative control); EDTA = (Group 2) NaOCl (15 seconds) before each file, final rinse NaOCl, EDTA (2 minutes each). Different letters indicate statistically significant difference ($p < .05$). Kruskal-Wallis test.

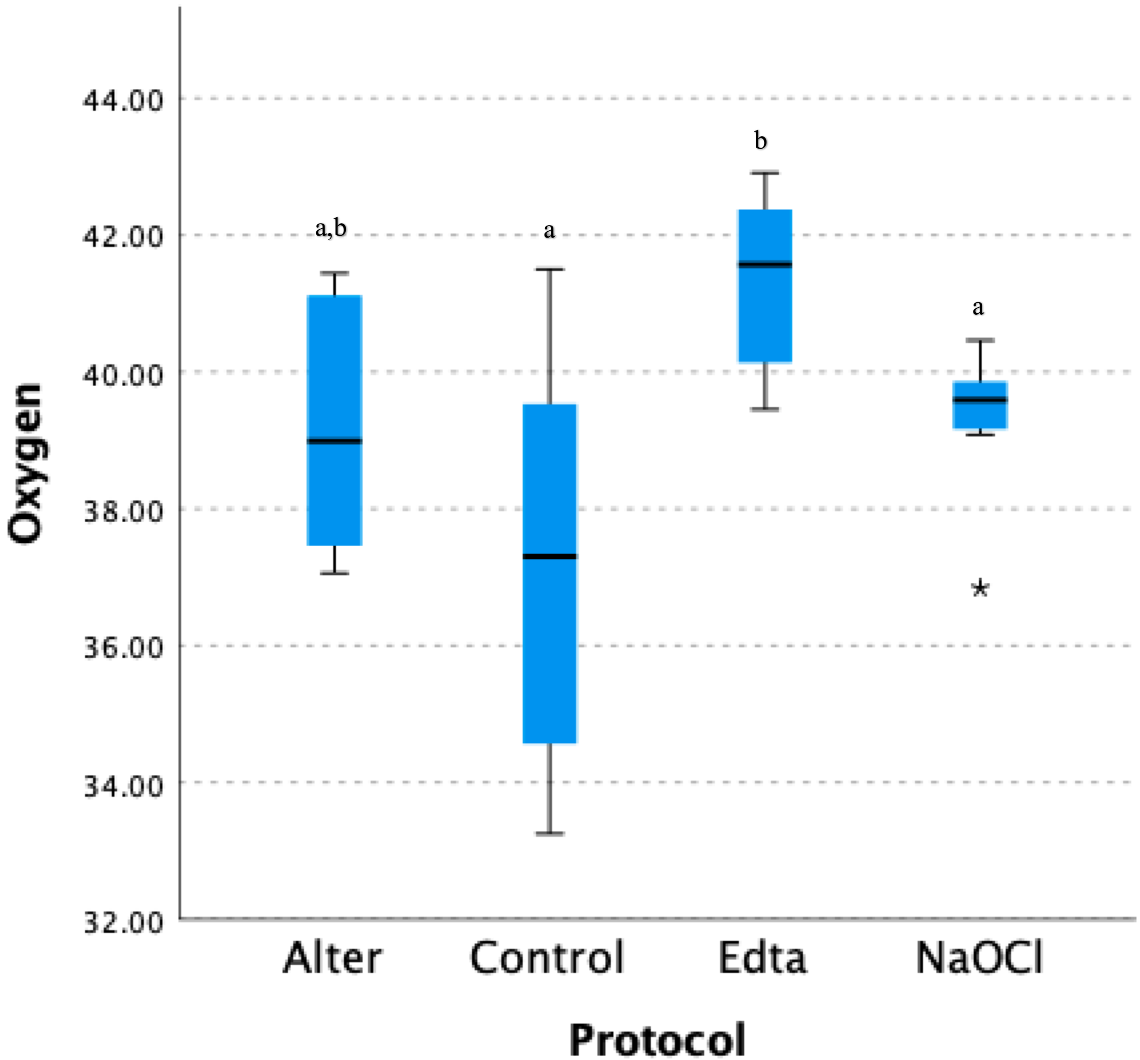

Figure 10: Relative atomic percentage of oxygen resulting from different irrigation protocols. Protocols: Alter = (Group 4) Alternating use of NaOCl and EDTA (15 seconds each) before each file, final rinse NaOCl, EDTA, NaOCl (15 seconds each); NaOCl = (Group 3) NaOCl (15 seconds) before each file, final rinse NaOCl, EDTA, NaOCl (2 minutes each). Control = (Group 1) Saline irrigation (negative control); EDTA = (Group 2) NaOCl (15 seconds) before each file, final rinse NaOCl, EDTA (2 minutes each). Different letters indicate statistically significant difference ($p < .05$). Kruskal-Wallis test.

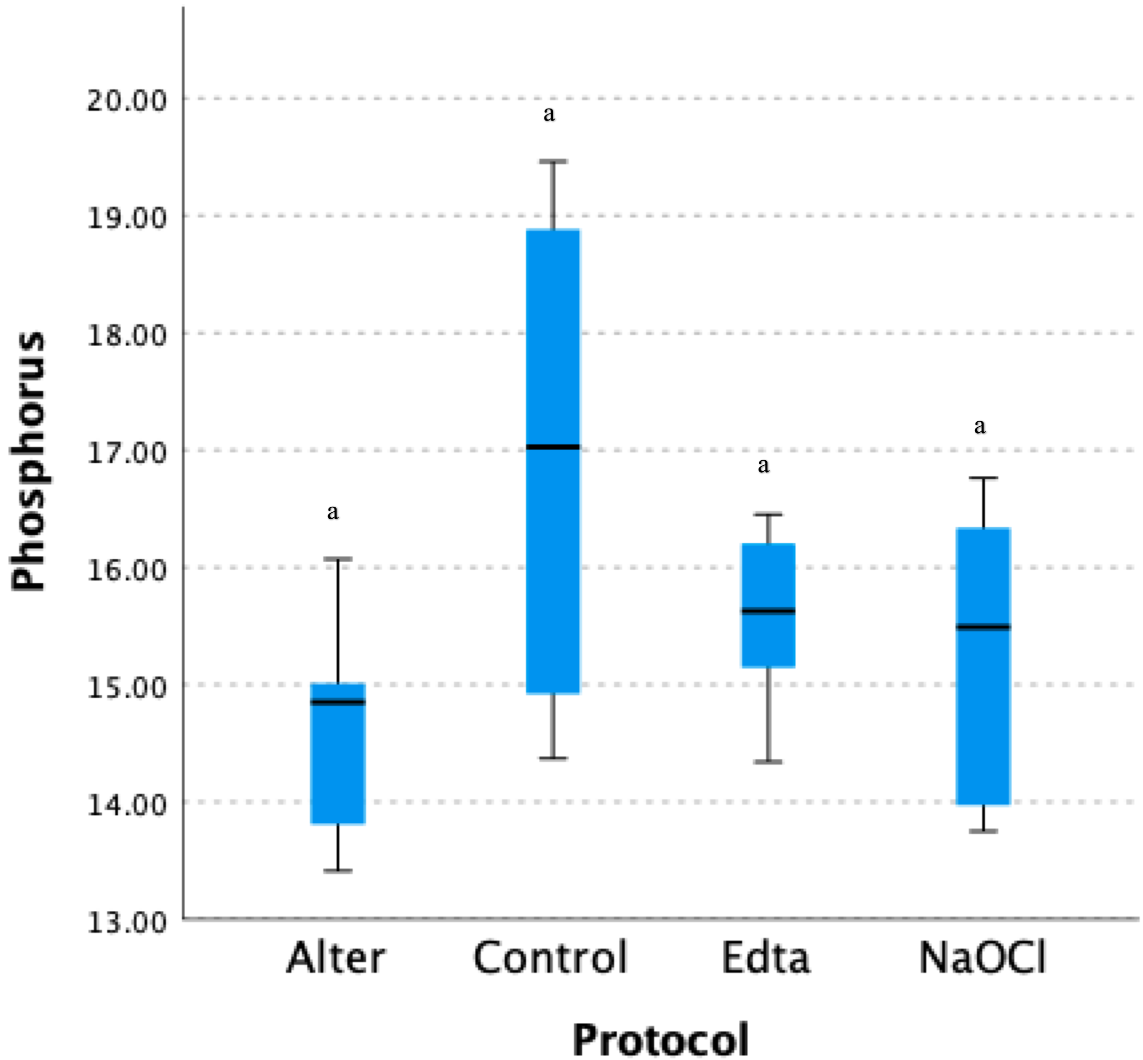

Figure 11: Relative atomic percentage of phosphorus resulting from different irrigation protocols.

Protocols: Alter = (Group 4) Alternating use of NaOCl and EDTA (15 seconds each) before each file, final rinse NaOCl, EDTA, NaOCl (15 seconds each); NaOCl = (Group 3) NaOCl (15 seconds) before each file, final rinse NaOCl, EDTA, NaOCl (2 minutes each). Control = (Group 1) Saline irrigation (negative control); EDTA = (Group 2) NaOCl (15 seconds) before each file, final rinse NaOCl, EDTA (2 minutes each). Different letters indicate statistically significant difference ($p < .05$). Kruskal-Wallis test.

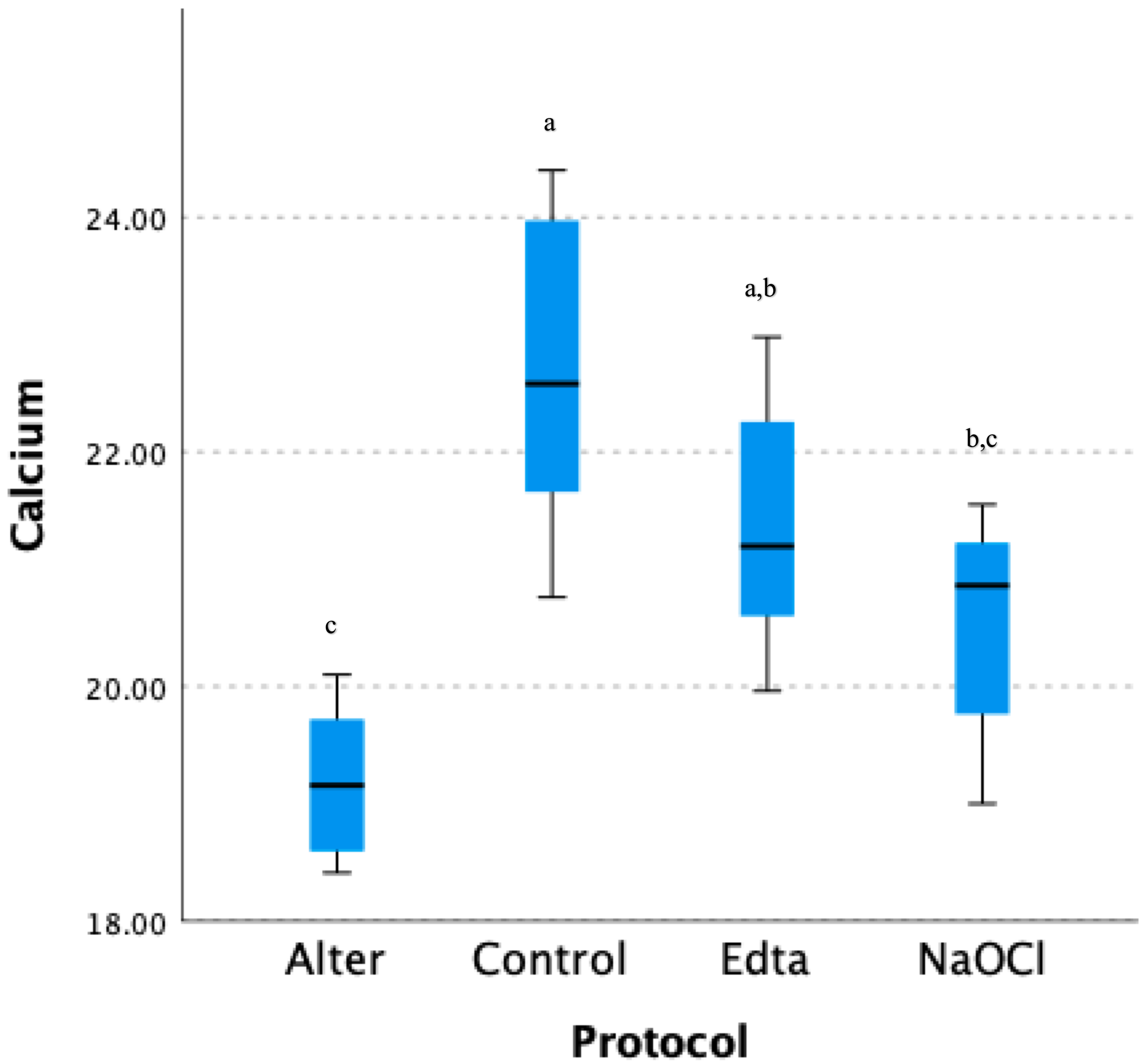

Figure 12: Relative atomic percentage of calcium resulting from different irrigation protocols. Protocols: Alter = (Group 4) Alternating use of NaOCl and EDTA (15 seconds each) before each file, final rinse NaOCl, EDTA, NaOCl (15 seconds each); NaOCl = (Group 3) NaOCl (15 seconds) before each file, final rinse NaOCl, EDTA, NaOCl (2 minutes each). Control = (Group 1) Saline irrigation (negative control); EDTA = (Group 2) NaOCl (15 seconds) before each file, final rinse NaOCl, EDTA (2 minutes each). Different letters indicate statistically significant difference ($p < .05$). Kruskal-Wallis test.

Chapter 5: Discussion

In the present study, atomic percentages of phosphorus and calcium in dentin were lowest when alternating irrigation with NaOCl and EDTA was carried out during instrumentation. It has been suggested that NaOCl and EDTA be used in an alternating manner in order to prevent accumulation of mineral debris from blocking accessory and lateral canals, as well as to facilitate the flow of irrigants (Sleiman & Khaled, 2005). However, evaluation of irrigation protocols should consider the possible impact on dentin the solutions come into contact with. Therefore, the aim of this study was to evaluate the effects of alternating EDTA and NaOCl on root canal dentin in a quantitative manner.

Previous studies have demonstrated that a final rinse of NaOCl after EDTA results in erosion of root canal dentin (Baumgartner & Mader, 1987; Qian et al, 2011). The chelating action of EDTA not only removes the smear layer after instrumentation, but also mineral from the surface and subsurface area of root canal dentin. This leads to exposed collagen fiber bundles. It is possible that as long as EDTA has not been used, NaOCl has limited effect on dentin collagen which is covered by hydroxyapatite. According to Zhang et al (2010), the demineralizing action of EDTA does not change the flexural strength of dentin. However, when demineralized dentin is flushed with NaOCl, the exposed collagen is aggressively attacked and dissolved by NaOCl. The resulting erosion leads to a decrease in the mechanical properties of the root canal dentin (Dotto et al, 2020).

When an alternating regimen of EDTA and NaOCl is used during instrumentation, it may seem intuitive that eroded dentin will be removed as the canal size increases as a result of files cutting into dentin. It may also be assumed that the smear layer produced by successive files acts to provide protection from erosion of the dentin wall. However, it is known that much of the canal wall remains untouched during rotary or hand instrumentation and therefore the eroding action of NaOCl after EDTA may be additive when performed after each file (Davis et al, 1972; Peters et al, 2001). Although the atomic percentages of calcium were not statistically significant between the alternating and NaOCl final rinse groups, the trend was toward lower calcium levels in the alternating group (mean reduction of 1.35%). This supports the possibility of an additive erosion effect. The lack of statistical significance is likely related to the small sample size that was analyzed in this study.

Most previous research on dentin erosion has used qualitative evaluation by SEM imaging. In the SEM study by Yamada et al (1983) it was shown that only when NaOCl was used after EDTA were the canals completely clear of both smear layer and soft tissue remnants. However, it should be noted that bacteria were not found to be present irrespective of whether EDTA or NaOCl was used as the final rinse (Yamada et al, 1983). When Baumgartner and Mader (1987) evaluated oblique views of irrigated dentin by SEM imaging, they observed samples that were prepared with alternating EDTA and NaOCl demonstrated irregular eroded dentin on uninstrumented portions of the canal walls. Although SEM studies demonstrate the presence of erosion, they are not quantitative without further analysis.

Previous studies that aimed at quantitative measurements of erosion after irrigation have been caried out using various techniques. In one study, mineral contents of root canal dentin chips obtained after exposure to different irrigants were analyzed using inductively coupled plasma-atomic emission spectrometry (ICP-AES) and reported as percentage weight (Ari & Erdemir, 2005). Another study estimated areas of dentinal tubule openings on SEM images using computer software (Qian et al, 2011). EDS allows for quantitative analysis of elemental atomic percentages. Doğan and Calt (2001) used EDS to demonstrate differences in calcium, phosphorus and magnesium following different irrigation regimens on surface dentin. Wang et al (2016) used EDS to measure atomic percentages in dentin at 5 µm increments up to 300 µm from the root canal wall. A depth of 300 µm from the canal lumen was chosen since previous research has shown the ability of NaOCl to penetrate up to that depth (Zou et al, 2010). In the current study, analysis of atomic percentages was carried out as described by Wang et al (2016).

Preparation of roots and irrigation protocols vary among studies assessing effects of irrigation solutions on root canal dentin. Many studies section roots before the dentin is exposed to irrigants (Doğan & Çalt, 2001; Marending et al, 2007; Sayin et al, 2007). Furthermore, it is common for the sectioned root surfaces to be submerged in the irrigation solution (Sim et al, 2001; Qian et al, 2011). This allows for maximal exposure of the root surface to the irrigant and sufficient volume to allow the irrigant to maintain its activity. Exposure time is an important consideration in study design. While Saito et al (2008) reported significantly greater smear layer removal when comparing a 1-minute final rinse with EDTA versus 15-second and 30-second groups, Calt and Serper (2002) found no difference in smear layer removal when time was increased over 1-minute. However, it was found that increasing time of EDTA exposure to 10 minutes resulted in significantly greater erosion of the peritubular and intertubular dentin (Çalt & Serper, 2002). In addition, it has been shown that immersing dentin bars in NaOCl for 2 hrs greatly reduces the elastic modulus and flexural strength of the samples (Sim et al, 2001).

The current study aimed to analyze the effects of irrigation protocols on dentin by utilizing a model that closely resembles the clinical situation. For this reason, extracted teeth were accessed and instrumented without prior sectioning. Orthodontic wax was also placed over the apical root surface to prevent the irrigant from exiting through the apex. Furthermore, the wax simulates the effect of the PDL and aids in producing similar fluid dynamics to the clinical scenario. The syringe needle was inserted as far as possible without binding, up to 1 mm from the working length. Vertical pumping motion, as well as rotation of the needle was performed in order to encourage similar exposure of irrigants along all parts of the canal wall.

The differences between atomic percentages in the current study may be conservative. It has been shown that increasing the heat of NaOCl can increase its tissue dissolving capability (Stojicic et al, 2010). Furthermore, sonic or ultrasonic activation, as well as increases in concentration, volume, and time, result in increased effectiveness of NaOCl (Moorer & Wesselink, 1982; Siqueira et al, 2000; Cullen et al, 2015). While high concentration NaOCl (6%) was used in this study, greater erosion of dentin may be observed clinically when methods are utilized to maximize the tissue dissolving and antibacterial capabilities of NaOCl. Additionally, final rinsing of canals in the alternating group was only caried out in 15-second intervals for each solution, compared to the 2-minute intervals of the other groups.

Single rooted teeth were used in the current study. After irrigation protocols, the three dentin discs were created by sectioning the roots perpendicularly to the long axis in the apical, middle and coronal thirds. However, due to limited access to the imaging laboratory as a result of the Covid-19 pandemic, a decision was made to only include middle root sections for EDS analysis. Wang et al (2016) found that there were no differences between samples obtained from the coronal, middle and apical sections of the root. Furthermore, they found no significant difference between samples obtained from single rooted teeth compared to those obtained from palatal roots of maxillary molars (Wang et al, 2016). Groups in the current study that had a similar irrigation protocol to the study by Wang et al (2016) produced similar values for atomic percentages of the analyzed elements.

Although differences in atomic percentages were observed between groups, a limitation of the current study is that no testing was carried out to investigate the clinical implications of the findings. Previous studies have related irrigation solutions to changes in dentin microhardness, flexural strength, and elastic modulus (Sim et al, 2001; Sayin et al, 2007; Zhang et al, 2010). It is possible that the changes in the structural and mechanical properties of dentin as a result of irrigating solutions contributes to vertical root fracture. However, evidence supporting this is lacking. Previous research suggested that excessive force during lateral condensation and tapping of inlays or dowels into place were the primary and secondary causes of vertical root fracture (Meister et al, 1980). Although these techniques have become far less common in current practice, vertical root fracture remains a significant cause for extraction of endodontically treated teeth (Fuss et al, 1999).

Minimally invasive endodontic treatment is aimed at preserving the strength of the treated tooth without compromising cleaning effectiveness. Maximal dentin preservation is a key consideration during the shaping of the root canal system for fracture prevention (Trope & Ray, 1992). With this philosophy in mind, it seems prudent to also consider the eroding effects of irrigation solutions on root canal dentin provided adequate eradication of microbes is achieved.

Chapter 6: Conclusion

Under the limitations of this study:

The results showed a statistically significant reduction in the atomic percentage of calcium when alternating NaOCl and EDTA during shaping of the root canal system compared to teeth in which EDTA was used as a final rinse as well as negative controls. Although not statistically significant, lower atomic percentages of calcium were observed in the alternating group compared to samples in which final irrigation with NaOCl followed EDTA (19.18% and 20.53% respectively). The sequence of irrigants used throughout instrumentation and as a final rinse should be considered as a way to minimize weakening of the remaining dentin structure during root canal treatment.

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