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A Comparative Evaluation of Endodontic Irrigation Methods for Removal of the Smear Layer

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By

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A thesis submitted to the College of Dental Medicine of Nova Southeastern University in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE IN DENTISTRY

Department of Endodontics

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Synopsis

The purpose of this in vitro study is to compare the effect of various irrigation systems on smear layer removal in curved root canals.

Root canal irrigation plays an important role in the debridement and disinfection of the root canal system. It has been well documented that the flushing component of the irrigants is as important as the tissue dissolving capability. Therefore, the efficacy of the irrigant might also be influenced by the method by which it is introduced.

Fifty-one recently extracted molar teeth with root curvatures of more than 30° were selected according to Schneider's method. The teeth were decoronated to obtain a standardized root length of 12 mm. The root tips were sealed with hot glue and embedded into a silicone mold. The canal preparations were performed by using ProTaper™ and ProFile™ systems up to #35,04. Sodium hypochlorite (NaOCl 6%) and ethylenediaminetetraacetic acid (EDTA 17%) were used as root canal irrigants according to Yamada protocol. To maintain irrigation consistency, a programmable syringe pump was connected to each system.

After finishing the cleaning and shaping of the curved canals, the final cleansing of the root canal space, with proper irrigation solutions, were accompanied by activation systems.

Five different treatment modalities were tested; Group 1: Traditional irrigation, Group 2: EndoActivator™, Group 3: Passive ultrasonic irrigation (PUI),

Group 4: EndoVac™, Group 5: Saline. The root halves (n=102) were imaged with the FEI Quanta 200 scanning electron microscope™ (SEM). Over 7000 magnified images were reviewed and scored by three board certified Endodontists in a double-blind manner. The data was analyzed by using the Cochran-Mantel-Haenszel method, Pairwise Comparisons and Intra-class correlation coefficients.

The EndoVac™ system (an apical negative pressure irrigation system) was found to be significantly more effective ($p<0.05$) than the other groups in all sections observed, this would include the apical, middle and coronal sections for the elimination of the smear layer as well as the debris removal and improved tubule visibility.

The negative pressure delivery systems may provide cleaner surfaces in the canals of curved roots of at least 30 degree or more.

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Abbreviations

μm	Micrometer
NiTi	Nickel-Titanium
ISO	International Standardization Organization
SEM	Scanning Electron Microscope
mm	Millimeter
AAE	American Association of Endodontists
TEM	Transmission Electron Microscope
PRC	Polymerase Chain Reaction
NaOCl	Sodium Hypochlorite
CHX	Chlorhexidine
EDTA	Ethylenediaminetetraacedic Acid
MTAD	Mixture of a Tetracycline, Acid, and Detergent
min	Minute
h	Hour
F	Fahrenheit
^{o}C	Degree Centigrade
CA	Citric Acid
STP	Sodium Triphosphate
PUI	Passive Ultrasonic Irrigation
UI	Ultrasonic irrigation

WL	Working length
MDT	Master Delivery Tip
ICC	Intra-Class correlation coefficients
C. albicans	Candida albicans
E. faecalis	Enterococcus faecalis
PSP	Programmable Syringe Pump

Glossary of Statistical Terms

P value	The probability of obtaining a result equal to or "more extreme" than what was actually observed when the null hypothesis is true. The p value significant level for this thesis was $p<0.05$.
Pairwise comparisons	Is a statistical tool to rank and compare entities in pairs to decide which of each entity is favorable or if they are alike or not.
Cochran-Mantel-Haenszel method	Is a technique utilized in the analysis between two categorical variables or groups to create an estimate for the relation between an exposure or treatment and the outcome.
Intra-class correlation coefficients	Is a statistical assessment for the reliability of findings to show how strongly the items in the identical groups are related to each other.

Products Used

Name	Manufacturer	City, State
Max-I-Probe needle	Dentsply	York, PA
EndoActivator	Dentsply	York, PA
EndoVac	Discus Dental	Culver, CA
Sodium Hypochlorite	Vista Dental	Racine, WI
Ethylenediaminetetraacetic acid	Elleman	New York, NY
Global Endodontic Microscope	Global Surgical Corporation	St. Louis, MO
ProTaper	Dentsply	York, PA
ProFile	Dentsply	York, PA
Programmable Syringe Pump	World Precision Instruments, Inc	Sarasota, FL

1. Introduction

1.1 Chemo-mechanical preparation:

Endodontic therapy is based on the removal of infected pulpal tissues and dentinal debris from the root canal system. The success of the endodontic treatment depends on many factors, commonly called the Endodontic triad, such as mechanical preparation, irrigation, and root canal obturation (1-4).

The Chemo-mechanical preparation is accomplished by combining the mechanical instrumentation with the antibacterial irrigation. The goals of this preparation are to reduce microorganisms from root canal system which are known to cause inflammatory reaction, to remove organic and inorganic tissues that may support microbial growth, and to reduce the risk of pushing the debris beyond the apical construction of the root canal system which may cause inflammation (5).

Infected root canal systems can harbor between $<10^2$ to $>10^8$ bacterial cells (6). These bacteria are able to penetrate into dentinal tubules up to 400 μm (7-9). Mechanical instrumentation is a key factor in microbial load reduction in the infected root canal system (5). Byström and Sundqvist found significant reduction in the number of bacterial in infected root canal by 100 to 1,000 fold after instrumentation with stainless steel hand files and irrigation with only 10 mL of physiologic saline solution per canal (10). However, canals could not be consistently rendered bacteria-free.

Dalton et al, compared bacterial reduction after instrumentation with either 0.04 tapered Nickel-Titanium (NiTi) rotary files to bacterial reduction with stainless steel K-file step back technique using only sterile saline. No significant difference was found between the two techniques with 72% of instrumented teeth still harboring positive culture (11).

Oval canals are one of the challenges during chemo-mechanical preparation, this was confirmed by Wu et al as they showed in their study a 42% unprepared canal walls after an even circumferential hand filing, this mechanical filling was only able to remove the inner layer of dentin from 58% of the circumference of the canal wall (12).

These studies clearly showed us the importance of irrigation along with the instrumentation. There is no doubt that mechanical instrumentation is not enough to disinfect the root canal system, yet, it plays an important part in bacterial reduction in infected root canal systems.

Different mechanical instrumentation techniques were developed from using ISO standardized 0.02 tapered stainless steel hand files to the engine-driven instrumentation since the introduction of NiTi instruments in 1988 (13). As previously mentioned, Mechanical instrumentation creates a path for the disinfectant solution to reach most of the enclosed infected parts of the root canals (14) and work towards shaping the canal and facilitate the placement of a biocompatible root filling material (5).

Mechanical instrumentation by itself is limited at completely eliminating residual bacteria and necrotic debris (10). According to Bystrom et al, mechanical

instrumentation eliminates bacteria in only 20-30% of cases (10,15). Peters et al confirmed these findings by using an advance technology, micro computed tomography (micro-ct). According to their findings, they reported that mechanical instrumentation left 35% or more of the canal surfaces untouched (16).

Classically, Mizrahi et al performed a scanning electron microscope (SEM) study using different instrumentation technique on 30 teeth and they found that one half of the root canal is most often better instrumented than the other half and no instrumentation technique were able to remove all the debris (17).

The root canal system consists of lateral and accessory canals, isthmuses, fins and other anatomical complexities that add significant limitations to complete elimination of debris from root canal system (18-20). Peters et al showed high percentage of root canal walls that were untouched with the current instrumentation technique (21).

Another classical study has shown that root canals morphology is very complex and that mechanically prepared canals contains areas not accessible by endodontic instruments (22). With that said, to achieve an enhanced disinfection of the root canal system, the mechanical instrumentation should be used in conjugations with an effective antimicrobial irrigant (23).

Chemo-mechanical preparation does not effectively debride the entire root canal system. These irrigation methods are effective and helpful in cleaning root canals coronally but show limitation at the apical part. To be effective, endodontic irrigants should ideally be delivered at 1-3 mm from the working length (24,25).

1.2 Dentin:

Dentin is a complex structure composed of odontoblast, odontoblastic processes, dentin tubules, non-collagenous proteins, and mineralized collagen forming the main dentinal corps. Dentin is classified into 3 types: Primary, secondary, and tertiary. Only primary and secondary dentin forms the dentin tissue of the normal non-carious teeth. Dentin tubules enclose and protect odontoblastic processes from environmental harmful stimuli. Odontoplastic process secretes the proteic matrix formed by collagen and non-collagen proteins (26,27).

Dentin is composed of nearly 22% hydrated organic matrix by weight. It is similar in composition to bone. Dentin has mainly Type-I collagen fibrils and reinforcing phase of nanocrystalline apatite mineral that contributes to its mechanical properties (28,29). Type I collagen is secreted by odontoblast into predentin at the proximal portion of the odontoblastic process. These process located within dentin tubule and secrets the dentin proteic and nonproteic components responsible for the dentinal biomineralization process (30).

Dentinal tubules, are microscopic channels which have a diameter of 2.5 μm close to the pulp and extend to the outer surface of cementum or enamel with a diameter of 0.9 μm at the dentino-enamel junction (31,32). The number of dentin tubules range between 18,000-21,000 per mm^2 (33). Dentin consists of about 70% inorganic materials mainly hydroxyapatite, 20% organic materials mainly type I collagen and 10% water.

There are three types of dentin present; **Primary dentin:** this type of dentin usually located between enamel and pulp chamber. During odontogenesis, odontoblasts play an important role in the formation of primary dentin until the tooth becomes functional. The outer layer closest to enamel is called **mantle dentin,** this is an atubular layer with thin and curved tubules. The diameter of this layer is between 15-30 mm thick and it is less mineralized. **Predentin** is the newly secreted unmineralized dentin which is between 10-47 micrometer and it is similar to the osteoid in bone.

Secondary dentin: this type of dentin form after the completion of root formation which is usually after the establishment of contacts between antagonistic cusps and will continues throughout life. This dentin is responsible for the shrinkage in the size of pulp chamber with patient age. The S-curve patter of the tubules looks more accentuated in this dentin due to space restriction.

Tertiary dentin: this type of dentin forms as a respond to an external stimulation such as decay or abrasion. It is either a reactionary type from a pre-existing odontoblast or reparative from newly differentiated odontoblast like cell. deposition of tertiary dentin can occur rapidly depending on the duration and intensity of the stimuli, which will usually result in irregular tubular configuration (32,34,35).

1.3 Smear layer:

A surface film of debris retained on dentin and other surfaces after instrumentation with either engine driven or hand endodontic files; consists of dentin particles, remnants of vital or necrotic pulp tissue, bacterial components and retained irrigant (36). Smear layer is not observed on un-instrumented surfaces and it is usually occluding the dentinal tubules orifices (37).

Lester and Boyde in 1963 described the smear layer as “organic matter trapped within translocated inorganic dentin” (38). Eick et al performed a Scanning Electron Microscope Study (SEM) study to examine the cut tooth surfaces, the results showed non homogenous debris particles with a size range between 0.5-0.15 μm (39). Another study showed a similar thin layer of debris that was 2 to 5 micrometers in thickness (40). McComb and Smith were the first to mention this layer in instrumented root canals. They concluded that the smear layer consists of dentin particles, remnants of vital or necrotic pulp tissue and bacterial remnant (organic and inorganic component) (41).

Smear layer has two main components, the **organic component** which is formed by vital or necrotic tissue, bacteria, blood cells, dentin collagen fibers and odontoblastic processes and **inorganic component** which is formed by hard tissue particles of the tooth composed of hydroxyapatite that were loosened during the instrumentation process (42).

In a SEM study to examine the morphological characteristics of the smear layer in teeth that were instrumented with K-type files and irrigated with 5.25%

solution of NaOCl, two confluent components were recognized: the smear layer on the canal wall surface which was about 1 to 2 μm in thickness and the smeared material in the dentinal tubules which was packed into some of the tubules for a distance up to 40 μm. This smeared layer has not been found on the un-instrumented walls and these findings strongly suggest that this layer results directly from instruments used to prepare the root canal walls. This layer appears to be friable and only loosely adherent (42).

The smear layer might harbor microorganisms, infect dentinal tubules, impede penetration or diffusion of antibacterial irrigants and medications into the dentinal tubules (43-48), as well as compromise the seal between the filling materials and dentinal wall (38,42,49-54). The smear layer also increases the micro leakage after canal obturation and interferes with the apical seal (55). In a study to examine the effect of the smear layer on the penetration depth of three different sealers (AH Plus, Apexit, and a Grossman Type-Roth 811) into the dentinal tubules. The smear layer prevented all the sealers from penetrating dentinal tubules & adversely affected the coronal and apical sealing ability of sealers (56). Another research study also confirmed the importance of removal of the smear layer and to establish a patent dental tubule for minimizing the time required to achieve the disinfecting effect of intracanal medications (7).

On the other hand, there are few studies that supported the concept of not removing the smear layer (57-59). During restorative procedures, the formation of the smear layer on dentin will form a protective diffusion barrier preventing the bacteria from entering the dentinal tubules. The permeability of the dentin will

increase by the removal of the smear layer with the acid etch (57). It has been well documented that the removal of the smear layer also facilitates the passive penetration of bacteria into the dentinal tubules (58). Furthermore, Love et al investigated in a study the penetration of Streptococcus gordonii into smeared and non-smeared dentin. This study has shown that smear layer is an effective barrier on the dentinal tubules as the non-smeared sample showed total bacterial penetration (59).

1.4 Biofilm:

Biofilm is the colonization and organization of microorganism at a surface and solution interface (36). It possesses the ability to self-organize (autopoiesis), withstand environmental perturbations (homeostasis), must be more effective in association than in isolation (synergy), and respond to environmental changes as a unit rather than a single individuals (community) (60,61). Biofilm major components are: Bacterial cells, solid surface, and a fluid medium. Bacteria has the ability to form biofilm on any surface that has nutrient containing fluid (62,63). Floating bacteria (planktonic microorganisms) which are in suspended form and are commonly seen within or outside the biofilm, are prerequisite for a biofilm formation (64). Approximately, 85% of the biofilm matrix material consist of protein, polysaccharides, nucleic acids, and salt. The other 15% is made up of microbial cells (65).

Bacteria in biofilm are protected from host defenses and antimicrobial agents by a special mechanism that is dissimilar than the “classic” genetic mechanism (gene mutation or genetic exchange). This mechanism is determined by some peculiarity of biofilm growth (66,67).

The different biofilm associated resistance mechanism can be summarized by: restricted penetration, antimicrobial destroying enzymes and gene transfer, quorum sensing, altered growth rate, Stress response to hostile environmental conditions leading to an overexpression of antimicrobial agent destroying enzymes and Intracellular biofilm (66-72).

Endodontic bacterial biofilms are classified as (73):

- Intracanal biofilms
- Extraradicular biofilms
- Periapical biofilms
- Biomaterial-centered infections

Intracanal biofilm: These are microbial biofilm formed on the root canal dentin of infected tooth. First identification of this type biofilm was reported by Nair under transmission electron microscopy (TEM) (74). The majority of these organism were loose collections of cocci, rods, filaments and spirochetes apart from these bacterial condensations were seen as palisade structure similar to dental plague on tooth surface (75). A SEM study by Sen et al showed that the bacteria formed dense colonies on the canal walls and in inter/intra tubular dentin as well. They also reported fungi forming dense but separate colonies on the canal walls (76).

Extraradicular biofilm: These are root surface biofilms formed adjacent to the root apex of endodontically infected teeth (77). Tronstad et al have shown in a research study on refractory endodontic cases, a smooth and structureless biofilm with a variety of bacterial forms (cocci, rods with presence of some fibrillary form) at the tip of the roots next to the apical foramen (78). Others, also reported a multilayered bacteria embedded in a heavy extracellular matrix in teeth with chronic apical periodontitis (79).

Ricucci et al reported two cases of unhealing fistula after conventional root canal treatment. In one of the cases the fistula did not heal even after an apical surgery. Histology of the apical biopsy revealed a calculus-like material on the external surface of the root apex after post treatment periapical periodontitis. They related the cause for the failure to the biofilm formation on these root surfaces. (80).

Using polymerase chain reaction (PCR)-based 16s RNA gene assay, Conrads showed F. nucleatum, Po. Gingivalis and Tannerella forsythensis to be associated with extraradicular biofilm (81).

Periapical biofilm: It is an isolated biofilm seen in the periapical region of endodontically infected teeth. This type of biofilm may or may not be dependent on the root canal infection (73). The bacteria in this biofilm have the ability to resist host defense and cause periapical lesions (82).

Actinomyces species and Propionibacterium propionicum have been seen in asymptomatic periapical lesions refractory to endodontic treatment (83). Sulphur granules have seen in these species which appear as ray fungus microscopically. (84).

Foreign body centered biofilm: It is usually found when bacteria adhere to both an artificial biomaterial surface and from biofilm structures which is also known as biomaterial-centered infection (85). It is a major complication associated with prosthesis and in implant supported prosthesis. Takemura et al showed that gram positive facultative anaerobes have the ability to colonize and produce extracellular polymeric matrix surrounding the gutta-percha points, while serum plays a crucial role in biofilm formation (86).

1.5 Irrigation solutions:

1.5.1 Sodium Hypochlorite (NaOCl):

Sodium Hypochlorite has a long history in medicine and dentistry. During World War I, chemist Henry Drysdale Dakin and surgeon Alexis Carrel utilized the buffered 0.5% NaOCl solution as antiseptic substance in the treatment of infected wounds (87).

Austin et al showed in their study that the chlorine concentration in Dakin's hypochlorite solution dropped quickly when the solution contacts necrotic tissues compared to the normal tissues. This fall in chlorine concentration will be

completed when the necrotic tissues particles are completely dissolved. Hypochlorite solution has an excellent cleansing ability on necrotic tissues. It also shows a much more irritating effect on the rabbit skin than Chloramine-T solution or the alkaline control solution (88).

NaOCl has many desirable features, its antimicrobial characteristics (89,90-93), its excellent ability to dissolve organic tissues (94-98) and its capability to denature endotoxins (99). Besides that, NaOCl solution is inexpensive, has a decent shelf life and it is easily available (100).

NaOCl display a dynamic balance as seen by the reaction:

$NaOCl + H2O \leftrightarrow NaOH + HOCl \leftrightarrow Na^{+} + OH^{-} + H^{+} + OCl^{-}$

NaOCl dissolve organic and fat components, it reduces the surface tension of the solution by degrading fatty acids and transforming them into fatty acids salts (soap) and glycerol (alcohol) (101). It also neutralizes amino acids forming water and salt (neutralization reaction). There is a reduction of pH with the exit of hydroxyl ions (101).

NaOCl contains hypochlorous acid. When this substance comes in contact with organic tissues, it acts as a solvent and release chlorine, which combines with the protein amino group to form chloramines (chloramination reaction) which interfere with the cell metabolism. Hypochlorous acid and hypochlorite ions cause amino acid degradation and hydrolysis (101). Chlorine (a strong oxidant) causes an irreversible oxidation of essential bacterial enzymes (cysteine) which produces an antimicrobial effect by inhibiting these enzymes (101-103).

Thus, the saponification, the neutralization of the amino acid, and chloramination reactions that occurs in the presence of microorganism and organic tissues produce the antimicrobial effect and cause tissue dissolution (101). Sodium hypochlorite is a strong base (pH > 11), the high pH value makes NaOCl a strong antimicrobial agent and similar to the mechanism of action of calcium hydroxide (104).

The effect of antimicrobial agents on biofilm results in different outcomes. Enumeration of the different effects on bacterial biofilms reveals that the possible outcomes are: complete dissolution of cells, bacterial cell disruption and separation from the biofilm (105).

Free floating bacteria presenting in an aqueous environment, so called planktonic microorganism, are prerequisite for biofilm formation (64). Microbial communities in biofilms are difficult to eradicate and resistant to the anti-microbial agents. Reports show this resistant could be 2-1,000 fold more than the corresponding planktonic form (106).

An in vitro evaluation of 2.25% NaOCl, 0.2% Chlorhexidine (CHX), 10% iodine, 5ppm colloidal silver and phosphate buffered saline (PBS) as control against biofilms of Prevotella intermedia, Peptostreptococcus micros, Streptococcus intermedius, Fusobacterium nucleatum and Enterococcus faecalis. The incubation period with these agents were 15 min to 1h, the results showed that NaOCl was the most effective agent followed by iodine and none of the agents were effective against F. nucleatum after 15 min (107).

According to Cvek et al and Bystrom & Sundqvist, NaOCl at 0.5% or 5% concentration has similar clinical efficiency in mechanical debridement of the root canal (108,109). Other studies also showed a 0.5%-1% concentration with neutral pH has an optimal antimicrobial effectiveness with minimal tissue irritating effect (110,111).

Microorganism communities in biofilm could be 1000-1500 times more resistant to antimicrobial agents (112). The biofilm community provides protection and resistant to these bacterial colonies against disinfecting agents.

In a different in vitro study, the effectiveness of 1%,3% and 6% NaOCl, 2% CHX and BioPure MTAD were tested on apical dentin biofilms. The intracanal contents were collected from patients diagnosed with chronic apical periodontitis. The results indicated that 6% NaOCl was the only irrigant capable of both rendering bacteria nonviable and physically removing the biofilm (113).

The effect of different concentrations of NaOCl on the growth and susceptibility of mono and dual-species biofilms of Fusobacterium nucleatum or Peptostreptococcus micros in vitro at 24 h or 96 h were compared by Ozok et al Their results showed that even though a 24 h dual-species biofilms had similar viable counts to those of mono-species biofilms, they still showed more resistance to NaOCl. Dual-species biofilms were more resistant and had more viable counts than monospecies biofilms at 96h. The resistance to NaOCl increased as the age of biofilms increased. Biofilms mixed species of F. nucleatum and P. micros showed a time-dependent synergy in growth and resistance to NaOCl (114).

The antimicrobial efficacy of 5.25% NaOCl, BioPure MTAD and Tetraclean (Ogna Laboratori Farmaceutici, Milano, Italy) against E. faecalis biofilms generated on cellulose nitrate membrane filters were compared by Giardino et al Only 5.25% NaOCl was effective in removing the biofilm on the surface of the membrane (115). A different study compared the effectiveness of 6% NaOCl, 1% NaOCl, smear clear ^{TM}, 2% CHX and BioPure^{TM} MTAD^{TM}. The results showed significant difference between 1% and 6% NaOCl and the other agents. 1% and 6% NaOCl were found to be more efficient against E. faecalis biofilm (91).

Sodium hypochlorite fragments long peptide chains and chlorinates protein terminal groups (116). Marending et al. showed that NaOCl caused a concentration-dependent reduction of elastic modulus and flexural strength in human root dentin which affected the mechanical properties of dentin. Also, the reduction of carbon and nitrogen was related to the hypochlorite concentration. NaOCl also altered intertubular dentine permeable to basic fuchsindye, although no effect of hypochlorite on inorganic dentin components under Scanning Electron Microscope. They also reported under SEM 3D reconstructions of exposed dentin surface a severely altered peripheral dentin matrix when exposed to 5% NaOCl (117). Sim et al. showed a reduction in the dentin flexural strength and elastic modulus when exposed to 5.25% NaOCl compared to saline solution (118).

Another study tested the effect of irrigating with 2.5% and 6% NaOCl for 5,10 or 20 min on root dentin microhardness. There was a significant difference in groups irrigated for 10 or 20 min and because of this effect, they recommended to limit the irrigation time to a period less than 10 min so not to weaken the tooth.

They also found that at a depth of 500 μm from lumen, 6% NaOCl has more effect on dentin microhardness than 2.5%, therefore, it is recommended not to use higher concentrations of NaOCl to preserve the physical properties of dentin (119).

Mountouris et al used both reflectance FTIR microspectroscopy and tapping mode atomic force microscopy to evaluate the deproteination potential of 5% aqueous NaOCl solution on the molecular composition and morphology of smear layer. The results showed that NaOCl reduced organic matrix (amid I, II, III peaks) but did not affect carbonates and phosphates. (120).

Another study treated the radicular segments from human teeth with 5% NaOCl for 2 min, control group was treated with distilled water. The specimens were processed for indirect immunofluorescence by using antitype I collagen and antichondroitin sulfate antibodies. The exposure to 5% NaOCl produced a drastic loss of immunoreactivity in the dentin surface with alteration in dentin collagen and glycosaminoglycans. It also showed the protective role of hydroxyapatite on organic matrix stability (121).

Saleh et al showed that irrigating the canal with 3% H_2O_2 / 5% NaOCl and 17% EDTA solutions significantly reduced the microhardness of root canal dentin. According to da Cunha et al. 10 min deproteination with 5% NaOCl reduced the push-out bond strength between dentin surfaces and fiber posts cemented (122). Sodium hypochlorite degenerate dentin by dissolution of dentinal collagen. The residual NaOCl may interfere with polymerization of the bonding resin due to oxygen generation. (123). A different study tested the effect of 10% NaOCl on the shear bond strength of different adhesive systems. The increase in the NaOCl

application time resulted in a progressive decrease in shear bond strengths for both dentin adhesives with the integrity of the collagen fibrils left exposed upon acid-etching which play a major role in the adhesive system (124).

Dentine bond strength and marginal adaptation of direct composite decrease significantly after NaOCl application by (-25%) and (-30%) respectively (125). Another study showed that after 24 months, the retention rates for Prime & Bond 2.1 system with and without 10% NaOCl pre-treatment were 80% and 63% respectively. No significant differences were found at any time between groups for retention or marginal staining (126).

Different study used SEM and CLSM-visualization of the dentin-composite interface and for bond strength measurements. The results showed that the removal of the collagen layer with 10% NaOCl can enhance or decrease bond strength depending on the bonding agent used (127).

The microtensile bond strength of four adhesive systems to root dentin with or without 5% NaOCl was tested in another study. Statistically significant differences were found among the NaOCl treated and non-treated groups. The 5% NaOCl reduced the bond strengths to dentin in almost all resin cements by 18% (128). In addition to that, Morris et al reported that 15-20 min of 5% NaOCl treatment reduced bond strength of C&B Metabond to root canal dentin by 67% (129). Same effect was also reported by Erdemir et al (130). On the other hand, Correr et al, showed that dentin surface treated with 10% NaOCl did not affect the resin-dentin bonding strength in primary teeth (131).

Potent anti-oxidants agents such as ascorbic acid or sodium ascorbate have been shown to completely reverse the reduction in bond strength. (132,133).

Preheating NaOCl solutions appears to improve its necrotic pulp tissue dissolution capacity and efficacy. A rise in temperature by 25^{o}C increased NaOCl efficacy by a factor of 100. The 1% NaOCl solution at 45^{o}C dissolve pulp tissues as effectively as the 5.25% solution at 20^{o}C (134). Cunningham et al reported that 2.6% NaOCl solution at a temperature of 37^{o} C. was equally effective as collagen dissolving agent when compared to 5.2% NaOCl at either 21^{o}C. or 37^{o}C. They also showed that warming of NaOCl solution to 37^{o}C produced a 4% and 9.5% reduction in the chlorine availability for the 2.5% and 5% solutions respectively after 24h (135). Another study tested the 2.6% and 5.25% concentration of NaOCl against rat connective tissue specimens. The results showed that regardless of the concentration, NaOCl heated to 140F was more effective than the same solution at 73.2F. Furthermore. 5.25% concentration was more effective than those at 2.6% at either temperature (136).

The dilution of NaOCl significantly reduces its necrotic tissue dissolution ability. Same study also reported that 5.25% NaOCl was the most effective antibacterial concentration and dilution of this concentration to 0.5% and 1% rendered NaOCl ineffective as necrotic tissue solvents (137). McComb and Smith evaluated the debridement property of 6% NaOCl and 1%. They concluded that during chemomechanical preparation, the 1% concentration was not as effective as the 6% in producing clean canals (37).

When NaOCl is added to the water, it undergoes the following reaction:

$NaOCl + H_2O \rightarrow NaOH + HOCl$ (hypochlorous acid) (1)

In aqueous solution, hypochlorous acid partially dissociates into the anion hypochlorite (OCl^-):

$HOCl \leftrightarrow H^+ + OCl^-$ (2)

The available chlorine is the sum of the $HOCl^+$ and OCl^- concentration in the solution (138).

In equation #2, hypochlorous acid dissociation depends on pH, as HOCl is consumed through its germicidal function, the clinical equilibrium between HOCl and OCl^- is maintained (108). According to Baker et al, at pH 10, all chlorine is in the OCl^- form, at 4.5 pH, where all chlorine is in the form of HOCl, the reverse occurs. With higher pH, the disinfecting properties decrease (139).

Bloomfield and Miles confirmed that hypochlorite at lower pH has greater antimicrobial activity (138). The availability of chlorine is dependent on the pH of the solution. Above pH of 7.6, the main form is hypochlorite and below this value is hypochlorous acid (140).

Another in-vitro study evaluated the effectiveness of different NaOCl concentration (1%, 2.5% and 5.25%) against E. faecalis. No significant difference between the three concentrations was found as all of these concentrations showed large zone of inhibition against E. faecalis. The study suggested that the use of large amount of irrigant compensated for the effect of concentration (141).

The antimicrobial efficacy of NaOCl is mainly due to its ability to oxidize and hydrolyse cell protein and to some extent, osmotically draw fluids out of cells due to its hypertonicity (142). At high concentration, NaOCl is very toxic and tends to induce tissue irritation on contact (110). In addition to that, NaOCl is a very alkaline solution with a pH of approximately 11-12. This makes the solution very hypertonic (~2800 mOsmol/kg) (142). When NaOCl contacts tissue protein, nitrogen, formaldehyde and acetaldehyde are formed within a short time and the peptide link are broken resulting in dissolution of proteins (110).

Sudden onset of pain is a landmark of tissue damage and may happen immediately or be delayed for couple of minutes or even hours (143). Involvement of the maxillary sinus will cause an acute sinusitis (144). bruising and ecchymosis of the surrounding mucosa and possibly the facial skin can also result due to bleeding into the interstitial tissues which may also include the formation of a hematoma (145,146).

Basically, NaOCl is cytotoxic to all cells except heavily keratinized epithelia. Pashley et al showed in their study that dilution as low as 1:1000 caused complete hemolysis of RBC's in vitro. Undiluted and 1:10 dilutions produced moderate to severe irritation to rabbit eyes which healed after 24 to 48 h. intradermal injections of undiluted 1:1, 1:2, and 1:4 dilutions of NaOCl produced skin ulceration (142).

A study was conducted to investigate different concentrations of NaOCl for antibacterial activity and tissue toxicity at 5,10,15, and 30 min. Concentration of NaOCl were 0.25%, 0.025% and 0.0125%. The results showed that a bactericidal effect were observed for concentrations as low as 0.025%. Tissue toxicity was

observed at concentration of 0.25%. Therefore, the 0.025% concentration preserves bactericidal properties and eliminates the harmful effect on wound healing (147). A different study by Becker et al. reported extreme pain, hematoma and ecchymosis with profuse hemorrhage when 5.25% NaOCl was forced beyond the apex of maxillary cuspid. Applying wet compresses to the face helped in reducing the burning sensation and relieved the pain. The patient was given antibiotics and analgesic and the tooth left open for drainage. There was an increase in the swelling during the next few days but the pain had subsided. Patient face had returned to normal after one month (148).

It is recommended that the clinician should look both clinically and radiographically for immature apices, resorption, apical perforation or any other conditions that result in irrigant extrusion beyond root canal to the surrounding tissues. Irrigation should be performed slowly and under gentle movement of the needle to prevent the binding of the needle into the canal wall (149). It was also recommended to keep a reservoir from the irrigant solution in the coronal chamber and to carry it into the canal during filling preparation to minimize the risk of extruding the irrigant beyond the root apex (110,150).

1.5.2 EDTA:

Ethylenediaminetetraacetic acid (EDTA) is a chelating agent with chemical formula $(HO_2CCH_2)_2NCH_2CH_2N(CH_2CO_2H)_2$.

This compound was first introduced by Ferdinand Munz in 1935 as an alternative to citric acid to use with dye solutions in the textile industry. He prepared it from ethylene diamine and chloroacetic acid (93).

Nygaard-Ostby in 1957 were the first to introduce EDTA to dentistry as an aid for the preparation of narrow and calcified root canals. EDTA is a polyprotic acid whose sodium salts are noncolloidal organic agents that can form nonionic chelates with metallic ions (94,95). It is usually used in a concentration between 10% and 17%, and to increase its chelating effectiveness, its pH was modified from 4 to a value between 7 and 8 (94-96).

One of the main inorganic components of the dentin is the calcium ion present in hydroxyapatite crystals. Any irrigant that will alter the calcium ion will affect the chemical composition of the dentin which in turn affect the permeability, microhardness and solubility of the dentin (151-153).

EDTA has a self-limiting property. This explain why its chelating action stopped once it reached equilibrium with calcium ions (97). Like other chelators, EDTA prompts the uptake of positive ions and will react with calcium ions in the hydroxyl apatite crystals, this reaction will change the microstructure of the dentin and soften it by changing the calcium-phosphorus ratio. These changes in the Ca-P ratio will affect the permeability and the hardness of the dentin and reduce the

torsional stress on the engine driven or rotary files that have been used for instrumentation (154).

The antibacterial property of EDTA is relatively limited (155), and its cleaning ability is mainly due to its ability to work as a chelator and detach the biofilm that adhere to the canal walls (94). Yoshida el al conducted a study on infected teeth and showed when 15% EDTA was used as a root canal irrigant with ultrasonic agitation, no bacteria could recover from 93 out of 129 root (156).

The biocompatibility of EDTA was also tested by Nygaard-Ostby, by evaluating the effect of forcing 15% EDTA into the periapical tissues of vital and necrotic teeth for up to 14 months, no tissue damage was detected. In addition to that, no pulpal necrosis was detected when EDTA was placed for up to 28 days after pulpotomy therapy (97).

The ability of EDTA to demineralize the dentin is due to its reaction with calcium ions in the hydroxyapatite crystals and forms soluble calcium chelates (97). EDTA also has the ability to decalcify dentin to a depth of 20-30 μm in five min (98). According to Calt et al, EDTA has the ability to remove the inorganic components of smear layer in less than 1 minute if its solution is able to reach to the surface of the root canal wall. Goldman et al. found that the smeared layer is mainly calcific in nature, and only chelating agents such as EDTA or citric acid can remove this layer (99). NaOCl by itself cannot remove the smear layer (37,44,157,158).

A SEM study was conducted to assess the final flush with different root canal irrigating solutions and showed that the combined use of 10 ml from 17%

EDTA solution buffered to pH 7.7 followed by 10 ml of 5.25% NaOCl solution was the most effective method in removing both superficial debris and smear layer components, and with this approach, the gold standard in the irrigation protocol was demonstrated (44).

Another study found that irrigating the canals walls with 17% EDTA and 6% NaOCl for 1 min is more effective in removing the smear layer than the 15 or 30 seconds groups (159). In addition to that, the use of an activation method (automated-dynamic activation with RinsEndo or the sonic-activation group with Endo-activator) with 17% EDTA and 3% NaOCl, were significantly better in removing the smear layer in the apical third than other groups (160).

Many studies were conducted to test the effect of EDTA and other chelating agents on the micohardness of the dentin (161-164). Cruz-Filho et al compared the effect of (15% EDTA, 10% citric acid, 5% malic acid, 10% sodium citrate, apple vinegar) on dentin lumen. EDTA and citric acid groups showed a sharp decrease in dentin microhardness compared to other group without a significant difference between each other (161).

Sayin et al conducted another study to evaluate the effect of single and combined use of EDTA with other agents on the micro-hardness of the dentin. Their results showed that the single and combined use of EDTA with 2.5% NaOCl significantly decreased the micro-hardness of the dentin. There is also a significant decrease only for EDTA and EDTA + NaOCl in the coronal region and for EDTAC and EDTAC + NaOCl in the apical and middle regions of the root canal (162). On the other hand, no significant difference was found between 17% EDTA and 7%

maleic acid in the reduction of microhardness but maleic acid significantly increased the surface roughness more than EDTA (163).

The effect of increasing the time of application of 17% EDTA, 17% EDTAC and 10% citric acid was also tested. No significant differences between initial microhardness after 1 min of application, however, EDTA produced a significantly greater reduction in microhardness after 3 min. EDTA and EDTAC showed no significant difference after 5 min while citric acid group showed less reduction (164).

Different studies have shown the antagonistic interactions occurring when NaOCl was used together with chelators. This interaction will cause a loss in the free available chlorine for NaOCl which consequently reduces the tissue dissolution ability of the NaOCl and its antimicrobial activities to a lesser extent (165,166).

A study by Baumgartner et al was conducted to measure the amount of chlorine gas that was evolved when 5.25% NAOCL was mixed with other irrigants. The study related the release of chlorine gas to the reduction of the PH values in the NaOCl solution. This chlorine gas was evolved significantly more when NaOCl was mixed with 50% citric acid than with 17% EDTA (167).

Both EDTA and citric acid strongly reduce the available chlorine in NaOCl solution which will result in a reduction in the effectiveness of the NaOCl and rendering the solution ineffective. (95,167). Zehnder et al tested the interaction of 17% EDTA with pH 8, 10% citric acid (CA), and other alternative chelators: 9% sodium triphosphate (STP), 15% amino tris methylenephosphonic acid (ATMA)

and 7% 1- hydroxyethylidene-1, 1-bisphosphonate (HEBP) with NaOCl. STP solution did not interact with NaOCl and showed 100% of free available chlorine, HEBP solution showed some reduction in the available chlorine, was dose dependent and continued over time. EDTA and ATMA groups caused an almost complete loss of chlorine and this effect was even more clear with CA group which showed a zero chlorine content in less than one min (168).

It has been shown in multiple studies that NaOCl does not really affect the chelating ability of the EDTA. Others, report a decrease in the tissue dissolving ability and the antimicrobial property of NaOCl when mixed with different chelators. (95,96,168).

1.6 Activation methods:

The root canal system has a very complex and irregular anatomy that mechanically instrumented canals harbor areas not attainable by currently utilized endodontic instruments (22).

According to Wu et al., in the apical areas of the oval canals, only 40% of the canal walls can be touched by the rotary instruments as both the balanced force (removed only 38.6% from inner layer of dentin) and circumferential filling (removed 57.7%) left large portions of the canal walls un-instrumented (12). Many studies showed that the canal fins, cul-de-sacs and isthmi all left untouched after instrumentation with nickel-titanium instruments, they also showed that these instruments work mainly on the central body of the canal (21,20,169,170).

Irrigation is an important part in root canal debridement however, there is no ideal irrigant which will combine all the ideal characteristics, even when its altered by changing the pH value (171,172), increasing the temperature (134,173,178), or increasing the wetting ability by adding a surfactant (174,175). Throughout the history of endodontics, many efforts have been made to develop more effective irrigant delivery and agitation systems and to improve root canal cleaning. These systems mainly divided into two broad categories: manual and machine assisted agitation devices (175).

Manual agitation technique (passive irrigation) involves the use of needles of different gauges and different designs (some are designs to dispense the irrigant through the most distal end, others have closed-ended tip that deliver the irrigant

laterally or side-vented channels.), to deliver the irrigant inside the canal. This can be done either passively or with agitation by moving the needle up and down (176). To reduce the chance of apical extrusion of the irrigant, the needle should remain loose inside the canal. The use of this technique will allow an easy control for the volume of the irrigant and the depth of the needle within the canal (175,177).

One of the critical factors that affect the efficiency of the manual irrigation is the depth of the needle. Grossman showed the importance of the adequate enlargement of the root canal to improve the irrigation efficiency. it was also reported that the irrigation with size 28G safety-ended needle will be less effective when the canal is enlarged to a size less than 40 at the apex (1,179,180).

The efficacy of the apical irrigation is directly proportional to the depth of the insertion of the needle (181). In order to allow the irrigant to reflux and move the debris coronally, the needle should fit loose in the canal. smaller gauged needle is recommended to establish deeper and more effective placement (182).

On the other hand, manual dynamic irrigation can be done by gently moving up and down a well-fitting gutta percha master cone in short 2-3 mm strokes. It has an effective hydrodynamic effect and improve the exchange of the irrigant (183-185).

McGill et al did a study to compare the efficacy of (static, manual-dynamic and automated-dynamic “RinsEndo”). The results showed that the automated-dynamic technique was significantly better (16%) than static irrigation but significantly showed lower value (5%) than manual-dynamic irrigation. When the

needle was placed closer to working length, the effectiveness of the irrigation increased by 7% (186).

Many factors could have affected the positive results of manual-dynamic irrigation: a- the push-pull action of a good-fitting gutta-percha cone in the canal may produce higher intra-canal pressure alteration similar to what a drain plunger does during the pushing action, resulting to a more efficient delivery of the irrigant to the untouched canal walls; b- the frequency of push-pull action of the gutta-percha point (33 Hz - 100 strokes per 30 seconds) is more than the frequency (1.6 Hz) of positive-negative hydrodynamic pressure produced by RinsEndo, possibly creating more turbulence inside the canal; and c- the push-pull action of the gutta-percha cone probably works by physically folding and dislodging the fluid under “viscously-dominated flow” (187) in the canal, which may allow a more efficient mixing of new unreacted solution with the spent, reacted molecules of the active NaOCl irrigant (186).

Ruddle demonstrated a machine-assisted type of agitation system by using a rotary handpiece-attached microbrush to remove debris from instrumented root canals. The microbrush rotates at 300rpm, causing the bristles to deform into the irregularities of the preparation, this action will displace the debris outside the canal. this device has not been commercially available since the patent was approved in 2001 (175,188). CanalBrush (Coltene Whaledent, Langenau, Germany) has recently made it commercially available. This highly flexible endodontic microbrush can be used manually with rotary action. However, when attached to a contra-angle handpiece running at 600 rpm, it becomes more

efficacious (175). Weise et al. reported an effective method of removing the debris from simulated canal extension and irregularities by using a flexible and small CanalBrush (189).

Tronstad et al in 1985, were the first to introduce the sonic instrument for endodontics use by using an air-driven sonic vibratory handpiece to which specially designed K-type files are attached. When activated, the instruments will vibrate in a whirling motion, and will graze the root canal wall when moved up and down (190).

Sonic instruments use a lower frequency (1000-6000 Hz) compared to ultrasonic instruments (25000 Hz) and produces smaller shear stresses (191). The vibration pattern of ultrasonic files is different from that of sonic instruments. Sonic files have a single node near the attachment of the file and one antinode at the tip of the instrument whereas ultrasonic activated files have numerous nodes and antinodes across the length of the instrument (192,193). In addition to that, the amplitude or the back and forth tip movement is significantly higher and greater with sonic energy (175).

Sonic endodontic instrument produces a large elliptical oscillation when operated in air, when the sonic file was loaded, the elliptical motion was eliminated leaving a pure longitudinal file oscillation. This oscillatory pattern of the sonic file may offer a useful mode of mechanically assisted root canal debridement as it is largely unaffected by loading and retains a large displacement amplitude (185). According to Sabins et al, passive sonic or ultrasonic irrigation for 30 sec resulted in significantly cleaner canals than hand filing alone. They also showed that

ultrasonically irrigated groups had significantly less debris at both 0-3 mm and 3-6 mm levels than the sonically irrigated group (194).

The EndoActivator system (Dentsply-Sirona, USA) is a sonically driven canal irrigation system with sonic vibration (up to 10,000 cpm). This battery-operated system comprised of a sonic handpiece and variously sized polymer tips (195). Due to the smooth structure and the flexible composition of these tips, their cutting efficiency into the dentin is very limited. A possible disadvantage to these tips is that they are radiolucent which will be difficult to identify if part of the tip separates inside the canal. This can possibly be improved by adding some radiopacifier to these polymer tips (195).

The use of EndoActivator facilitates the removal of the debris from lateral canals, remove the smear layer when used with demineralizing agents like EDTA and dislodge clumps of simulated biofilm within the curved canals of molar teeth (184,196).

A cloud of debris can be seen within a fluid-filled pulp chamber when the tip of the EndoActivator is activated. The main function of the EndoActivator is to produce vigorous intracanal fluid agitation through acoustic streaming and cavitation (195). According to Guerisolo et al., this hydrodynamic activation improves the penetration, circulation and flow of irrigant into the more inaccessible regions of the root canal system (197).

According to Van der Sluis et al., EndoActivator is an effective system to remove $Ca(OH)_2$ from experimental grooves within a prepared canal (198).

Furthermore, this system can also be used to deliver MTA (Dentsply-Sirona) into immature teeth with blunderbuss canals, or into perforation defects (195).

The use of ultrasonic was first introduced into dentistry by Catuna for cavity preparations using an abrasive slurry (322). Zinner reported the use of an ultrasonic instrument to remove plaque and calculus deposits from the tooth surfaces.

In 1957, Richman was the first to introduce the concept of ultrasonic instrumentation to endodontics. Martin et al. introduced the use of ultrasonically activated K-type files to remove dentin in the preparation of root canals before obturation (199,200) and in 1980, ultrasonic unit was commercially available for endodontic application (201).

The term **"endosonic"** was introduced by Martin and Cunningham as an ultrasonic synergistic system which combine both instrumentation and canal disinfection (202).

Mechanical agitation or fluid flow is more important in the ability of NaOCl to dissolve tissue than the initial percentage of available chlorine (203). The introduction of ultrasonic vibration is directly associated with the cleaning effectiveness of the irrigant to the canal space (175).

Cesar de Gregorio et al showed that ultrasonic and sonic activation resulted in a more efficient irrigation to the lateral canals at 4.5 and 2 mm from working length and the addition of EDTA did not improve the penetration of the irrigants into the lateral canals (204).

It was demonstrated that passive ultrasonic irrigation with a nickel-titanium tip has superior necrotic tissue-dissolving ability over sonic irrgant activation while preserving the simulated canal anatomy (205).

Ultrasound is a sound energy with a frequency above the range of human hearing, which is 20 kHz (206). Low frequency ultrasonic handpieces (1-8 kHz) were developed to produce lower shear stresses which cause less alteration to the tooth surface (207-210).

Two main methods for production of ultrasonic wave were identified: the first is **magnetostriction** which converts the electromagnetic energy into mechanical energy. This method has elliptical movement and oscillate in figure-eight pattern which generate heat, thus, adequate cooling is necessary.

The second method is based on the piezoelectric principle, in which a crystal is used that changes dimension when an electrical charge is applied. The deformation of this crystal is converted into mechanical oscillation without producing heat (206,211-213). The tip of piezoelectric unit moves in a linear back and forth, piston-like motion which is ideal for Endodontics use. According to Lea et al, the position of nodes and antinodes of the endosonic file activated by a 30 kHz piezon generator was along the file length and does not increase linearly with increasing generator power. This is helpful in surgical Endodontics and when troughing to look for hidden canals or when removing separated instruments or posts (214,215).

Two types of ultrasonic irrigation have been described in the literature. The first one is incorporation of simultaneous ultrasonic instrumentation and irrigation (UI). The second type is the passive ultrasonic irrigation (PUI) (198).

Due to the difficulty of controlling the cut of dentin and the possibility of making aberrant conformation and alteration to the canal shape, the first type has less popularity in dental practice. Therefore, literatures supported the use of ultrasound for passive irrigation (216-219).

Weller et al. in 1980, was the first to use the term Passive Ultrasonic Irrigation (PUI) to describe a passive irrigation approach where there was no instrumentation, or contact of the canal walls with an Endodontic file or instrument “noncutting” action (175,219).

Passive Ultrasonic Irrigation depends on the transmission of acoustic energy by means of ultrasonic waves from an oscillating file or smooth wire to an irrigant in the root canal. This action can produce acoustic streaming and cavitation of the irrigant (191,209,220).

During PUI, two flushing methods might be used, a continuous flush of irrigant from the ultrasonic handpiece or an intermittent flush by using syring delivery which allow more control on the amount of irrigant flowing through the apical area of the canal (175,177).

Many studies have shown the effectiveness of PUI in removing remnants of pulp tissue and canal debris (221-224), with the ability to significantly reduce the amount of planktonic bacteria (225-227) and its superiority over syringe irrigation (226). Moorer & Wesselink showed the significant increase in tissue dissolving

ability of organic material by NaOCl when NaOCl is agitated by ultrasonic (203). Other studies also explained the enhancement in this effect due to the increase in NaOCl temperature by ultrasound effect (173,228,229).

Acoustic streaming is the rapid movement of fluid in a circular or vortex-like motion around a vibrating file. Acoustic microstreaming is the acoustic streaming inside the root canal during ultrasonic irrigation which usually occurs during PUI (207,209,211,230).

The tip of the file represents the maximum displacement amplitude, a reduction in this amplitude will occurs when the file touches the canal walls at an antinode compared with when it touches at a node (198,230). When the file is unable to vibrate freely, acoustic microstreaming will become less intense, however, it will not stop completely (198,209,230).

Pre-shaping the file in a curve canal will cause more powerful acoustic streaming (198,217,220). Studies have shown that the thinner the file, the higher the frequency, streaming velocity and the displacement amplitude of the file. This will also result in a stronger acoustic microstreaming (198). The shear flow produced by the acoustic microstreaming produces shear stresses along the canal wall, which can remove debris and bacteria from the canal (198,209). Jensen et al. recommended the use of a vibrating file with small size under high power setting to reduce the chances of the file to contact the canal walls (231).

Cavitation in a fluid can be described as the impulsive formation of cavities in a liquid through tensile forces induced by high speed flows or flow gradients.

This action will cause the bubbles to expand and then rapidly collapse producing a focus of energy (198).

Acoustic cavitation can be defined as the formation of new bubbles or the expansion, contraction and/or distortion of pre-existing bubbles, so called (nuclei) in a liquid, the process being coupled to acoustic energy (198,233).

According to Roy et al. two types of cavitation can occur during PUI: transient and stable. Transient cavitation occurs when the vapor bubbles undergo highly energetic pulsations and when the file can vibrate freely or slightly touches the canal wall. This type of cavitation was more visible at the end and along the length of the file. Also in their study, they reported that a smooth file with sharp edges and a square cross-section produced significantly more transient cavitation than a regular K-file (198,230).

Stable cavitation is a linear pulsation of gas-filled bodies in a low amplitude ultrasound field. When the file comes in contact with the canal wall, stable cavitation was affected less than transient cavitation and was seen at the midpoint of the file (198,230).

Air entrapment by an advancing liquid front in closed-end microchannel is a well-recognized physical phenomenon and has been referred to as vapor lock effect in the Endodontic literatures (234-236). The contact angle of the liquid and the depth and size of the channel will determine the ability of the liquid to penetrate through these closed-end channels. In Endodontics, root canal irrigation is usually performed within time frame of minutes, air entrapment in the apical area of the

root canal might prevent the irrigant from adequately contact and disinfect this area (175, 237, 238, 235).

Senia et al. showed that NaOCl did not reach any closer that 3 mm from working length even after enlarging the apical part of the root to a size 30. This can be related to the fact that NaOCl will quickly forms micro gas bubbles at the apical end of the root canal after reacting with the organic tissues inside the root. these gas bubbles coalesce into apical vapor lock with subsequent instrumentation (175,236).

This vapor lock will prevent any irrigants from reaching into the apical area. Acoustic microstreaming and cavitation can only occur in a liquid phase, therefore once the ultrasonically activated tip leaves the irrigant and goes inside the apical vapor lock, acoustic microstreaming/cavitation becomes impossible (175,239). Boutsioukis et al. suggested a brief insertion of the needle to working length whilst irrigating at a flow rate of 0.083 mL s^{-1} and delivering irrigant at 0.260 Ml s^{-1} without moving the needle were capable of removing an established apical vapor lock (240).

Other studies suggested the use of a hand-activated well-fitting root filling material (eg, a size 40, 0.06 taper gutta-percha point) to working length after instrumentation with the corresponding rotary instrument. This simple method will help in eliminating the vapor lock at the space previously occupied by air and will be replaced by root filling material that will carry with it a film of irrigant to working length of the canal (175,241).

Vera et al suggested to maintain the apical patency of the root canal with a size #10 ISO file to improve the apical penetration of the irrigant and overcome the vapor lock effect, this approach can be achieved after preparing the canal with an apical diameter of greater than #30 0.06 file. The results of this approach did not significantly improve the apical penetration of the irigant (242,243).

The EndoVac system (Discus Dental, Culver City, CA, USA) is a new irrigation system based on a negative pressure approach. This system consists of master delivery tip, macro-cannula and micro-cannula (239).

The master delivery tip is responsible to deliver and evacuate the irrigant. This tip is connected to a syringe of irrigant and the evacuation hood is connected via tubing to the high speed suction of the dental unit. The macro-cannula is used to suction irrigant from the chamber toward the coronal and middle sections of the root canal. the macro-cannula or micro-cannula is connected by tubing to the high-speed suction of the dental chair (239).

The macro-cannula is responsible for the initial flushing of the coronal part of the canal. The tip of the macro-cannula has a size of 0.55 mm and 0.02 taper (239).

During irrigation, irrigant will be delivered to the pulp chamber with the master delivery tip, this tip will also suction the access irrigant to prevent over flow. The cannula inside the canal will pull the irrigant from the pulp chamber down the canal by negative pressure, then the irrigant will be suctioned into the cannula and out through the suction hose. The negative pressure mechanism will enable the

irrigant to reach to the apical potion of the canal (working length) and to avoid the apical vapor lock (239,244).

Nielsen et al. compared the efficacy of EndoVac system with that of needle irrigation using NaOCl and EDTA at 1 and 3 mm from working length. No significant differences were noticed at the apical 3 mm of root canal, but EndoVac system was significantly better and resulted in less remaining debris at 1mm level from apex. They also showed that during same time, the volume of the irrigant delivered by EndoVac system was significantly higher than the volume delivered by conventional syringe irrigation with the advantage of reducing the risk of irrigant extrusion to the periapex area (245,246).

One of the disadvantages of EndoVac is the clogging of the micro-cannula holes with debris which may affect the efficacy of the system. This can be overcome by replacing the cannula or using positive pressure rinse to open the blocked holes (247).

Our research was designed to observe the different irrigation protocols, that are available today in Endodontics, to compare the results of smear layer removal, the amount of remaining debris and visibility of the dentinal tubules. Our hypothesis is that there should be no significant difference.

2. Materials and Methods

Two hundred and seven (207) recently extracted molar teeth (maxillary and mandibular) were obtained from the teeth Databank at Nova Southeastern University College of Dental Medicine. The inclusion criteria included teeth with: intact coronal structure, no evidence of previous root canal therapy, no signs for any internal, external or cervical resorption, no restorations or carious lesions and single canal roots with a curvature of 30° or more. The angle of curvature was selected according to Schneider's method. In this method, first point was marked at the middle of a file at the level of the canal orifice, a straight line was drawn parallel to the file image from the first point to the level where the file starts to deviate from this line, this will represent the second point. The third point was marked at the apical foramen of the root and another line was drawn from this point to the second point. The angle formed by the intersection of the lines represents the canal curvature (248).

Only fifty-one roots of the two hundred and seven molars met the criteria.

The teeth were decoronated to obtain a standardized root length of 12 mm (261). After decoronation, patency and working length were verified by placing a number 10 K-file into the canal. A Global Endodontic Microscope (Global Surgical Corporation - St. Louis, MO, U.S.A) at 2.00x magnification power was utilized to visualize the file at the apex. The working length (WL) was determined by subtracting 0.5 mm from the canal length (249) as determined by the #10 K-file.

Before the instrumentation, root tips were sealed with hot glue and roots were embedded into silicone mold.

Instrumentation:

Instrumentation was performed by using NiTi rotary instruments (ProTaper system, Dentsply-Sirona). The sequence of the rotary files: SX, S1, S2, F1. The apical preparation was performed by using ProFile #25/.04, #30/.04 and finally #35/.04 hand files (ProFile system, Dentsply-Sirona). Between each instrument, canals were irrigated with 1 ml of 6% NaOCl solution using a syringe and a 30-gauge needle (Max-I-Probe needle, Dentsply-Sirona) (n= 39). For EndoVac group (n=12); the pulp chamber was flushed with 1 ml of 6% NaOCl solution using MDT (Master delivery tip).

To maintain irrigation consistency, a programmable syringe pump (PSP) (Alladin, AL 1000; World Precision Instruments, Inc, Sarasota, FL) set to deliver 3.0 ml / min was connected to each system.

After finishing the cleaning and shaping, the teeth were randomly distributed to the following groups (Figure 1);

1. **Traditional irrigation** with a 30-gauge needle (Max-I-Probe needle, Dentsply Tulsa Dental). (Max-I-Probe needle, Dentsply Tulsa Dental). Needle tip will be positioned at Working Length– 2 mm. (n=12)

a. 6% NaOCl application for 30 secs, followed by a passive wait of 60 secs,

b. 17% EDTA for 30 secs, followed by a passive wait of 60 sec

c. Final irrigation with 6% NaOCl for 30 secs, followed by a passive wait of 60 sec.

2. **EndoActivator** and a 30-gauge needle (Max-I-Probe needle, Dentsply Tulsa Dental). Needle tip will be positioned at working Length – 2 mm. (n=12)

a. 6% NaOCl application for 30 secs, followed by an activation for 60 sec

b. 17% EDTA application for 30 secs, followed by an activation for 60 sec

c. Final irrigation with 6% NaOCl for 30 secs, followed by a passive wait of 60 seconds.

3. **PUI** (Passive Ultrasonic Irrigation)

Needle tip will be positioned at working Length – 2 mm. (n=12)

a. 6% NaOCl application for 30 secs, followed by a PUI activation for 60 seconds

b. 17% EDTA application for 30 secs, followed by an activation for 60 seconds

c. Final irrigation with 6% NaOCl for 30 secs, followed by an activation for 60 seconds.

4. **EndoVac irrigation**. (n=12)

a. Macro Cycle – 20 seconds of rapid apical/coronal movement from as

deep as the macro will go apically to the pulp chamber floor.

b. Micro Cycle

I. 6% NaOCl for 30 secs, followed by a passive wait for 60 seconds.

II. 17% EDTA application for 30 secs, followed by a passive wait for 60 seconds

III. Final irrigation with 6% NaOCl for 30 secs, followed by a passive wait for 60 seconds.

5. Saline irrigation with the same protocol as group 1 with a 30-gauge needle. (n=3)

After the final irrigation, teeth were removed from the silicone mold and fixed by submerging in a 10 percent neutral-buffered formalin solution at 18^{o}C for 24 hours. After the fixation, the teeth were dehydrated and bisected longitudinally in a buccolingual direction. A chisel was used to expose the root interiors. One half of each root was coated with sputter coat 2X 30sec under argon gas using a gold target with Cressington 108 sputter coater. After this process those specimens were imaged under low vacuum conditions with the FEI Quanta 200 SEM.

The root halves (n=102) were evaluated under Scanning Electron Microscope (SEM) (Figure 2).

Scanning electron microscope was introduced by McMullan. This type of electron microscope scans the sample with a focused beam and creates an image

for it. Different signals, which carry information about the sample's surface structure and composition, will be produced when the electron interacts with atoms at different levels within the sample (250,251). Ardenne in 1937 was the first to invent a true microscope with high magnification (252). SEM micrograph has the ability to produce a three-dimensional appearance due to the large depth of field produced by the very narrow electron beam.

The amount of smear layer presents on the surfaces of the root canal wall at the coronal, middle, and apical portion was scored according to Nova Grid System (Nova Southeastern University-College of Dental Medicine Scoring System) (Figure 3). This system permits random scoring from 3 calibrated, independent Board C ertified Endodontists in order to provide a fair and reliable evaluation of each system used.

The scoring system for the amount of dentinal tubules opened, will be as follows (Figure 4):

Score 1- All dentinal tubules visible.

Score 2- More than 50% of the tubules visible (>50%).

Score 3-Less than 50% of the tubules visible (<50%).

Score 4-No tubules visible.

For smear layer removal the results will be recorded as follows (Figure 2):

Score 1- No smear layer present.

Score 2- Less than 50% of the surface covered with smear layer (<50%).

Score 3- More than 50% of the surface covered with smear layer (>50%).

Score 4- All surface covered with smear layer.

For the amount of debris and canal cleanliness the scale will consist of the following:

Score 1- No debris present.

Score 2- Less than 50% of the surface covered with debris (>50%).

Score 3- More than 50% of the surface covered with debris (>50%).

Score 4- All surfaces covered with debris.

The scores given to each individual square was averaged to get one score per slide. A form was provided to the evaluator for data collection (Figure 5). Data was analyzed by using the Cochran-Mantel-Haenszel method.

The teeth were randomly distributed to the following groups:	
#	Groups
1	Traditional irrigation with a 30-gauge needle (Max-I-Probe needle, Dentsply Tulsa Dental). (Max-I-Probe needle, Dentsply Tulsa Dental). (n=12)
2	EndoActivator and a 30-gauge needle (Max-I-Probe needle, Dentsply Tulsa Dental). Needle tip will be positioned at working Length – 2 mm. (n=12)
3	PUI (Passive Ultrasonic Irrigation). Needle tip will be positioned at working Length – 2 mm. (n=12)
4	Endovac irrigation. (Discus Dental, Culver City, CA) (n=12)
5	Saline irrigation with the same protocol as group 1 with a 30-gauge needle. (n=3)

Figure 1. Treatment groups

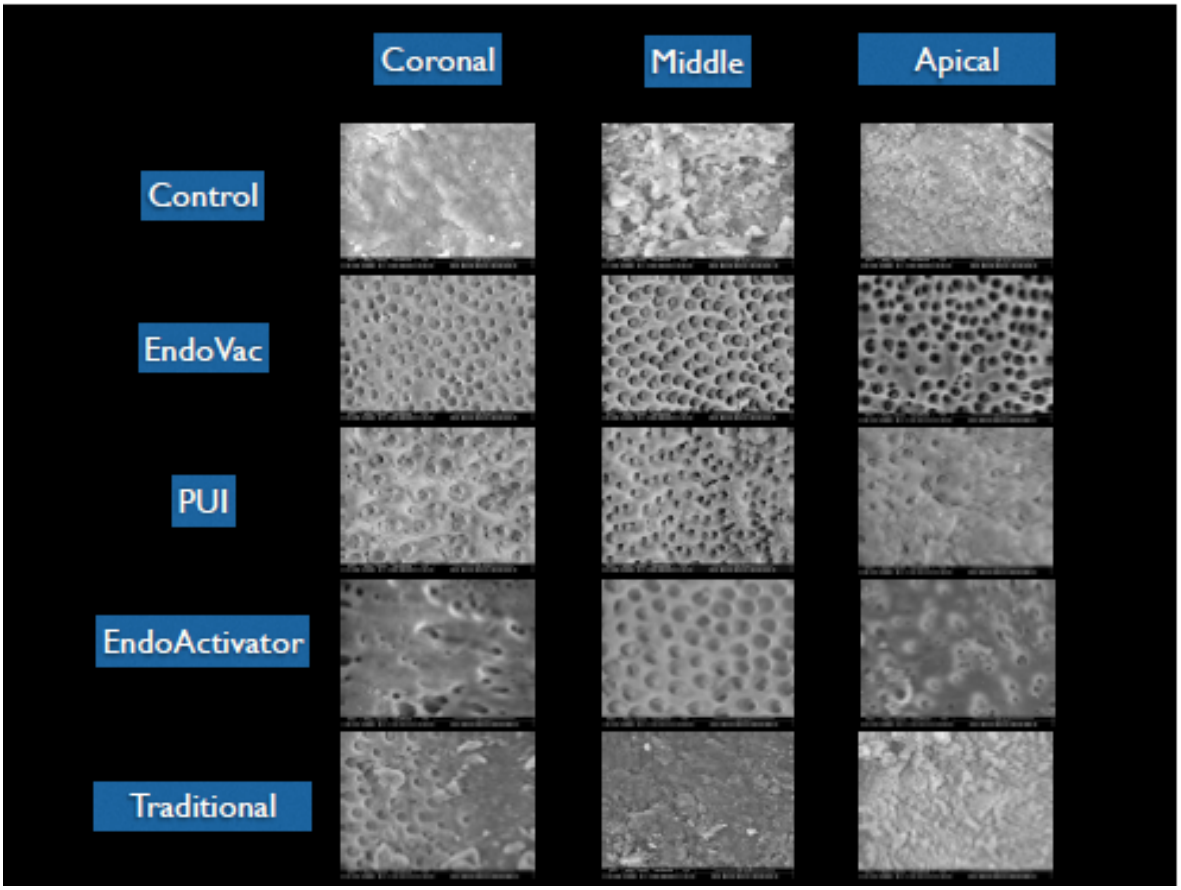

Figure 2. Representative SEM micrographs taken from the coronal, middle and the apical third of the canals.

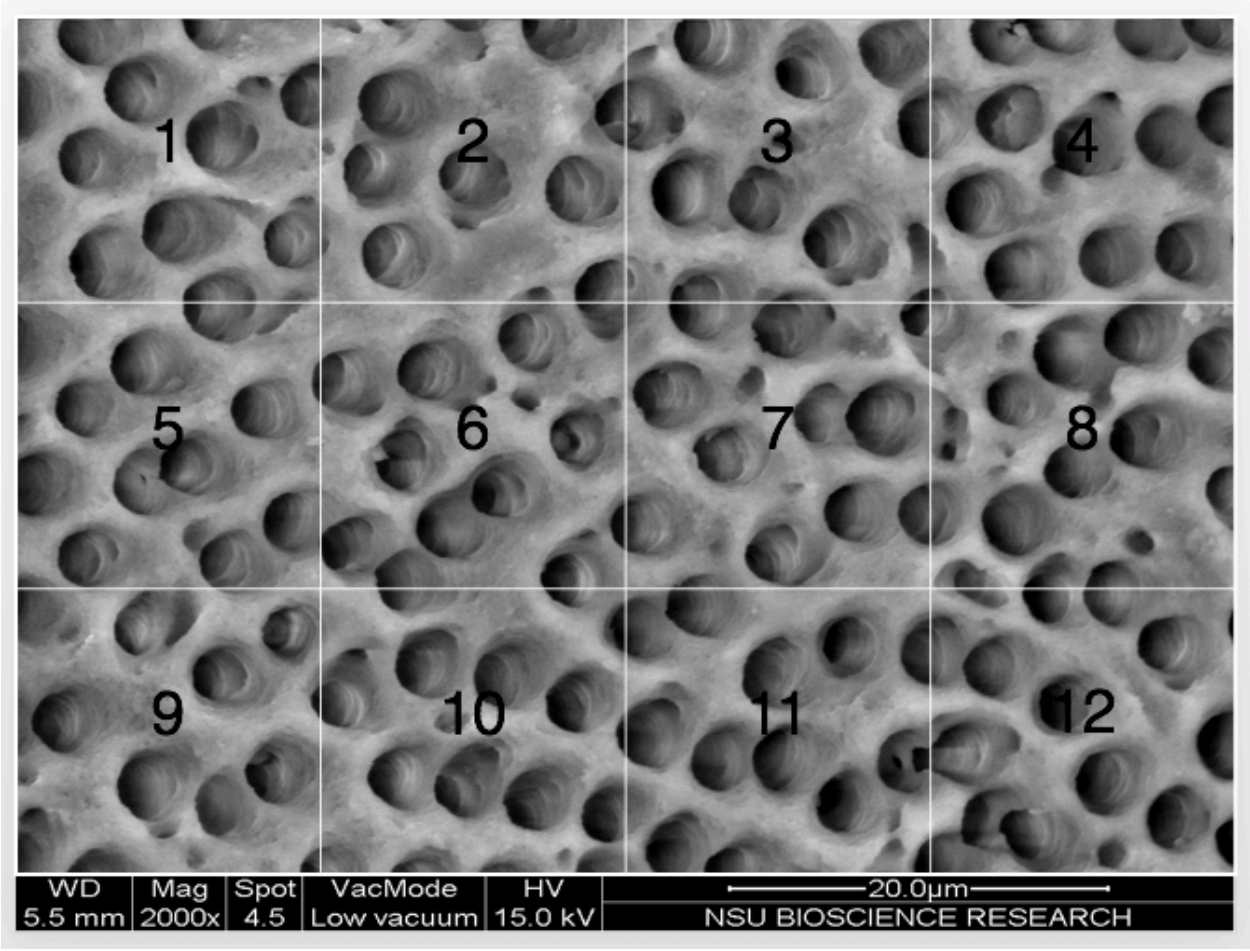

Figure 3. Sample SEM micrograph to illustrate Nova Grid Scoring System taken from one of the EndoVac specimen.

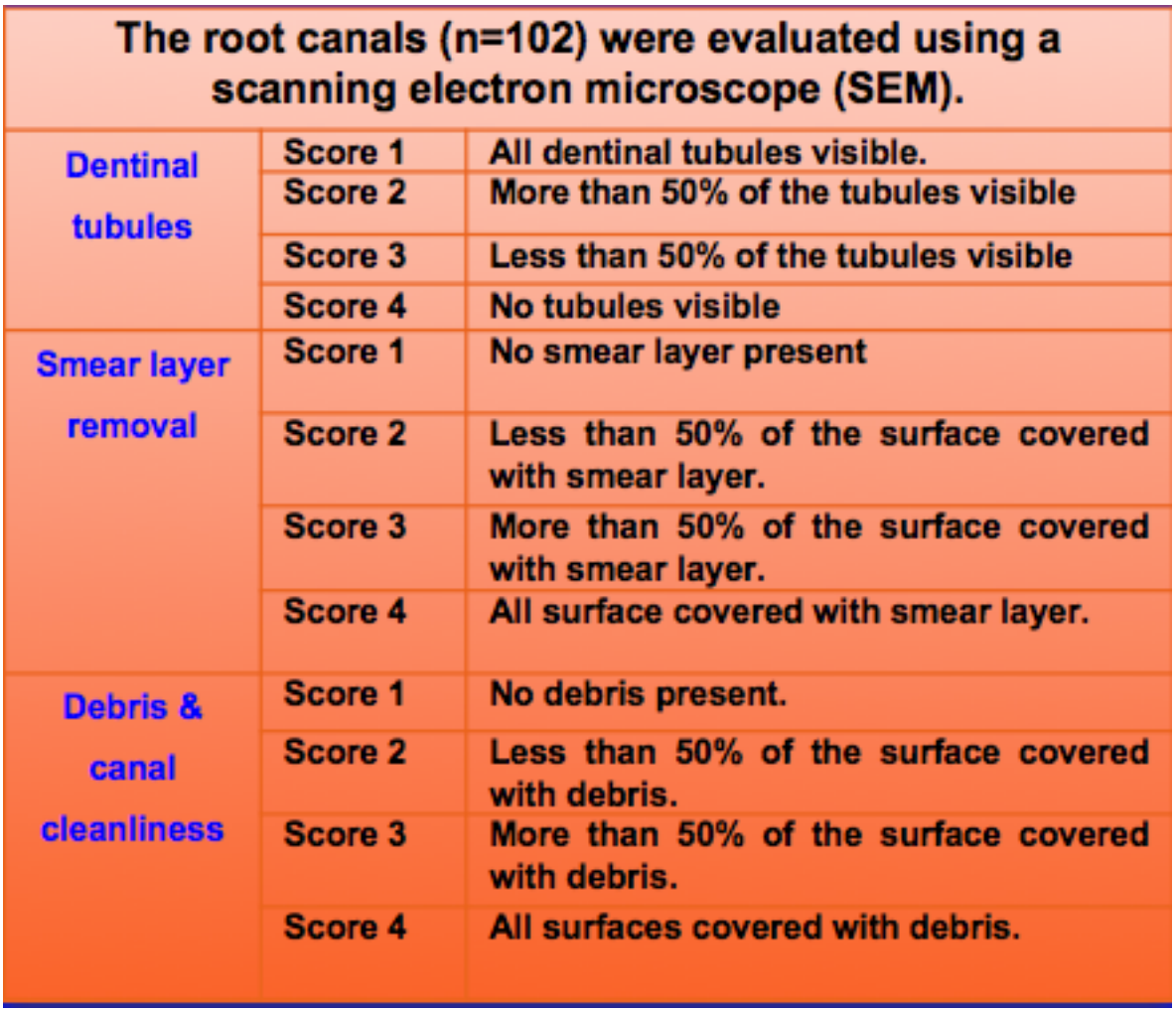

Figure 4. Scoring system

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	o	P	Q	R	S	T	U	V	W	X	Y	Z	A	AB	AC	AD	AE	AF	AG	AH	AI	AJ	AK	AL	AM
1	Sample	Dentin Tubules													Smear Layer													Debris												
2		1	2	3	4	5	6	7	8	9	10	11	12		1	2	3	4	5	6	7	8	9	10	11	12		1	2	3	4	5	6	7	8	9	10	11	12
3	1	1	2	4	4	2	2	3	4	2	2	4	4		1	1	4	4	1	1	3	4	1	2	4	4		2	2	3	2	2	2	2	2	2	2	2	3
4	2	3	3	3	2	3	3	4	3	2	3	3	4		3	3	3	3	3	3	3	3	3	3	4	4		4	4	4	4	4	4	4	4	4	4	3	3
5	3	3	3	3	3	3	3	3	3	3	3	3	3		3	3	3	3	3	3	3	3	3	3	3	3		2	2	2	1	1	2	3	2	2	2	2	2
6	4	3	3	4	3	4	4	4	4	4	4	4	3		3	3	3	3	3	3	3	3	3	3	3	3		2	2	2	3	2	2	3	2	2	2	3	3
7	5	3	3	3	3	3	3	3	3	3	3	3	3		3	3	3	3	3	3	3	3	3	3	3	3		2	2	2	1	3	2	2	2	2	2	1	1
8	6	4	4	4	4	4	4	4	4	4	4	4	4		4	4	4	4	4	4	4	4	4	4	4	4		3	2	2	2	2	2	2	2	3	3	2	2
9	7	4	4	4	4	4	4	4	4	4	4	4	4		4	4	4	4	4	4	4	4	4	4	4	4		2	2	2	2	2	2	2	2	3	2	2	2
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22	20	3	3	3	3	3	3	3	3	3	3	3	3		3	3	3	3	3	3	3	3	3	3	3	3		2	2	2	2	2	2	2	2	2	2	2	2
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28	26	3	3	3	3	3	3	2	2	2	2	3	3		3	3	3	3	3	3	2	3	3	3	3	3		2	2	2	1	2	2	2	2	2	2	2	2
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30	28	3	3	3	3	3	3	3	3	3	3	3	3		3	3	3	3	3	3	3	3	3	3	3	3		1	1	1	2	1	1	1	1	2	1	1	2

Figure 5. Sample from the form that was provided to the evaluator for data collection

3. Results

The average scores of SEM evaluation of each group are shown in (Table 1). In the control group (Saline), smear layer covered the entire surface of the root canal dentin. There were relatively very few open dentinal tubules.

Nested-random effects model was used to look for differences by group, and to group by sections of the tooth for each dependent variable (tubules, smear layers, and remaining debris). Pairwise comparisons were conducted using Bonferroni adjustments. Consistency between raters was calculated using intra-class correlation coefficients (ICC).

The traditional irrigation group demonstrated more samples with a moderate smear layer (Fig. 2), whereas the EndoVac, EndoActivator and PUI groups demonstrated more samples with visible dentinal tubules. The EndoVac system removed significantly more smear layer and debris than other groups in every segment of the roots. There was no significant difference between EndoActivator and PUI except the debris removal in the apical third. EndoActivator removed significantly more debris compared to PUI in the apical third. The highest scores, indicating the presence of more debris and smear layer were recorded in the apical third of the canals. This was followed by the middle third, and, then the coronal third (Fig. 2, 6).

Statistical significance was found at $p<0.05$, and results are presented below using marginal means. Marginal means are the means for that factor averaged across all levels of the other factor.

	Tubule visibility			Smear layer			Debris		
Group	Coronal	Middle	Apical	Coronal	Middle	Apical	Coronal	Middle	Apical
Traditional	3.09	2.83	3.46	3.11	2.81	3.42	2.40	2.21	2.55
	0.75	0.81	0.87	0.74	0.79	0.90	0.69	0.59	1.01
Passive Ultrasonic	2.62	2.88	3.30	2.69	2.90	3.23	1.88	1.76	2.14
	0.96	0.71	0.62	0.91	0.80	0.73	0.79	0.69	0.93
Endoactivator	2.46	2.64	2.73	2.51	2.74	2.92	1.79	1.75	1.87
	0.83	0.94	0.96	0.71	0.82	0.82	0.72	0.81	0.81
Endovac	1.93	1.78	1.84	2.16	2.00	2.11	1.44	1.41	1.40
	0.83	0.83	0.81	0.68	0.74	0.72	0.54	0.51	0.49
Control	4.00	3.98	3.99	3.93	3.99	4.00	2.66	2.72	2.74
	0.00	0.15	0.21	0.25	0.10	0.00	1.06	0.99	1.21

Table 1. Average scores of SEM evaluation of tubule visibility, smear layer and remaining debris. Scores 1-2 (clean canal wall) versus 3-4.

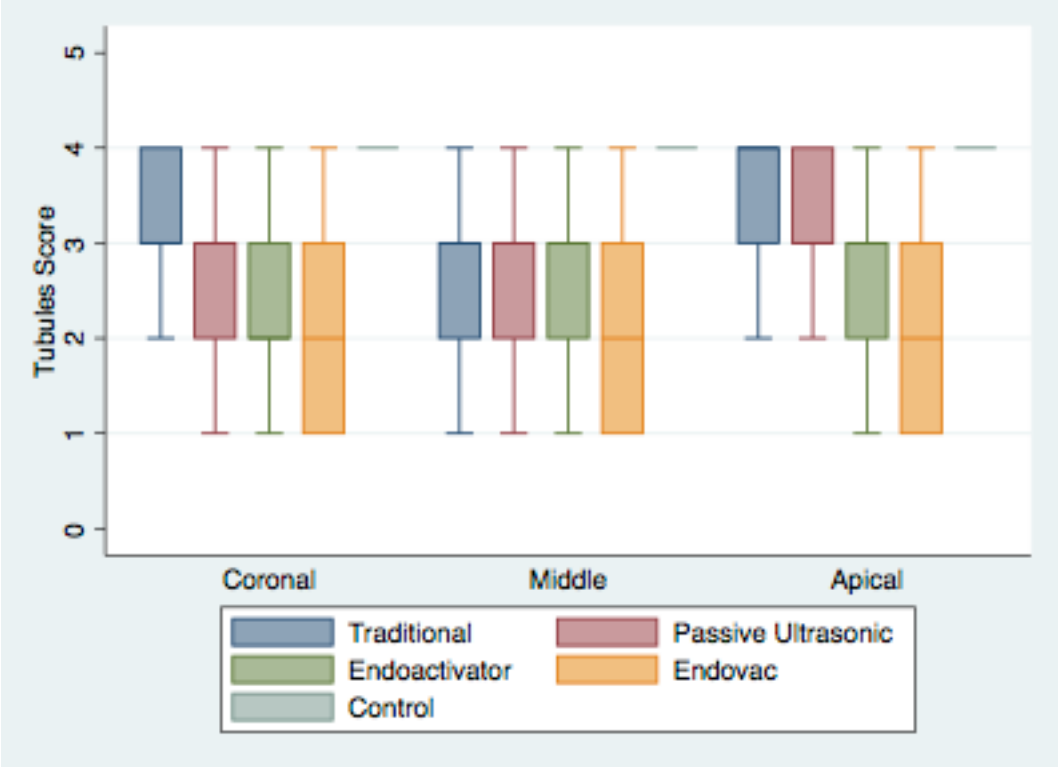

Figure 6.1. Distribution of tubule visibility scores at coronal, middle and apical levels of all three examiners.

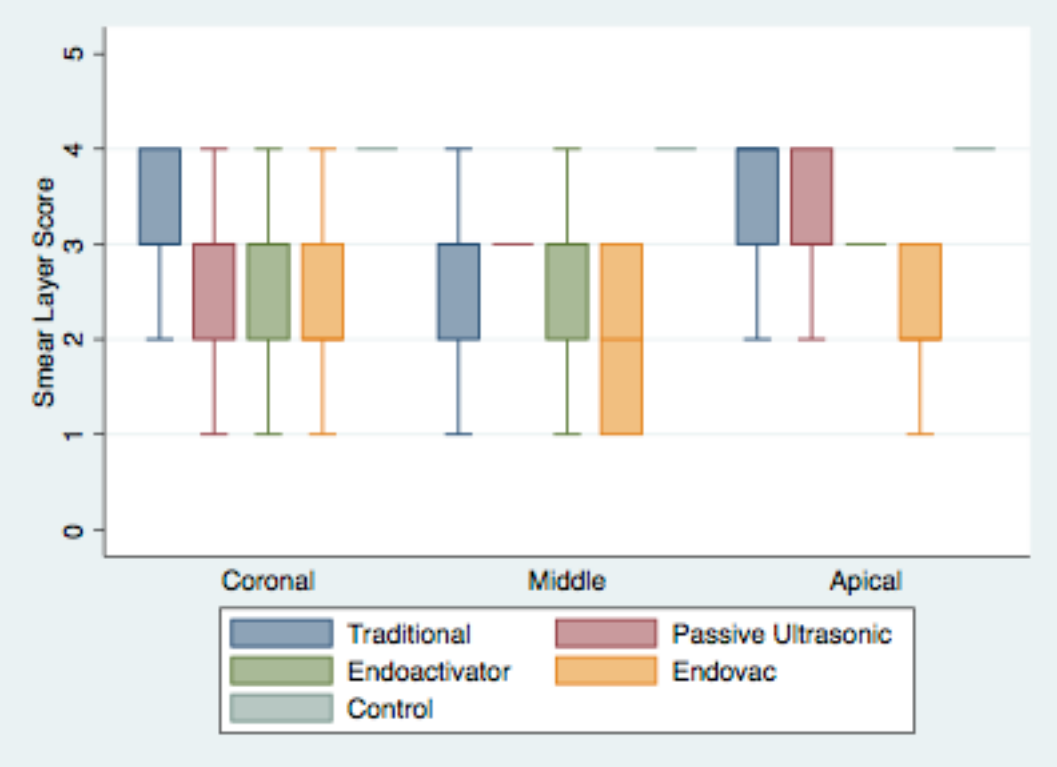

Figure 6.2. Distribution of smear layer scores at coronal, middle and apical levels of all three examiners.

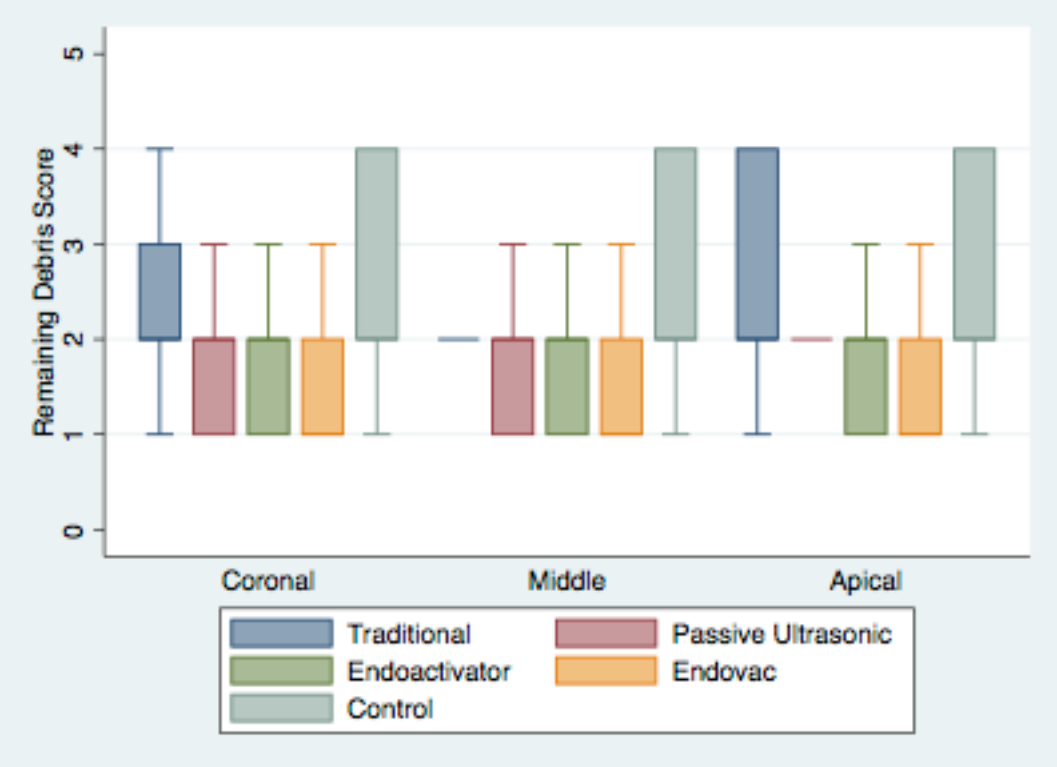

Figure 6.3. Distribution of remaining debris scores at coronal, middle and apical levels of all three examiners.

Tubules:

Table 2. Tubules Descriptive Statistics*

Measure	Intervention Group	Coronal	Middle	Apical
Tubules	Traditional	3.09	2.83	3.46
		0.75	0.81	0.87
	Passive Ultrasonic	2.62	2.88	3.30
		0.96	0.71	0.62
	Endoactivator	2.46	2.64	2.73
		0.83	0.94	0.96
	Endovac	1.93	1.78	1.84
		0.83	0.83	0.81
	Control	4.00	3.98	3.99
		0.00	0.15	0.21

Table 2.1. Tubules Intra Class Correlation Coefficient - Rater Agreement*

ICC	Measure	Lower 95% CI	Upper 95% CI
Average	0.98	0.98	0.99

* The tables are pairwise comparisons between the means for each group. The difference is the mean difference. Significance represent a statistical difference if $p < 0.05$.
Table 2.1 tells us how similar the raters were in rating the measures. An ICC of .98 is excellent.

Table 2.2. Tubules Pairwise Comparisons for ALL Sections*

Group		Group	Difference	Lower 95% CI	Upper 95% CI	Significant
Passive Ultrasonic	vs	Traditional	-0.20	-0.51	0.12	NS
Endoactivator	vs	Traditional	-0.51	-0.87	-0.15	$p < 0.05$
Endovac	vs	Traditional	-1.27	-1.53	-1.02	$p < 0.05$
Control	vs	Traditional	0.87	0.65	1.09	$p < 0.05$
Endoactivator	vs	Passive Ultrasonic	-0.31	-0.67	0.05	NS
Endovac	vs	Passive Ultrasonic	-1.08	-1.34	-0.82	$p < 0.05$
Control	vs	Passive Ultrasonic	1.07	0.84	1.29	$p < 0.05$
Endovac	vs	Endoactivator	-0.76	-1.08	-0.44	$p < 0.05$
Control	vs	Endoactivator	1.38	1.09	1.67	$p < 0.05$
Control	vs	Endovac	2.14	2.01	2.28	$p < 0.05$

Table 2.3. Tubules Pairwise Comparisons for Coronal Section*

Group		Group	Difference	Lower 95% CI	Upper 95% CI	Significant
Passive Ultrasonic	vs	Traditional	-0.47	-1.04	0.10	NS
Endoactivator	vs	Traditional	-0.62	-1.09	-0.15	$p < 0.05$
Endovac	vs	Traditional	-1.16	-1.54	-0.78	$p < 0.05$
Control	vs	Traditional	0.91	0.60	1.22	$p < 0.05$
Endoactivator	vs	Passive Ultrasonic	-0.15	-0.75	0.44	NS
Endovac	vs	Passive Ultrasonic	-0.69	-1.22	-0.16	$p < 0.05$
Control	vs	Passive Ultrasonic	1.38	0.90	1.86	$p < 0.05$
Endovac	vs	Endoactivator	-0.54	-0.94	-0.13	$p < 0.05$
Control	vs	Endoactivator	1.54	1.18	1.89	$p < 0.05$
Control	vs	Endovac	2.07	1.85	2.29	$p < 0.05$

Table 2.4. Tubules Pairwise Comparisons for Middle Section*

Group		Group	Difference	Lower 95% CI	Upper 95% CI	Significant
Passive Ultrasonic	vs	Traditional	0.05	-0.48	0.57	NS
Endoactivator	vs	Traditional	-0.19	-0.73	0.35	NS
Endovac	vs	Traditional	-1.05	-1.53	-0.57	$p < 0.05$
Control	vs	Traditional	1.14	0.73	1.56	$p < 0.05$
Endoactivator	vs	Passive Ultrasonic	-0.24	-0.72	0.25	NS
Endovac	vs	Passive Ultrasonic	-1.10	-1.51	-0.68	$p < 0.05$
Control	vs	Passive Ultrasonic	1.10	0.76	1.44	$p < 0.05$
Endovac	vs	Endoactivator	-0.86	-1.29	-0.43	$p < 0.05$
Control	vs	Endoactivator	1.33	0.97	1.70	$p < 0.05$
Control	vs	Endovac	2.19	1.94	2.45	$p < 0.05$

Table 2.5. Tubules Pairwise Comparisons for Apical Section

Group		Group	Difference	Lower 95% CI	Upper 95% CI	Significant
Passive Ultrasonic	vs	Traditional	-0.16	-0.63	0.31	NS
Endoactivator	vs	Traditional	-0.73	-1.27	-0.18	$p < 0.05$
Endovac	vs	Traditional	-1.62	-2.11	-1.13	$p < 0.05$
Control	vs	Traditional	0.54	0.15	0.93	$p < 0.05$
Endoactivator	vs	Passive Ultrasonic	-0.57	-1.04	-0.09	$p < 0.06$
Endovac	vs	Passive Ultrasonic	-1.46	-1.88	-1.04	$p < 0.05$
Control	vs	Passive Ultrasonic	0.70	0.40	1.00	$p < 0.05$
Endovac	vs	Endoactivator	-0.90	-1.40	-0.39	$p < 0.05$
Control	vs	Endoactivator	1.27	0.87	1.66	$p < 0.05$
Control	vs	Endovac	2.16	1.83	2.49	$p < 0.05$

Smear layer:

Table 3. Smear Layer Descriptive Statistics*

Measure	Intervention Group	Coronal	Middle	Apical
Smear	Traditional	3.11	2.81	3.42
		0.74	0.79	0.90
	Passive Ultrasonic	2.69	2.90	3.23
		0.91	0.80	0.73
	Endoactivator	2.51	2.74	2.92
		0.71	0.82	0.82
	Endovac	2.16	2.00	2.11
		0.68	0.74	0.72
	Control	3.93	3.99	4.00
		0.25	0.10	0.00

Table 3.1. Smear Layer Intra Class Correlation Coefficient - Rater Agreement*

ICC	Measure	Lower 95% CI	Upper 95% CI
Average	0.98	0.97	0.99

Table 3.2. Smear Layer Pairwise Comparisons for ALL Sections*

Group		Group	Difference	Lower 95% CI	Upper 95% CI	Significant
Passive Ultrasonic	vs	Traditional	-0.17	-0.46	0.12	NS
Endoactivator	vs	Traditional	-0.39	-0.69	-0.09	$p < 0.05$
Endovac	vs	Traditional	-1.02	-1.30	-0.74	$p < 0.05$
Control	vs	Traditional	0.87	0.66	1.08	$p < 0.05$
Endoactivator	vs	Passive Ultrasonic	-0.21	-0.51	0.08	NS
Endovac	vs	Passive Ultrasonic	-0.85	-1.13	-0.56	$p < 0.05$
Control	vs	Passive Ultrasonic	1.04	0.82	1.26	$p < 0.05$
Endovac	vs	Endoactivator	-0.63	-0.92	-0.34	$p < 0.05$
Control	vs	Endoactivator	1.26	1.03	1.48	$p < 0.05$
Control	vs	Endovac	1.89	1.70	2.08	$p < 0.05$

Table 3.3. Smear Layer Pairwise Comparisons for Coronal Section*

Group		Group	Difference	Lower 95% CI	Upper 95% CI	Significant
Passive Ultrasonic	vs	Traditional	-0.42	-0.90	0.06	NS
Endoactivator	vs	Traditional	-0.60	-0.96	-0.24	$p < 0.05$
Endovac	vs	Traditional	-0.96	-1.28	-0.63	$p < 0.05$
Control	vs	Traditional	0.82	0.51	1.13	$p < 0.05$
Endoactivator	vs	Passive Ultrasonic	-0.18	-0.65	0.30	NS
Endovac	vs	Passive Ultrasonic	-0.54	-1.00	-0.07	$p < 0.05$
Control	vs	Passive Ultrasonic	1.24	0.80	1.68	$p < 0.05$
Endovac	vs	Endoactivator	-0.36	-0.68	-0.03	$p < 0.05$
Control	vs	Endoactivator	1.42	1.12	1.72	$p < 0.05$
Control	vs	Endovac	1.78	1.53	2.02	$p < 0.05$

Table 3.4. Smear Layer Pairwise Comparisons for Middle Section*

Group		Group	Difference	Lower 95% CI	Upper 95% CI	Significant
Passive Ultrasonic	vs	Traditional	0.09	-0.45	0.63	NS
Endoactivator	vs	Traditional	-0.07	-0.56	0.42	NS
Endovac	vs	Traditional	-0.81	-1.29	-0.32	$p < 0.05$
Control	vs	Traditional	1.18	0.77	1.59	$p < 0.05$
Endoactivator	vs	Passive Ultrasonic	-0.16	-0.63	0.30	NS
Endovac	vs	Passive Ultrasonic	-0.90	-1.36	-0.44	$p < 0.05$
Control	vs	Passive Ultrasonic	1.09	0.70	1.47	$p < 0.05$
Endovac	vs	Endoactivator	-0.74	-1.13	-0.35	$p < 0.05$
Control	vs	Endoactivator	1.25	0.97	1.53	$p < 0.05$
Control	vs	Endovac	1.99	1.73	2.24	$p < 0.05$

Table 3.5. Smear Layer Pairwise Comparisons for Apical Section*

Group		Group	Difference	Lower 95% CI	Upper 95% CI	Significant
Passive Ultrasonic	vs	Traditional	-0.19	-0.70	0.32	NS
Endoactivator	vs	Traditional	-0.50	-1.00	0.00	NS
Endovac	vs	Traditional	-1.31	-1.79	-0.83	$p < 0.05$
Control	vs	Traditional	0.59	0.18	1.00	$p < 0.05$
Endoactivator	vs	Passive Ultrasonic	-0.31	-0.73	0.11	NS
Endovac	vs	Passive Ultrasonic	-1.12	-1.53	-0.72	$p < 0.05$
Control	vs	Passive Ultrasonic	0.78	0.46	1.11	$p < 0.05$
Endovac	vs	Endoactivator	-0.81	-1.18	-0.45	$p < 0.05$
Control	vs	Endoactivator	1.09	0.81	1.38	$p < 0.05$
Control	vs	Endovac	1.90	1.66	2.15	$p < 0.05$

Debris:

Table 4. Debris Descriptive Statistics*

Measure	Intervention Group	Coronal	Middle	Apical
Debris	Traditional	2.40	2.21	2.55
		0.69	0.59	1.01
	Passive Ultrasonic	1.88	1.76	2.14
		0.79	0.69	0.93
	Endoactivator	1.79	1.75	1.87
		0.72	0.81	0.81
	Endovac	1.44	1.41	1.40
		0.54	0.51	0.49
	Control	2.66	2.72	2.74
		1.06	0.99	1.21

Table 4.1. Debris Intra Class Correlation Coefficient - Rater Agreement*

ICC	Measure	Lower 95% CI	Upper 95% CI
Average	0.98	0.97	0.98

Table 4.2. Debris Pairwise Comparisons for ALL Sections*

Group		Group	Difference	Lower 95% CI	Upper 95% CI	Significant
Passive Ultrasonic	vs	Traditional	-0.46	-0.74	-0.19	$p < 0.05$
Endoactivator	vs	Traditional	-0.58	-0.80	-0.37	$p < 0.05$
Endovac	vs	Traditional	-0.97	-1.16	-0.78	$p < 0.05$
Control	vs	Traditional	0.32	-0.17	0.80	NS
Endoactivator	vs	Passive Ultrasonic	-0.12	-0.38	0.15	NS
Endovac	vs	Passive Ultrasonic	-0.50	-0.76	-0.25	$p < 0.05$
Control	vs	Passive Ultrasonic	0.78	0.27	1.29	$p < 0.05$
Endovac	vs	Endoactivator	-0.39	-0.55	-0.22	$p < 0.05$
Control	vs	Endoactivator	0.90	0.42	1.38	$p < 0.05$
Control	vs	Endovac	1.28	0.84	1.72	$p < 0.05$

Table 4.3. Debris Pairwise Comparisons for Coronal Section*

Group		Group	Difference	Lower 95% CI	Upper 95% CI	Significant
Passive Ultrasonic	vs	Traditional	-0.53	-0.92	-0.13	$p < 0.05$
Endoactivator	vs	Traditional	-0.61	-0.94	-0.28	$p < 0.05$
Endovac	vs	Traditional	-0.96	-1.24	-0.69	$p < 0.05$
Control	vs	Traditional	0.26	-0.22	0.74	NS
Endoactivator	vs	Passive Ultrasonic	-0.09	-0.46	0.29	NS
Endovac	vs	Passive Ultrasonic	-0.44	-0.77	-0.10	$p < 0.05$
Control	vs	Passive Ultrasonic	0.78	0.27	1.29	$p < 0.05$
Endovac	vs	Endoactivator	-0.35	-0.62	-0.08	$p < 0.05$
Control	vs	Endoactivator	0.87	0.40	1.34	$p < 0.05$
Control	vs	Endovac	1.22	0.81	1.64	$p < 0.05$

Table 4.4. Pairwise Comparisons for Middle Section*

Group		Group	Difference	Lower 95% CI	Upper 95% CI	Significant
Passive Ultrasonic	vs	Traditional	-0.45	-0.79	-0.11	p < 0.05
Endoactivator	vs	Traditional	-0.46	-0.79	-0.14	p < 0.05
Endovac	vs	Traditional	-0.80	-1.05	-0.54	p < 0.05
Control	vs	Traditional	0.51	0.02	0.99	p < 0.05
Endoactivator	vs	Passive Ultrasonic	-0.01	-0.36	0.35	NS
Endovac	vs	Passive Ultrasonic	-0.34	-0.64	-0.05	p < 0.05
Control	vs	Passive Ultrasonic	0.96	0.43	1.49	p < 0.05
Endovac	vs	Endoactivator	-0.34	-0.62	-0.05	p < 0.05
Control	vs	Endoactivator	0.97	0.45	1.49	p < 0.05
Control	vs	Endovac	1.30	0.83	1.78	p < 0.05

Table 4.5. Debris Pairwise Comparisons for Apical Section*

Group		Group	Difference	Lower 95% CI	Upper 95% CI	Significant
Passive Ultrasonic	vs	Traditional	-0.41	-0.93	0.11	NS
Endoactivator	vs	Traditional	-0.68	-1.19	-0.16	p < 0.05
Endovac	vs	Traditional	-1.15	-1.60	-0.70	p < 0.05
Control	vs	Traditional	0.17	-0.61	0.95	NS
Endoactivator	vs	Passive Ultrasonic	-0.26	-0.75	0.22	NS
Endovac	vs	Passive Ultrasonic	-0.74	-1.13	-0.34	p < 0.05
Control	vs	Passive Ultrasonic	0.58	-0.18	1.34	NS
Endovac	vs	Endoactivator	-0.47	-0.83	-0.11	p < 0.05
Control	vs	Endoactivator	0.85	0.11	1.59	p < 0.05
Control	vs	Endovac	1.32	0.67	1.97	p < 0.05

Data were analyzed by Pairwise comparisons. Endovac™ system was significantly more effective ($p<0.05$) than the other groups at the apical, middle and coronal sections for elimination of smear layer as well as debris removal and improved tubule visibility. Negative pressure delivery systems may provide better cleaning in curved root canals.

4. Discussion

One of the main goals of endodontic treatment is to optimize root canal disinfection which involves a thorough chemo-mechanical debridement of pulp tissues, canals debris and eliminate infective microorganisms (1-4).

As mentioned previously, chemo-mechanical preparation combined the action of root canal instrument with the action of different irrigants in an important step to remove the smear layer and achieve optimal disinfection of the root canal system (253).

The teeth selected in this study had intact coronal structures to avoid any possible detrimental effects on: the physical properties, the composition of root canal dentin and dentinal tubules, which may cause some variations in the outcome.

The teeth that were selected were mainly molars with roots that have a curvature of 30 or more degrees, as these teeth are more challenging to instrument and clean (254,255). In addition to that, curved canals have higher risks of instrument separation due to torsional stress and cyclic fatigue (256,257). These canals are usually narrow, which make the contact angle between the file and the dentinal wall bigger and increase the fatigue of the file (258).

The degree of curvature was determined by using Schneider's method. This method as explained in materials and methods is simple, practical and has already been adopted by many studies (248).

Plastic teeth/models were avoided in this study as it was important to create an environment that is similar as much as possible to the actual challenge in the clinical setting as shown in many studies (259,260).

Decoronation of the teeth was accomplished to create a straight line access and to remove any obstacles that may interfere with the instrumentation and cleaning process. In this study, decoronation was performed to standardize root length at 12 mm as this tends to create an artificial scenario for which the access opening cannot influence the intracanal procedures and to eliminate any possible discrepancy that may affect the final results of this study.

In addition to that, it has been shown that the average molar root length from cervical line to apex is about 12-13 mm (261).

As part of the chemo-mechanical preparation, the root canals in the present study were instrumented with ProTaper and ProFile rotary system up to size #35/.04 at the apical portion of the canal. These instruments have been shown to remove significantly more smear and debris than hand instrumentation (262). However, the efficacy of the irrigation delivery systems also depends on the root canal preparation size and taper. It is an ongoing debate that while some studies report that larger apical preparation sizes reduce the bacterial population (263,264), others indicate the risk of perforations or possible root fractures (265,266).

Many studies have showed the importance of the depth of the needle insertion and how far the irrigant can penetrate apically. These studies were also conducted to determine the ideal size for the root canal preparation and how the

canal enlargement will improve the access of the irrigant to the microorganism that have penetrated deeply into the dentin.

Studies by Ørstavik et al (267,268) and Matsumiya and Kitamura (269) concluded that the size of apical instrumentation may play an important role in removal of canal bacteria. They also reported that with larger instrumentation, fewer bacteria remained in the canal and the healing was more rapid. Recently, Wu and Wesselink (179) concluded that instrumentation of the molar canals to size #45 apical file with 0.02 taper will result in a much cleaner canals (270).

In addition to that, for the needle tip to function efficiently, a proper enlargement of the root canal is recommended. Ram et al recommended preparation of the canal up to size #40 /.02 taper for effective delivery of the irrigant (271). Other study by Salzgeber and Brilliant et al. showed that the irrigant can reach to the root canal apex when the canal was instrumented to file size #30 /.02 taper (272). Even though larger preparation sizes with large tapers provide a constant increase in hydrodynamic flow during irrigation, in this study, the apical preparation size was limited to #35 /.04 mm taper in order to conserve radicular dentin, to minimize the risk of procedural errors and to establish a path for the irrigants to reach into the most infected areas of the root canal (14). This will ensure adequate apical flushing and proper chemical disinfection.

The largest irrigation needle in the present study was the stainless steel micro-cannula for EndoVac system, which has an external diameter size of 0.32mm with 0.04mm taper. This tip can be used in the canals that are enlarged

to size #35 and is responsible for the irrigation of the apical part of the canal by placing it close to working length to suction irrigant and debris (239).

Therefore, it was important to make sure all the tips were able to move loosely in the canal systems (245), to avoid insufficient preparation and enlargement of the root canal. This will restrict the activation of the ultrasonic irrigant and limit the free oscillation of the file, which can limit the cleaning efficacy of the root canal irrigant (202,226).

Many studies also showed that a canal preparation to size #30-35/ .04 taper is required for NaOCl to be effective (276-275), and an apical preparation to ISO size 0.35 or larger is recommended (276).

The depth of the activator tip or the irrigation needle and the size of the canal preparation influence the cleaning and shaping process. This plays an important role on the extent of irrigant replacement and the amount of pressure at the apical part of the root canal (277).

Nielsen and Baumgartner reported that the needle depth in the standard irrigation technique should be limited to 2 mm from WL (245). This measurement was applied in the present study. This depth was also applied to the PUI and EndoActivator, while the EndoVac system was positioned to WL (280,246) Moreover, the rate of irrigation solution delivery into the canal system was set at 3.0 ml / min for each irrigation group except EndoVac. The EndoVac system delivers more irrigant into the root canal system. Unfortunately, there was no plausible means to measure the rate of irrigant delivery for the EndoVac group (278).

For this study, a 30-guage side vented (Max-I-Probe needle, Dentsply-Sirona) needle was used. Studies have shown that the 30G needles were more efficient in cleaning the apical part of the root canal (176,279). The needle has a luer lock connector to provide a secure attachment and better removal from any disposable syringe.

Manufacturer claimed that the rounded tip reduces the chance of perforating the apex and the side-port in the cannula allows a unique upward turbulent flow which reduces the chance of the irrigant from passing through the apical foramen. This needle has a tip size of 0.3mm, which allows deeper penetration of the needle inside the root canal and its side vented design allows a more effective flow for the irrigant (279). This needle size is convenient for a canal space prepared up to size #35/.04 apical size to allow the needle to get into the last 2 mm of working length (181,280).

The needle allows a decent control of the depth of penetration of the irrigant. However, the delivery of the irrigant was restricted to only 1mm deeper from the tip and the flushing mechanism is relatively weak with limited ability to access isthmuses and lateral accessary canals (175,271).

A study by Dalton et al. showed that 72% of instrumented teeth still contain a positive culture (11) and instrumentation only eliminates 20-30% of bacteria (15). Therefore, the introduction and the use of different chemicals is a very critical part in cleaning and disinfecting root canal systems.

In this study, NaOCl was used, as it is the most common Endodontic irrigant with a superior tissue dissolution property and a well-documented antimicrobial, sporicidal and virucidal characteristic (23,280).

As earlier explained, the antimicrobial effectiveness of NaOCl depends on its high pH value and the potency of hypochlorous acid, which is a strong non-radical oxidant. When hypochlorous acid get in contact with the organic tissues, chlorine will be released. This chlorine will interfere with the DNA synthesis and cause the cell proliferation to cease (101,131).

Giardino et al showed that 5.25% NaOCl was the only irrigant to remove and desegregate E. faecalis biofilm generated on cellulose nitrate membrane filters compared to BioPure MTAD (Dentsply Tulsa Dental, Johnson City, TN) and Tetraclean (Ogna Laboratori Farmaceutici, Milano, Italy) (115). Luis E. Chavez et al exposed biofilms of E. faecalis, Lactobacillus paracasei, S. anginosus, and S. gordonii isolated from the infected root canals after 5 min exposure to the following: alkali (pH=12), 2.5% chlorhexidine digluconate, EDTA, and 1% NaOCl. The results showed that 1% NaOCl affected the membrane integrity of all organisms and removed most biofilm cells while the 2.5% CHX had a mild effect and removed only 50% of its biofilm cells (281).

A SEM and biofilm assay was used in another study to show that biofilm grown on dentin harbored more cells than polystyrene. The study also showed that biofilms of starved E. faecalis cells were more resistant to 5.25% NaOCl than in stationary cells and the effect of 5.25% NaOCl will decrease as the biofilm mature. This may contribute to the predominant role of E. faecalis in persistent periapical

infections (282). Ozdemir et al. showed that the combination of 2.5% NaOCl and 17% EDTA significantly reduced intracanal E. faecalis biofilm in young and old individuals however, the bacterial count in the old group were higher (283).

Other research study demonstrated that 2% CHX does not improve the biofilm dissolution or increase the cleaning of the dentin and 30 min application of NaOCl is necessary to achieve a higher value of cleaning and biofilm dissolution independent of the concentration (284). A study by Seet et al. showed that sonic or laser activation of 4% NaOCl resulted in greater E. faecalis reduction compared with syringe irrigation (285).

In this study 6% concentration of NaOCl was used. Previous studies have shown that dilution of NaOCl will significantly lower its tissue dissolution ability and the 6% concentration has more effective antimicrobial properties and better debridement quality compared to lower concentrations (137,286,287). As discussed, Clegg el al showed that 6% NaOCl was the only irrigant to remove biofilm compared to 1%, 3% NaOCl and 2% CHX (288).

Due to the limitation of NaOCl to act on the inorganic particles of the smear layer after instrumentation, EDTA was used as another main chemical and powerful chelating agent. This approach was justified by the recommendation of Yamada et al. when they set up the gold standard of the irrigation protocol and showed the necessity to use a chelating agent (17% EDTA) in combination with a tissue solvent irrigant (5.25% NaOCl) to remove both superficial debris and smear layer more efficiently (44).

Ethylenediaminetetraacetic Acid reacts with calcium ions in the hydroxyapatite crystal and removes them from the dentin matrix. It may also detach biofilm from the walls of the root canal (156).

The antimicrobial activity of EDTA is very limited and some studies related this activity to the chelation of cations from the bacteria outer membrane (155). A study by Ballal NV. et al. to evaluate the antimicrobial efficacy of 7% maleic acid and 17% EDTA against E. faecalis and Staphylococcus. No significant different were found (289). Ordinola-Zapata et al. reported in their study that a contact time of 5 min of 17% EDTA and 10% citric acid had no effect on the biofilm viability (290). These finding were similar to study by Arias-Moliz et al that showed EDTA has no effect on the E. faecalis even after 60 min (291).

Urea peroxide was introduced by Stewart et al. and showed the ability of urea peroxide solution to retain it is antimicrobial effect in the presence of blood. They also advocated the addition of EDTA to RC-Prep (15% EDTA, 10% urea peroxide and carbowax) to combine the chelating and the antimicrobial actions together (292).

EDTA was used at a concentration of 17% as many articles demonstrated its effectiveness at this level (44,293). In addition, a 1 min EDTA application with ultrasonic activation found to be very effective on smear layer removal at the apical area of the root canal (294).

Besides that, EDTA can cause erosion of the dentin structure with a significant reduction in the microhardness if left more than 3 min. For this reason, irrigation with EDTA was done for 30 secs with passive wait of no more than 1 min

to limit the amount of dentin destruction and according to other studies, EDTA has the ability to remove smear layer if applied for one min (295).

Another chelating agent known as EDTAC is produced by adding quaternary ammonium bromide (Cetavlon or Cetrimide) to EDTA to reduce its surface tension. Although, it has been shown no significant difference in the effectiveness between EDTA and EDTAC in removing smear layer, other studies have showed significantly lower efficacy of EDTAC than that of EDTA with reduced ability to remove calcium ions from dentin. Also, reducing the surface tension of EDTA did not significantly improve its effectiveness (296,297,298). Based on these facts, a decision was made to use EDTA in this study.

The method of delivery of the irrigant solutions also play an important part in root canal disinfection. Many studies have shown that traditional needle irrigation was insufficient to clean and reach all the anatomical complexities of the root canals (22,20) and the necessity to activate the endodontic irrigant appears to be an important approach to achieve a better cleaning and disinfection of the root canal system (194).

As mentioned earlier in this study, the main advantage of using ultrasonic in cleaning and shaping along with root canal irrigation is the acoustic streaming (209). This is a state of steady streaming patterns in a rapid vortex-like motion associated with a vibrating file. The agitation of the irrigant, by this method, has the advantage of increasing the penetration and the effectiveness of the irrigant through hydrodynamic shear stress (219,299,300). By taking the 6% NaOCl solution as an example , this irrigant has 300 μm maximum depth of penetration

after 20 min of application (301) whereas E. faecalis can go up to 800-1000 μm inside dentinal tubules (7).

The other physical effect that can be observed in the irrigant with free ultrasonic vibration is cavitation. This term reflects the growth and the collapse of gas bubbles due to rapid changes in pressure during oscillation in the fluid (302). A forced collapse in these bubbles creates some structure deformity in the surface due to the heavy impact which can be helpful in the endodontic application to disrupt bacterial biofilm and remove smear layer debris (303).

Under usual clinical conditions, the power of dental ultrasonic units is too low to produce significant cavitation effects on the dentinal walls (229).
EndoActivator is a sonically driven root canal irrigation system, which can produce a strong hydrodynamic phenomenon and vigorously agitate the irrigant once activated. This system operates at a lower frequency and produces less shear stresses compare to ultrasonic irrigation (209). Numerous researches have shown its effectiveness in removing the smear layer and displacing the clumps of simulated biofilm in curved canals (184,196).

In the current study, and specially at the coronal and apical thirds of the canal, our results agreed with the numerous researchers that have shown the effectiveness of EndoActivator in removing smear layer (184,195,196). This could be related to the improved flow rate and to the acoustic streaming action in creating implosions that radiates miniature tsunamis or shockwaves that dissipate at 25,000-30,000 times per second (195). The oscillation of the file shows large

displacement amplitudes and is unaffected by loading (when the file contacts the walls) (185).

As previously discussed, PUI depends on the transmission of the acoustic energy by means of ultrasonic waves from an oscillating file to the irrigant inside the root canal (220,209). This action improved the irrigant flow into the irregularities of the root canal and increased its volume (204).

Couple of studies have demonstrated that the ultrasonic activation method of the irrigant is more effective than traditional needle irrigation against canal debris and smear layer (194,304,305). This could be related to the cavitation effect and the increase in the irrigant temperature which improved the dissolution action of NaOCl inside the canal (209). This is in accordance with the results of the current study.

However, One of the PUI drawback in the present study, is the limited ability to show its effectiveness at the apical part of the root canal compared to the EndoVac system. Similar findings applied to the EndoActivator groups. This could be related to the present findings, such as the difficulty to standardize the position of the ultrasonically activated file in the middle of the canal and the vapor lock effect with air entrapment at the apical area of the root canal. This may prevent the adequate contact of the irrigant to this inaccessible are, and thus limit its effectiveness (237,238,235).

The present results did not agree with previous studies that have shown no significant differences in the removal of dentin debris between syringe irrigation and PUI (223,226,306).

Martin and Cunningham introduced Cavi-Endo as an untrasonic endodontic device. They conducted couple of studies on using ultrasound in root canal treatment. In these studies, they showed the efficacy of endosonic in root canal preparation and disinfection with the ability of ultrasonically activated irrigant to improve the cleaning of these canals. Martin and Cunningham also related the success of ultrasonic instrument to the synergistic effect which combine the ultrasonic energy with the irrigation solution (199-202).

The amount of time required to activate the irrigant is another critical factor in achieving the optimal cleaning results. A study was performed by Cameron et al. to compare different ultrasonic irrigation periods on smear layer removal. They illustrated that both 3 and 5 min ultrasonic irrigation produced smear free canals, while the 1 min irrigation was not effective. Other studies found the ultrasonic irrigation was ineffective against smear layer (307,308).

When the ultrasonic debridement efficacy in vital mandibular molar was compared histologically, the results showed significantly cleaner isthmuses and canals after only 1 min application of ultrasonic needle with no larger than a size #30 file compared to 3 min application in previous studies (309).

Similar results to the previous in vivo study were reported in necrotic human mandibular molars, as ultrasonic application for 1 min after hand/rotary preparation of the root canal showed significantly cleaner isthmuses and improved biofilm removal at the apical part of the root canal (310).

Ultrasonic was also promising in curved canals. Blank-Goncalves et al. reported a better removal of the smear layer in the apical third of curved root canals

when sonic and ultrasonic irrigation were used (311). According to Ahmad et al., better results can be achieved by pre-bending the file (217,220,312).

Other studies reported significantly cleaner isthmuses when PUI was used compared to syringe irrigation and PUI was able to remove debris in areas untouched by endodontic instruments (221,224).

On the other hand, Siqueira et al. tested the effectiveness of 4% NaOCl used in three irrigation methods in eliminating E. faecalis from the root canals, the results showed no significant differences between hand files and ultrasonic agitation (313).

Due to the limitation of conventional needle irrigation to replenish and exchange the irrigant (especially at the apical part of the root canal and the vapor lock effect that results in trapped air), the debridement efficiency of the irrigant and its ability to get in direct contact with the canal walls will be restricted (181,314). Therefore, a new system with different mechanism was introduced by Schoeffel GJ (239).

The EndoVac system is a root canal irrigation device which utilize an apical negative pressure (ANP) mechanism to deliver the irrigant with higher flow as close as 1 mm from working length, with the ability to suction out the canal debris with lower risk of irrigant extrusion accident (239,245,246).

In fact, the negative apical pressure system showed significantly less tendency for apical extrusion of the irrigant compared to the side vented needle (Max-I-Probe), whereas the size of the apical enlargement did not significantly affect the apical extrusion of the root canal irrigant (315).

The effectiveness of EndoVac system in cleaning canal debris is well documented (246). A study by Nielsen et al. showed a significant better debridement of EndoVac system compared with needle irrigation at 1 mm from working length using a 30-guage irrigation needle and canal enlargement to ISO size 36/.04 taper or larger (245). EndoVac system also achieved better control against E. faecalis than traditional positive pressure with no relation to the size of the canal preparation (#35 or #45), nor to the taper of the preparation (316). This system was also very successful in reducing intracanal levels of Candida Albicans (317).

In contrast, an in-vitro study by Townsend et al. compared the effect of different agitation techniques against E. faecalis. Their results showed that the ultrasonic agitation was significantly better than EndoVac and needle irrigation against intra-canal bacteria (196). Our study did not agree with their findings as these results could be related to the use of plastic simulated canals which do not have any actual similarities to natural teeth in the clinical setting. In addition, the present results did not agree with Brito et al findings either. These findings reported no significant differences between conventional irrigation with: NaviTip needles inserted up to 3 mm short of working length, EndoActivator and EndoVac system as they reported that all of these techniques showed a highly significant reduction in bacterial population (318).

A recent micro computed tomography analysis showed that EndoVac was not very successful in eliminating hard tissues debris from the isthmus area in the

mesial root of the mandibular molars. However, the apical negative pressure approach showed a much lower percentage of debris compare to conventional irrigation (319).

In this study, EndoVac™ system was significantly more effective ($p<0.05$) than the other groups at the apical, middle and coronal sections for elimination of smear layer, debris removal and improved tubule visibility.

Although Uroz-Torrez et al. did not find any significant difference between the EndoActivator system and standard irrigation protocols (320), the current results demonstrated that the use of sonic and ultrasonic activation methods were actually more efficient in cleaning the root canal system compared to conventional irrigation. This is in agreement with other studies (294,321). In the recent study there was no significant difference between PUI and EndoActivator systems in curved canals. Jensen et al. confirmed these findings in their study comparing PUI to sonic activation (312).

In summary, Negative pressure delivery systems appears to provide better cleaning in curved root canals. Introducing the micro-cannula to the working length, the irrigant was able to reach safely to the complex anatomy of the root canal system in adequate volume and flow and suctioned out with canal debris to achieve excellent results in removing smear layer from dentinal walls.

5. Conclusions:

Irrigation regimes play an important role in the success of endodontic treatment. Up to date, no single irrigant protocol could achieve all the tasks required by irrigation. The advances in technology like positive and negative irrigation techniques have brought to fruition new devices that depend on different mechanism of irrigant delivery to the most apical part of the root canal system, tissue debridement and removal of both debris and smear layer.

The Endovac™ apical negative pressure irrigation system was found to clean the root canal systems significantly better in all of the root canal sections we observed. This system was also superior to positive pressure devices in preventing apical extrusion of the irrigant, eliminating vapor lock effect, and providing adequate irrigant volume.

We recommend the use of the negative pressure irrigation system to improve the removal of smear layer and debris from root canals without the risk of irrigant extrusion.

6. References

1- Grossman, L.I. Irrigation of root canals. JADA 30:1915 Dec 1943.

2- Steward, GG. T. The importance of chemomechanical preparation of the root canal. Oral Surg 8:993 Sept 1955.

3- Ingle, J.I. Root canal obturation. JADA 53:47 July 1956.

4- Nicholls, E. the efficacy of cleansing of the root canal. Br Dent J 112:167 Feb 20,1962.

5- GR Young, P Parashos, HH Messer. The principles of techniques for cleaning root canals. Australian Dental Journal Supplement 2007;52:(1 Suppl): S52-S63.

6- Figdor D, Sundqvist G. A big role for the very small—understanding the endodontic microbial flora. Aust Dent J. 2007;52(suppl 1): S38-S51.

7- Haapasalo M, Orstavik D. In vitro infection and disinfection of dentinal tubules. J Dent Res. 1987; 66:1375-9.

8- Perez F, Calas P, Falguerolles A, Maurette A. Migration of a Streptococcus sanguis strain through the root dentinal tubules. J Endod 1993; 19(6):297-301.

9- Peters LB, Wesselink PR, Buijs JF, Winkelhoff AJ. Viable bacteria in root dentinal tubules of teeth with apical periodontitis. J Endod. 2001; 27:76-81.

10- Byström A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. Scand J Dent Res. 1981; 89:321-328.

11- Dalton BC, Ørstavik D, Phillips C, Pettiette M, Trope M. Bacterial reduction with nickel-titanium rotary instrumentation. J Endod 1998; 24:763-767.

12- Wu MK, van der Sluis LW, Wesselink PR. The capability of two hand instrumentation techniques to remove the inner layer of dentine in oval canals. Int Endod J. 2003; 36:218-224.

13- Walia HM, Brantley WA, Gerstein H. An initial investigation of the bending and torsional properties of Nitinol root canal files. J Endod 1988; 14:346-351.

14- Baugh D, Wallace J. The role of apical instrumentation in root canal treatment: a review of the literature. J Endod. 2005; 31:333-40.

15- Ebru O D, Elif K, Esra D, Ahmet Y C, Yeliz T C. Efficacy of different instrumentation techniques on reducing Enterococcus faecalis infection in experimentally infected root canals. Journal of Dental Science 2014; 9(1):23-28.

16- Peters OA, Schonenberger K, Laib A. Effects of four Ni-Ti preparation techniques on root canal geometry assessed by micro computed tomography. Int Endod J 2001; 34:221-230.

17- Mizrahi, S.J, Tucher, j.w., Seltzer, S. A scanning electron microscopic study of the efficacy of various endodontic instruments. J Endod 1:324 Oct. 1975.

18- Skidmore AE, Bjorndal AM. Root canal morphology of the human mandibular first molar. Oral Surg Oral Med Oral Pathol. 1971; 32:778–784.

19- Vertucci FJ. Root canal anatomy of the human permanent teeth. Oral Surg Oral Med Oral Pathol. 1984; 58:589–599.

20- Peters OA. Current challenges and concepts in the preparation of root canal systems: a review. J Endod. 2004; 30:559–567.

21- Peters OA, Peters CI, Schonenberger K, Barbakow F. ProTaper rotary root canal preparation: Effects of canal anatomy on final shape analyzed by micro CT. Int Endod J. 2003;36:86–92.

22- Davis SR, Brayton S, Goldman M. The morphology of the prepared root canal: A study utilizing injectable silicone. Oral Surg Oral Med Oral Pathol 1972; 34:642-8.

23- Bystrom A, Sundqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. Int Endod J 1985; 18:35-40.

24- Moodnik RM, Dorn SO, Feldman MJ, Levey M, Borden BG. Efficacy of biochemical instrumentation: a scanning electron microscopic study. J Endod 1976; 2:261– 6.

25- Svec TA, Harrison JW. Chemomechanical removal of pulpal and dentinal debris with sodium hypochlorite and hydrogen peroxide vs normal saline solution. J Endod. 1977 Feb;3(2):49-53.

26- Foreman PC, Soames JV. Comparative study of the composition of primary and secondary dentine. Caries Res 1989; 23:1–4.

27- Maricela Garces-Ortiz, Constantino Ledesma-Montes, and Jose Reyes-Gasga. Scanning electron microscopic study on the fibrillary structures within tubules of human dentin. JOE, Vol. 41, issue 9, p1510-1514. July 2015.

28- Marshall GW, Marshall SJ, Kinney JH, Balooch M. The dentin substrate: structure and properties related to bonding. J Dent 1997; 25:441–58.

29- Pashley D H. Dentin: a dynamic substrate—a review. Scanning Microscopy, 3 (1989), pp. 161–174 [discussion 174–176].

30- Sasaki T, Garant PR. Structure and organization of odontoblasts. Review. Anat Rec. 1996 Jun;245(2):235-49.

31- Ross, Michael H., Gordon I. Kaye, and Wojciech Pawlina, 2003. Histology: a text and atlas. 4th edition. Page 450.

32- Cate, A.R. Ten. Oral Histology: development, structure, and function. 5th ed. 1998. Page 152.

33- Schilke R, Lisson JA, Bauss O, Geurtsen W. Comparison of the number and diameter of dentinal tubules in human and bovine dentine by scanning electron microscopic investigation. Arch Oral Biol. 2000 May; 45(5):355-61.

34- Michel Goldberg, Askok Kulkarni, Marian Young and Adele Boskey. Dentin: Structure, composition and mineralization. Front Biosci (Elite Ed); Jan 2011; 3:711-735.

35- J.H. Kinneya, R.K. Nallab, J.A. Poplec, T.M. Breunigd, R.O. Ritchieb, Age-related transparent root dentin: mineral concentration, crystallite size, and mechanical properties, Biomaterials. (2005) 3363–3376.

36- AAE Glossary of endodontic terms, ninth edition.

37- McComb D, Smith DC. A preliminary scanning electron microscopic study of root canals after endodontic procedures. J Endod 1975; 7:238-242.

38- Lester KS, Boyde A. Scanning electron microscopy of instrumented, irrigated and filled root canals. Br Dent J 1977; 143:359-66.

39- Eick JD et al. Scanning electron microscopy of cut tooth surfaces and identification of debris by use of the electron microprobe. Journal of Dental Research 1970.49:1359-1368.

40- Brännström M, Johnson G. Effects of various conditioners and cleaning agents on prepared dentin surfaces: A scanning electron microscopic investigation. J Prosthet Dent 1974; 31:422-430.

41- McComb & Smith:A preliminary Scanning electron microscopy study of root after endodontic procedures. J endod 1975; 1:238-242.

42- Mader CL, Baumgartner C, Peters DD: Scanning electron microscopic investigation of the smeared layer on root canal walls. J Endodon, 1984; 10: 477-483.

43- Baker NA, Eleazer PD, Averbach RE, Seltzer S. Scanning electron microscopic study of the efficacy of various irrigating solutions. J Endod. 1975; 1:127–35.

44- 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; 9:137–42.

45- Meryon SD, Brook AM. Penetration of dentine by three oral bacteria *in vitro* and their associated cytotoxicity. Int Endod J. 1990; 23:196–202.

46- Meryon SD, Jakeman KJ, Browne RM Penetration in vitro of human and ferret dentine by three bacterial species in relation to their potential role in pulpal inflammation. Int Endod J. 19, 213–20, 1986.

47- Goldberg F, Abramovich A. Analysis of the effect of EDTAC on the dentinal walls of the root canal. J Endod. 1977 Mar;3(3):101-5.

48- Baumgartner JC, Mader CL. A scanning electron microscopic evaluation of four root canal irrigation regimens. J Endod. 13, 147–57, 1987.

49- White RR, Goldman M, Lin PS. The influence of the smeared layer upon dentinal tubule penetration by plastic filling materials. J Endod. 10, 558–62. 1984.

50- Kennedy WA, Walker WA III, Gough RW. Smear layer removal effects on apical leakage. J Endod. 12, 21–7, 1986.

51- Cergneux M, Ciucchi B, Dietschi JM, Holz J. The influence of the smear layer on the sealing ability of canal obturation. Int Endod J. 20, 228–32, 1987.

52- White RR, Goldman M, Lin PS. The influence of the smeared layer upon dentinal tubule penetration by endodontic filling materials. Part II. J Endod. 13, 369–74, 1987.

53- Saunders WP, Saunders EM. The effect of smear layer upon the coronal leakage of gutta-percha root fillings and a glass ionomer sealer. Int Endod J. 25, 245–9, 1992.

54- Oksan T, Aktener BO, Sen BH, Tezel H. The penetration of root canal sealers into dentinal tubules. A scanning electron microscopic study. Int Endod J. 26, 301–5, 1993.

55- Clark-Holke D, Drake D, Walton R, Rivera E, Guthmiller JM. Bacterial penetration through canals of endodontically treated teeth in the presence or absence of the smear layer. Journal of Dentistry 31, 275–81,2003.

56- Kokkas AB, Boutsioukis ACh, Vassiliadis LP, Stavrianos CK. The influence of the smear layer on dentinal tubule penetration depth by three different root canal sealers. J Endod 2004; 30:100-102.

57- Pashley et al. Dentin permeability: Effect of smear layer removal. Prosthetic Dent. 1981; 46:531-37.

58- Safavi KE, Spangberg L, Langeland K. Smear layer removal effects on root canal dentin tubule infection. J Endod 1989; 15:175.

59- Love et al. Penetration of smear or non-smeared dentin by Streptococcus gordonii.IEJ,1996;29:2-12.

60- Mohammadi Z, Palazzi F, Giardino L, Shalavi S. Microbial biofilms in endodontic infections: an update review. Biomed J. 2013 Mar-Apr; 36(2):59-70.

61- Costerton J W, Caldwell D, Lewandowski Z, DeBeer D, Korber D and James G. Biofilms, the customized microniche. J Bacteriol 1994; 176:2137-42.

62- Costerton JW, Cheng KJ, Geesey GG, Ladd TI, Nickel JC, Dasgupta M, Marrie TJ. Bacterial biofilms in nature and disease. Annu Rev Microbiol. 1987; 41():435-64.

63- Henrici, A.T. Studies of Freshwater Bacteria: I. A Direct Microscopic Technique. J. Bacteriol. 25, 277–287 (1933).

64- Bowden GH, Hamilton IR. Survival of oral bacteria. Crit Rev Oral Biol Med 1998; 9:54-84.

65- Whitchurch CB, Tolker-Nielsen T, Ragas PC, Mattick JS. Extracellular DNA required for bacterial biofilm formation. Science. 2002 Feb 22; 295(5559):1487.

66- Anderl, J.N., Franklin, M.J. & Stewart, P.S. Role of antibiotic penetration limitation in Klebsiella pneumoniae biofilm resistance to ampicillin and ciprofloxacin. Antimicrob. Agents Chemother. 44, 1818–1824 (2000).

67- Pozo del JL and Patel R. The challenge of treating biofilm-associated bacterial infections. Translational Medicine 2007; 82:204-209.

68- Tack, K.J. & Sabath, L.D. Increased minimum inhibitory concentrations with anaerobiasis for tobramycin, gentamicin, and amikacin, compared to latamoxef, piperacillin, chloramphenicol, and clindamycin. Chemotherapy 31, 204–210 (1985).

69- Dong, Y.H. & Zhang, L.H. Quorum sensing and quorum-quenching enzymes. J. Microbiol. 43 (Spec No): 101–109 (2005).

70- Bagge, N., Hentzer, M., Andersen, J.B., Ciofu, O., Givskov, M. & Hoiby, N. Dynamics and spatial distribution of beta-lactamase expression in Pseudomonas aeruginosa biofilms. Antimicrob. Agents Chemother. 48, 1168–1174 (2004).

71- Brooun, A., Liu, S. & Lewis, K. A dose-response study of antibiotic resistance in Pseudomonas aeruginosa biofilms. Antimicrob. Agents Chemother. 44, 640–646 (2000).

72- Anderson, G.G., Palermo, J.J., Schilling, J.D., Roth, R., Heuser, J. & Hultgren, S.J. Intracellular bacterial biofilm-like pods in urinary tract infections. Science 301, 105–107 (2003).

73- Ingle JI, Bakland LK, Baumgartner JC. Endodontics. 6th edition. 2008. pp. 85 –268.

74- Ramachandran Nair PN. Light and electron microscopic studies of root canal flora and periapical lesions. J Endod. 1987 Jan; 13(1):29-39.

75- Listgarten M. Formation of dental plaque and other biofilms In: Newman HN, Wilson M eds. Dental Plaque Revisited. Oral Biofilms in Health and Disease. Cardiff: Bioline, 1999: 187-210.

76- Sen BH, Piskin B, Demirci T. Observation of bacteria and fungi in infected root canals and dentinal tubules by SEM. Endod Dent Traumatol. 1995 Feb; 11(1):6.

77- Frank SA, Barbour AG. Within –host dynamics of antigenic variation. Infect Genet Evol 2006;6(2): 141-6.

78- Tronstad L, Barnett F, Cervone F. Periapical bacterial plaque in teeth refractory to endodontic treatment. Endod Dent Traumatol 1990; 6:73-77.

79- Lomacali G, Sen BH, Cankaya H. Scanning electron microscopic observations of apical root surfaces of teeth with apical perriodontitis. Endod Dent Traumatol 1996; 12:70-76.

80- Ricicci D, Martorano M, Bate AL, Pascon EA. Calculus-like deposit on the apical external root surface of teeth with post treatment apical periodontitis: report of two cases. Int Endod J 2005; 38:262-71.

81- Conrads, G., S. E. Gharbia, K. Gulabivala, F. Lampert, and H. N. Shah. The use of a 16S rDNA directed PCR for the detection of endodontopathogenic bacteria. J Endod. 1997; 23:433-438.

82- Medvedev AE,Sabroe I, Hasday JD, Vogel SN. Tolerance to microbial TLR ligands: molecular mechanisms and relevance to disease. J Endotoxin Res 2006;12(3):133-50.

83- Siqueira JF. Periapical actinomycosis and infection with Propionibacterium propionicum. Endodontic Topics. 2003;6(1):78–95.

84- Jontell M, Gunraj MN, Bergenholtz G. Immunocompetent cells in the normal dental pulp. J Dent Res 1987;66(6):1149-53.

85- Wilson M. Susceptibility of oral bacterial biofilms to antimicrobial agents. J Med Microbiol. 1996 Feb; 44(2):79-87.

86- Takemura N, Noiri Y, Ehara A, Kawahara T, Noguchi N, Ebisu S. Single species biofilm-forming ability of root canal isolates on gutta-percha points. Eur J Oral Sci. 2004 Dec; 112(6):523-9.

87- Dakin HD. On the use of certain antiseptic substances in treatment of infected wounds. BMJ 1915 2: 318-320.

88- Austin JH, Taylor HD. Behavior of hypochlorite and chloramines- T solutions in contact with necrotic and normal tissue In vivo. J Exp Med 1918 27: 627-633.

89- Abdullah M, Ng YL, Gulabival K, Moles DR, Spratt DA. Susceptibilties of two Enterococuus faecalis phenotypes to root canal medications. J Endod 2005; 31:30-6.

90- Torabinejad M, Shabahang S, Aprecio RM, Kettering JD. The antimicrobial effect of MTAD: an in vitro investigation. J Endod 2003; 29:400 –3.

91- Dunavant TR, Regan JD, Glickman GN, Solomon ES, Honeyman AL. J Endod Comparative evaluation of endodontic irrigants against Enterococcus faecalis biofilms. 2006 Jun; 32(6):527-31.

92- Stojicic S, Shen Y, Qian W, Johnson B, Haapasalo M Antibacterial and smear layer removal ability of a novel irrigant, QMiX. Int Endod J. 2012 Apr; 45(4):363-71.

93- Carolina EC, Laura BL, Viviana GR, Valencia ME. Development and validation of a method for the determination of EDTA in non-alcoholic drinks by HPLC. J Food Compost Anal 2007; 20:248-52.

94- Zehnder M. Root canal irrigants. J Endod 2006; 32:389–98.

95- Grawehr M, Sener B, Waltimo T, et al. Interactions of ethylenediamine tetraacetic acid with sodium hypochlorite in aqueous solutions. Int Endod J 2003; 36:411–5.

96- Saquy PC, Maia Campos G, Sousa Neto MD, et al. Evaluation of chelating action of EDTA in association with Dakin's solution. Braz Dent J 1994; 5:65–70.

97- Nygaard-Ostby B. Chelation in root canal therapy: ethylenediaminetetraacetic acid for cleansing and widening of root canals. Odontol Tidskr 1957; 65:3-11.

98- Von der Fehr FR, Nygaard-Ostby B. E ect of EDTAC and sulfuric acid on root canal dentine. Oral Surg Oral Med Oral Pathol 1963; 16:199-205.

99- Goldman M. Kronman JH, Goldman LB, Clausen H. Grady J. New method of irrigation during endodontic treatment. J Endodon 1976; 2:257-60.

100- Frais S, Ng YL, Gulabivala K. Some factors affecting the concentration of available chlorine in commercial sources of sodium hypochlorite. Int Endod J 2001 34: 206-215.

101- Estrela C, Estrela CR, Barbin EL, Spanó JC, Marchesan MA, Pécora JD. Mechanism of action of sodium hypochlorite. Braz Dent J. 2002; 13(2):113-117.

102- Lapenna D, Cuccurullo F. Hypochlorous acid and its pharmacological antagonism: an update picture, Gen. Pharmacol 1996; 27:1145-1147.

103- Hidalgo E, Bartolome R, Dominguez C. Cytotoxicity mechanisms of sodium hypochlorite in cultured human dermal fibroblasts and its bactericidal effectiveness. Chem boil interact 2002; 139:265-82.

104- Estrela C, Sydney GB, Bammann LL, Junior OF. Mechanism of action of calcium and hydroxyl ions of calcium hydroxide on tissue and bacteria. Braz Dent J 1995 6: 85-90.

105- Bryce G, Donnell DO, Ready D. Contemporary root canal irrigants are able to disrupt and eradicate single- and dual-species biofilms. J Endod. 2009; 35:1243–8.

106- Svensater G, Bergenholtz G. Biofilms in endodontic infections. Endod Topics 2004 9: 27-36.

107- Spratt DA, Pratten J, Wilson M, Gulabivala K. An in vitro evaluation of the antimicrobial efficacy of irrigants on biofilms of root canal isolates. Int Endod J. 2001 Jun; 34(4):300-7.

108- Byström A, Sundqvist G. Bacteriologic evaluation of the effect of 0.5% sodium hypochlorite in endodontic therapy. Oral Surgery, Oral Medicine and Oral Pathology 1983;55, 307–12.

109- Cvek M, Nord CE, Hollender L. Antimicrobial effect of root canal debridement in teeth with immature root. A clinical and microbiologic study. Odontologisk Revy (1976a);27, 1–10.

110- Hauman CH, Love RM. Biocompatibility of dental materials used in contemporary endodontic therapy: a review. Part 1. Intracanal drugs and substances. Int Endod J 2003 36: 75-85.

111- Dakin HD. On the use of certain antiseptic substances in treatment of infected wounds. British Medical Journal (1915);2, 318–20.

112- Svensäter G, Bergenholtz G. Biofilms in endodontic infections. Endodontic Topics. 2004;9(1):27–36.

113- Clegg MS, Vertucci FJ, Walker C et al. The effect of exposure to irrigant solutions on apical dentine biofilms in vitro. J Endod 2006 32: 434-437.

114- Ozok AR, Wu MK, Luppens SB, Wesselink PR. Comparison of growth and susceptibility to sodium hypochlorite of mono- and dual-species biofilms of Fusobacterium nucleatum and Peptostreptococcus (micromonas) micros. J Endod. 2007 Jul; 33(7):819-22.

115- Giardino L, Ambu E, Savoldi E, Rimondini R, Cassanelli C, Debbia EA. Comparative evaluation of antimicrobial efficacy of sodium hypochlorite, MTAD, and Tetraclean against Enterococcus faecalis biofilm. J Endod 2007 33: 852-855.

116- J.M. Davies, D.A. Hortwitz, K.J. Davies. Potential roles of hypochlorous acid and N-chloroamines in collagen breakdown by phagocytic cells in synovitis. Free Radical Biology & Medicine, 15 (1993), pp. 637–643.

117- Marending M, Luder HU, Brunner TJ, Knecht S, Stark WJ, Zehnder M. Effect of sodium hypochlorite on human root dentine--mechanical, chemical and structural evaluation. Int Endod J. 2007 Oct;40(10):786-93. Epub 2007 Aug 14.

118- T.P. Sim, J.C. Knowles, Y.L. Ng, J. Shelton, K. Gulabivala. Effect of sodium hypochlorite on mechanical properties of dentine and tooth surface strain. International Endodontic Journal, 34 (2001), pp. 120–132.

119- Slutzky-Goldberg I, Maree M, Liberman R, Helling I. Effect of sodium hypochlorite on dentin microhardness. J Endod 2004 30: 880-882.

120- 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: 175-182.

121- Oyarzun A, Cordero AM, Whittle M. Immunohistochemical evaluation of the effects of sodium hypochlorite on dentine collagen and glycosaminoglycans. J Endod 2002 28: 152-156.

122- Da Cunha LF, Furuse AY, Mondelli RF, Mondelli J.Compromised bond strength after root dentin deproteinization reversed with ascorbic acid. J Endod 2010; 36:130–4.

123- Ishizuka T, Kataoka H, Yoshioka T, Suda H, Iwasaki N, Takahashi H, Nishimura F. Effect of NaOCl treatment on bonding to root canal dentin using a new evaluation method. Dent Mater J 2001 20: 24-33.

124- Perdigao J, Lopes M, Geraldeli S, Lopes GC, Garcia-Godoy F. Effect of a sodium hypochlorite gel on dentin bonding. Dent Mater 2000 16: 311-323.

125- Frankernberger R, Kramer N, Oberschachtsiek H, Petschelt A. Dentin bond strength and marginal adaption after NaOCl pre-treatment. Oper Dent 2000 25: 40-45.

126- Saboia Vde P, Almeida PC, Ritter AV, Swift EJ Jr, Pimenta LA. 2-year Clinical evaluation of sodium hypochlorite treatment in the restoration of non-carious cervical lesions: a pilot study. Oper Dent 2006 31:530-535.

127- Pioch T, Kobaslija S, Schagen B, Gotz H. Interfacial micromorphology and tensile bond strength of dentin bonding systems after NaOCl treatment. J Adhes Dent 1999 1: 135-142.

128- Ari H, Yasar E, Belli S. Effects of NaOCl on bond strengths of resin cements to root canal dentin. J Endod 2003 29: 248-251.

129- Morris MD, Lee KW, Agee KA, Bouillaguet S, Pashley DH. Effects of sodium hypochlorite and RC-prep on bond strengths of resin cement to endodontic surfaces. J Endodon 2001; 27:753–7.

130- Erdemir A, Eldeniz AU, Belli S, Pashley DH. Effect of solvents on bonding to root canal dentin. J Endod 2004 30: 589-592.

131- Correr GM, Puppin-Rontani RM, Correr-Sobrinho L, Sinhoret MA, Consani S. Effect of sodium hypochlorite on dentin bonding in primary teeth. J Adhes Dent 2004 6: 307-312.

132- Lai SC, Mak YF, Cheung GS, Osorio R, Toledano M, Carvalho RM, Tay FR, Pashley DH. J. Reversal of compromised bonding to oxidized etched dentin. Dent Res. 2001 Oct; 80(10):1919-24.

133- Vongphan N, Senawongse P, Somsiri W, Harnirattisai C. Effects of sodium ascorbate on microtensile bond strength of total-etching adhesive system to NaOCl treated dentine. J Dent 2005 33: 689-695.

134- Sirtes G, Waltimo T, Schaetzle M, Zehnder M. The effects of temperature on sodium hypochlorite short-term stability, pulp dissolution capacity, and antimicrobial efficacy. J Endod. 2005; 31:669–71.

135- Cunningham WT, Balekjian AY. Effect of temperature on collagen-dissolving ability of sodium hypochlorite endodontic irrigant. Oral Surg Oral Med Oral Pathol 1980 49: 175-177.

136- Abou-Rass M, Oglesby SW. The effects of temperature, concentration, and tissue type on the solvent ability of sodium hypochlorite. J Endod. 1981 Aug; 7(8):376-7.

137- Hand RE, Smith ML, Harrison JW. Analysis on the necrotic tissue dissolution property of sodium hypochlorite. J Endod. 1978; 4,60 – 4.

138- Bloomfield SF, Miles G. The relationship between residual chlorine and disinfection capacity of sodium hypochlorite and sodium dichlorisocyanurate solutions in the presence of E. coli and milk. Microbios 1979; 10:33– 43.

139- Baker RJ. Types and significance of chlorine residuals. J Am Water Works Assoc. 1959; 51:1185–90.

140- Smith RM, Martell AE. Critical stability constants. New York: Plenum Press; 1976.

141- Siqueira JF Jr, Rocas IN, Favieri A, Lima KC. Chemomechanical Reduction of the Bacterial Population in the Root Canal after Instrumentation and Irrigation with 1%,2.5%, and 5.25% Sodium Hypochlorite. J Endod. 2000 Jun;26(6):331-4.

142- Pashley EL, Birdsong NL, Bowman K, Pashley DH. Cytotoxic effects of NaOCl on vital tissue. J Endod. 1985;11, 525–8.

143- Witton R, Henthorn K, Ethunandan M, Harmer S. and Brennan P.A. Neurological complications following extrusion of sodium hypochlorite solution during root canal treatment. Int Endod J 2005; 38: 843-848.

144- Kavanagh C P, Taylor J. Inadvertent injection of sodium hypochlorite into the maxillary sinus. Br Dent J 1998; 185: 336-337.

145- Mehra P, Clancy C, Wu J. Formation of a facial hematoma during endodontic therapy. J Am Dent Assoc 2000; 131: 67-71.

146- Gernhardt C R, Eppendorf K, Kozlowski A, Brandt M. Toxicity of sodium hypochlorite used as an endodontic irrigant. Int Dent J 2004; 37: 272-280.

147- Heggers JP, Sazy JA, Stenberg BD, Strock LL, McCauley RL, Herndon DN, Robson MC. Bactericidal and wound-healing properties of sodium hypochlorite solutions: the 1991 Lindberg Award. Journal of Burn Care and Rehabilitation 1991;12, 420–4.

148- Becker GL, Cohen S, Borer R. The sequelae of accidentally injecting sodium hypochlorite beyond the root apex. Oral Surgery, Oral Medicine and Oral Pathology 1974;38, 633-8.

149- Ehrich DG, Brian JD Jr, Walker WA. Sodium hypochlorite accident: inadvertent injection into the maxillary sinus. J Endod. 1993;19, 180–2.

150- Brown DC, Moore BK, Brown CE Jr, Newton CW. An in vitro study of apical extrusion of sodium hypochlorite during endodontic canal preparation. J Endod. 1995;21, 587–91.

151- Marshall GW. Dentin: microstructure and characterization. Quintessence Int 1993; 24:606-17.

152- Rotstein I, Dankner E, Goldman A, Heling I, Stabholz A, Zalkind M Histochemical analysis of dental hard tissues following bleaching. J Endod. 1996; 22:23– 6.

153- Ari H,Erdemir A.Effects of endodontic irrigation solutions on mineral content of root canal dentin using ICP-AES technique. J Endod 2005; 31:187–9.

154- Hülsmann M, Heckendorff M, Lennon A. Chelating agents in root canal treatment: mode of action and indications for their use. Int Endod J. 2003 Dec; 36(12):810-30.

155- Patterson SS. In vivo and in vitro studies of the effect of the disodium salt of ethylenediamine tetra-acetate on human dentine and its endodontic implications. Oral Surg Oral Med Oral Pathol 1963; 16:83–103.

156- Yoshida T, Shibata T, Shinohara T, Gomyo S, Sekine I. Clinical evaluation of the efficacy of EDTA solution as an endodontic irrigant. J Endod 1995; 21:592-3.

157- Goldman LB. Goldman M. Kronman JH. Lin PS. The efficacy of several endodontics irrigating solutions: a scanning electron microscopic study. Oral surg 1981:52:199-204

158- Wayman BE, Kopp WM, Pinero GJ, Lazzari EP. Citric and lactic acids as root canal irrigants in vitro. J endodon 1979; 5:258-65.

159- Saito K, Webb TD, Imamura GM, Goodell GG. Effect of shortened irrigation times with 17% ethylene diamine tetra-acetic acid on smear layer removal after rotary canal instrumentation. J Endod 2008; 34:1011-4.

160- Caron G, Nham K, Bronnec F, Machtou P. E activeness of different final irrigant activation protocols on smear layer removal in curved canals. J Endod 2010; 36:1361-6.

161- Cruz-Filho AM, Sousa-Neto MD, Savioli RN, Silva RG, Vansan LP, Pécora JD. effect of chelating solutions on the microhardness of root canal lumen dentin. J Endod 2011; 37:358-62.

162- Sayin TC, Serper A, Cehreli ZC, Otlu HG. The effect of EDTA, EGTA, EDTAC, and tetracycline-HCl with and without subsequent NaOCl treatment on the microhardness of root canal dentin. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007 Sep; 104(3):418-24.

163- Ballal NV, Mala K, Bhat KS. Evaluation of the effect of maleic acid and ethylenediaminetetraacetic acid on the microhardness and surface roughness of human root canal dentin. J Endod 2010; 36:1385-8.

164- De-Deus G, Paciornik S, Mauricio MH. Evaluation of the effect of EDTA, EDTAC and citric acid on the microhardness of root dentine. Int Endod J 2006; 39:401-7.

165- Rossi-Fedele G, Doğramaci EJ, Guastalli AR, Steier L, de Figueiredo JA. Antagonistic interactions between sodium hypochlorite, chlorhexidine, EDTA, and citric acid. J Endod 2012; 38:426-31.

166- Irala LED, Grazziotin-Soares R, Azevedo Salles A, Munari AZ, Pereira JS. Dissolution of bovine pulp tissue in solutions consisting of varying NaOCl concentrations and combined with EDTA. Braz Oral Res 2010; 24:271–6.

167- Baumgartner JC, Ibay AC. The chemical reactions of irrigants used for root canal debridement. J Endod 1987; 13:47-51.

168- Zehnder M, Schmidlin P, Sener B, Waltimo T. Chelation in root canal therapy reconsidered. J Endod 2005; 31:817-20.

169- Haga CS. Microscopic measurements of root canal preparations following instrumentation. J Br Endod Soc 1968; 2:41–6.

170- Tan BT, Messer HH. The quality of apical canal preparation using hand and rotary instruments with specific criteria for enlargement based on initial apical file size. J Endod 2002; 28:658–64.

171- Cotter JL, Fader RC, Lilley C, Herndon DN. Chemical parameters, antimicrobial activities, and tissue toxicity of 0.1 and 0.5% sodium hypochlorite solutions. Anti- microb Agents Chemother 1985; 28:118–22.

172- Christensen CE, McNeal SF, Eleazer P. Effect of lowering the pH of sodium hypo-chlorite on dissolving tissue in vitro. J Endod 2008; 34:449–52.

173- Cunningham WT, Joseph SW. Effect of temperature on the bactericidal action of sodium hypochlorite endodontic irrigant. Oral Surg Oral Med Oral Pathol 1980; 50:569–71.

174- Lui JN, Kuah HG, Chen NN. Effect of EDTA with and without surfactants or ultra- Sonics on removal of smear layer. J Endod 2007; 33:472–5.

175- Li-sha Gu, Jong Ryul Kim, Junqi Ling, Kyung Kyu Choi, David H. Pashley, Franklin R. Tay. Review of Contemporary Irrigant Agitation Techniques and Devices**.** Journal of Endodontics, Vol. 35, Issue 6, p791–804. 2009.

176- Kahn FH, Rosenberg PA, Gliksberg J. An in vitro evaluation of the irrigating charac- teristics of ultrasonic and subsonic handpieces and irrigating needles and probes. J Endod 1995; 21:277–80.

177- Van der Sluis LW, Gambarini G, Wu MK, Wesselink PR. The influence of volume, type of irrigant and flushing method on removing artificially placed

dentine debris from the apical root canal during passive ultrasonic irrigation. Int Endod J 2006;39: 472–6.

178- Abou-Rass M, Oglesby SW. The effects of temperature, concentration, and tissue type on the solvent ability of sodium hypochlorite. J Endod. 1981;7, 376 –7.

179- Wu MK, Wesselink PR. Efficacy of three techniques in cleaning the apical portion of curved root canals. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1995; 79:492–6.

180- Falk KW, Sedgley CM. The influence of preparation size on the mechanical efficacy of root canal irrigation in vitro. J Endod 2005; 31:742–5.

181- Chow TW. Mechanical effectiveness of root canal irrigation. J Endod 1983; 9:475–9.

182- Gulabivala K, Ng YL, Gilbertson M, Eames I. The fluid mechanics of root canal irrigation. Physiol Meas. 2010;31(12): R49–84.

183- Machtou P. Irrigation investigation in endodontics. Paris VII University, Paris, France: Masters thesis; 1980.

184- Caron G. Cleaning efficiency of the apical millimeters of curved canals using three different modalities of irrigant activation: an SEM study. Paris VII University, Paris, France: Masters thesis; 2007.

185- Walmsley AD, Lumley PJ, Laird WR. Oscillatory pattern of sonically powered endodontic files. Int Endod J 1989; 22:125–32.

186- McGill S, Gulabivala K, Mordan N, Ng YL. The efficacy of dynamic irrigation using a commercially available system (RinsEndo) determined by removal of a collagen ‘bio-molecular film’ from an ex vivo model. Int Endod J 2008;41: 602–8.

187- Wiggins S, Ottino JM. Foundations of chaotic mixing. Philos Transact A Math Phys Eng Sci 2004; 362:937–70.

188- Ruddle CJ. Microbrush for endodontic use. Washington, DC: United States Patent. 6,179,617; 2001.

189- Weise M, Roggendorf MJ, Ebert J, Petschelt A, Frankenberger R. Four methods for cleaning simulated lateral extensions of curved root canals: a SEM evaluation. Int Endod J 2007; 40:991–2.

190- Tronstad L, Barnett F, Schwartzben L, Frasca P. Effectiveness and safety of a sonic vibratory endodontic instrument. Endod Dent Traumatol 1985; 1:69–76.

191- Ahmad M, Pitt Ford TR, Crum LA. Ultrasonic debridement of root canals: an insight into the mechanisms involved. J Endod 1987; 13:93–101.

192- Plotino G, Pameijer CH, Grande NM, Somma F. Ultrasonic in endodontics: a review of the literature. J Endod. 2007;33(2):81–95.

193- Zahed Mohammadi, Sousan Shalavi, Luciano Giardino, Flavio Palazzi and Saeed Asgary. Impact of Ultrasonic Activation on the Effectiveness of Sodium Hypochlorite: A review. Iran endod J. 2015 Fall; 10(4): 216-220.

194- Sabins RA, Johnson JD, Hellstein JW. A comparison of the cleaning efficacy of short-term sonic and ultrasonic passive irrigation after hand instrumentation in molar root canals. J Endod 2003; 29:674–8.

195- Ruddle CJ. Endodontic disinfection: tsunami irrigation. Endod Practice 2008. Feb:7–15.

196- Townsend C, Maki J. An in vitro comparison of new irrigation and agitation techniques to ultrasonic agitation in removing bacteria from a simulated root canal. J Endod 2009; 35:1040–3.

197- Guerisoli DM, Marchesan MA, Walmsley AD, Lumley PJ. Evaluation of smear layer removal by EDTAC and sodium hypochlorite with ultrasonic agitation. Int Endod J 2002;35(5): 418-421

198- Van der Sluis LWM, Versluis M, Wu MK, Wesselink PR. Passive ultrasonic irrigation of the root canal: a review of the literature. Int Endod J 2007;40(6): 415-426.

199- Martin H. Ultrasonic disinfection of the root canal. Oral Surg Oral Med Oral Pathol 1976; 42:92–9.

200- Martin H, Cunningham WT, Norris JP. A quantitative comparison of the ability of diamond and K-type files to remove dentin. Oral Surg Oral Med Oral Pathol. 1980; 50:566 – 8.

201- Martin H, Cunningham WT, Norris JP, Cotton WR. Ultrasonic versus hand filing of dentin: a quantitative study. Oral Surg Oral Med Oral Pathol 1980; 49:79–81.

202- Martin H, Cunningham W. Endosonic endodontics: the ultrasonic synergistic system. Int Dent J. 1984; 34:198–203.

203- Moorer WR, Wesselink PR. Factors promoting the tissue dissolving capability of sodium hypochlorite. International Endodontic Journal 15, 187-96. 1982.

204- De Gregorio C, Estevez R, Cisneros R, et al. Effect of EDTA, sonic, and ultrasonic activation on the penetration of sodium hypochlorite into simulated lateral canals: an in vitro study. J Endod 2009; 35:891–5.

205- Al-Jadaa A, Paqué F, Attin T, Zehnder M. Acoustic hypochlorite activation in simulated curved canals. J Endod. 2009 Oct; 35(10):1408-11.

206- Stock CJ. Current status of the use of ultrasound in endodontics. Int Dent J. 1991 Jun; 41(3):175-82.

207- Ahmad M, Roy RA, Kamarudin AG, Safar M. The vibratory pattern of ultrasonic files driven piezoelectrically. Int Endod J. 1993; 26:120–124.

208- Lumley PJ, Walmsley AD, Marquis PM. Effect of air inlet ring opening on sonic hand piece performance. J Dent. 1994; 22:376–379.

209- Ahmad M, Pitt Ford TR, Crum LA. Ultrasonic debridement of root canals: acoustic streaming and its possible role. J Endod (1987a) ;14, 490-9.

210- Layton CA, Marshall JG, Morgan LA, Baumgartner JC. Evaluation of cracks associated with ultrasonic root-end preparation. J Endod. 1996; 22:157–160.

211- Walmsley AD. Ultrasound and root canal treatment: the need for scientific evaluation. Int Endod J. 1987; 20:105–111.

212- Lumley PJ, Walmsley AD, Laird WRE. An investigation into the occurrence of cavitational activity during endosonic instrumentation. J Dent. 1988; 16:120–122.

213- Laird WRE, Walmsley AD. Ultrasound in dentistry: Part 1 (Biophysical interactions) J Dent. 1991; 19:14–17.

214- Lea SC, Walmsley AD, Lumley PJ, Landini G. A new insight into the oscillation characteristics of endosonic files used in dentistry. Phys Med Biol 2004;49: 2095–102.

215- Gianluca Plotino, Cornelis H. Pameijer, Nicola Maria Grande, Francesco Somma. Ultrasonics in Endodontics: A Review of the Literature. J Endod. 2007;33(2):81-95.

216- Klyn SL, Kirkpatrick TC, Rutledge RE. In vitro comparisons of debris removal of the EndoActivator system, the F file, ultrasonic irrigation, and NaOCl

irrigation alone after hand-rotary instrumentation in human mandibular molars. J Endod. 2010 Aug; 36(8):1367-71.

217- Lumley PJ, Walmsley AD, Walton RE, Rippin JW. Effect of precurving endosonic files on the amount of debris and smear layer remaining in curved root canals. J Endod 1992; 18:616–9.

218- Walmsley AD, Murgel C, Krell KV. Canal markings produced by endosonic instruments. Endod Dent Traumatol 1991; 7:84–9.

219- Weller RN, Brady JM, Bernier WE. Efficacy of ultrasonic cleaning. J Endod 1980; 6:740–3.

220- Ahmad M, Roy RA, Kamarudin AG, Observations of acoustic streaming fields around an oscillating ultrasonic file. Endodontics Dental Traumatology, Oct. 1992.

221- Goodman A, Reader A, Beck M, Melfi R, Meyers W. An in vitro comparison of the efficacy of the step-back technique versus a step-back/ultrasonic technique in human mandibular molars. J Endod 11,249-56. 1985.

222- Cameron JA. The use of 4 percent sodium hypochlorite, with or without ultrasound, in cleansing of uninstrumented immature root canals; SEM study. Australian Dental Journal, 32, 204-13. 1987a.

223- Cheung GSP, Stock CJR. in vitro cleaning ability of root canal irrigant with and without endosonics. Int Endod J, 26, 334-43. 1993.

224- Gutarts R, Nusstein J, Reader A, Beck M. In vivo debridement efficacy of ultrasonic irrigation following hand-rotary instrumentation in human mandibular molars. J Endod. 2005;31, 166-70.

225- Sjogren U. Sundqvist G. Bacteriologic evaluation of ultrasonic root canal instrumentation. Oral Surgery. Oral Medicine and Oral Pathology 63, 336-70, 1987.

226- Huque J, Kota K, Yamaga M, Iwaku M, Hoshino E. Bacterial eradication from root dentine by ultrasonic irrigation with sodium hypochlorite. International Endodontic Journal, 31, 242-50. 1998.

227- Spoleti P, Siragusa M, Spoleti MJ. Bacteriological evaluation of passive ultrasonic activation. J Endod. 2003;29, 21-4.

228- Cameron JA. The effect of ultrasonic endodontics on the temperature of the root canal wall. J Endod 1998;14, 554-8.

229- Ahmad M, Measurements of temperature generated by ultrasonic file in vitro. Endodontics and Dental Traumatology 1990;6, 230-1.

230- Roy RA, Ahmad M, Crum LA, Physical mechanisms governing the hydrodynamic response of an oscillating ultrasonic file. Int Endod J. 1994; 27,197-207.

231- Walmsley AD, Williams AR. Effects of constraint on the oscillatory pattern of endosonic files. J Endod. 1989; 15,189-94.

232- Jensen SA, Walker TL, Hutter JW, Nicoll BK. Comparison of cleaning efficacy of passive sonic activation and passive ultrasonic activation after hand instrumentation in molar root canals. J Endod 1999; 25:735– 8.

233- Leighton TG. The Acoustic Bubble. Chapter 1 and 2. New York: Academic Press. 1994.

234- Pesse AV, Warrier GR, Dhir VK. An experimental study of the gas entrapment process in closed-end microchannels. Int J Heat Mass Transfer 2005; 48:5150–65.

235- Dovgyallo GI, Migun NP, Prokhorenko PP. The complete filling of dead-end conical capillaries with liquid. J Eng Phy 1989; 56:395–7

236- Senia ES, Marshall FJ, Rosen S. The solvent action of sodium hypochlorite on pulp tissue of extracted teeth Oral Surg Oral Med Oral Pathol 1971 31:96-103.

237- Cameron J. The use of ultrasonics in the removal of the smear layer: a scanning electron microscope study. J Endod. 1983;9(7):289–92.

238- Bankoff SB. Entrapment of gas in the spreading of a liquid over a rough surface. AICHE J 1958; 4:24–6.

239- Schoeffel GJ. The EndoVac method of endodontic irrigation: part 2—efficacy. Dent Today 2008; 27:82,84,86–87.

240- Boutsioukis C, Kastrinakis E, Lambrianidis T, Verhaagen B, Versluis M, van der Sluis LW. Formation and removal of apical vapor lock during syringe irrigation: a combined experimental and computational fluid dynamics approach. Int Endod J. 2014; 47, 191-201.

241- Huang TY, Gulabivala K, Ng Y-L. A bio-molecular film ex-vivo model to evaluate the influence of canal dimensions and irrigation variables on the efficacy of irrigation. Int Endod J 2008; 41:60–71.

242- Vera J, Hernandez EM, Romero M, Arias A, van der Sluis LW. Effect of maintaining apical patency on irrigant penetration into the apical two millimeters of large root canals: an in vivo study. J Endod. 2012; 38(10): 1340-3.

243- Vera J, Arias A, Romero M. Effect of maintaining apical patency on irrigant penetration into the apical third of root canals when using passive ultrasonic irrigation: an in vivo study. J Endod. 2011;37(9):1276-8.

244- Shin SJ, Kim HK, Jung IY, Lee CY, Lee SJ, Kim E. Comparison of the cleaning efficacy of a new apical negative pressure irrigating system with conventional irrigation needles in the root canals. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010 Mar;109(3):479-84.

245- Nielsen BA, Baumgartner J. Comparison of the EndoVac system to needle irrigation of root canals. J Endod. 2007 33(5):611-15.

246- Desai P, Himel V. Comparative safety of various intracanal irrigation systems. J Endod 2009; 35:545–9.

247- Kurtzmann GM. Improving endodontic success through the use of the EndoVac irrigation system. Endod Pract. 2009; 10:17–20.

248- Schneider SW. Comparison of canal preparation in straight and curved root canals. Oral Surg Oral Med Oral Pathol. 1971; 32:271-5.

249- Kuttler Y. Microscopic investigation of root apexes. J. Am Dent Assoc 1955; 50:544-52.

250- McMullan, D. “Scanning electron microscope 1928-1965. Scanning. 2006;17 (3): 175-185.

251- McMullan, D. "Von Ardenne and the scanning electron microscope". Proc Roy Microsc Soc. 1988;23: 283–288.

252- Von Ardenne M. Improvements in electron microscopes. convention date (Germany) 18 February 1937.

253- Young GR, Parashos P, Messer HH. The principles of techniques for cleaning root canals. Australian Dental Journal Supplement.. 2007;52:(1 Suppl): S52-S63.

254- Amato M, Vanoni-Heineken I, Hecker H, Weiger R. Curved versus straight root canals: the benefit of activated irrigation techniques on dentin debris removal. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2011; 111:529-34.

255- Rödig T, Döllmann S, Konietschke F, Drebenstedt S, Hülsmann M. Effectiveness of different irrigant agitation techniques on debris and smear layer removal in curved root canals: a scanning electron microscopy study. J Endod. 2010; 36:1983-7.

256- Grossman LI. Guidelines for the prevention of fracture of root canal instruments. Oral Surg Oral Med Oral Pathol. 1969; 28:746–52.

257- Choksi D, Idnani B, Kalaria D, Patel RN. Management of an Intracanal Separated Instrument: A Case Report. Iran Endod J. 2013; 8:205–07.

258- Madarati AA, Watts DC, Qualtrough AJE. Factors contributing to the separation of endodontic files. Br Dent J. 2008; 204:241–45.

259- Yang GB, Zhou XD, Zhang H, Wu HK. Shaping ability of progressive versus constant taper instruments in simulated root canals. Int Endod J. 2006; 39:791–9.

260- Calberson FL, Deroose CA, Hommez GM, Raes H, De Moor RJ. Shaping ability of GTTM Rotary Files in simulated resin root canals. Int Endod J. 2002; 35:607–14.

261- Jesus D. P., Manoel D. S., Paulo C. S., Julian B. W. Morphologic Study of the Maxillary Molars Part 1: External Anatomy. Braz Dent J (1991) 2 (1): 45-50.

262- Heard F, Walton RE. Scanning electron microscope study comparing four root canal preparation techniques in small curved canals. Int Endod J 1997; 30:323–31.

263- Card SJ, Sigurdsson A, Orstavik D, Trope M. The effectiveness of increased apical enlargement in reducing intracanal bacteria. J Endod 2002; 28:779–83.

264- Rollison S, Barnett F, Stevens RH. Efficacy of bacterial removal from instrumented root canals in vitro related to instrumentation technique and size. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002; 94:366–71.

265- Albrecht LJ, Baumgartner JC, Marshall JG. Evaluation of apical debris removal using various sizes and tapers of ProFile GT files. J Endod 2004; 30:425–8.

266- Wilcox LR, Roskelley C, Sutton T. The relationship of root canal enlargement to finger-spreader induced vertical root fracture. J Endod. 1997; 23(8):533-4

267- Dalton BC, Ørstavik D, Phillips C, Pettiette M, Trope M. Bacterial reduction with nickel-titanium rotary instrumentation. J Endod 1998; 24:763-767.

268- Ørstavik D, Kerekes K, Molven O. Effects of extensive apical reaming calcium hydroxide dressing on bacterial infection during treatment of apical periodontitis: a pilot study. Int Endod J, 24 (1991), pp. 1–7.

269- Matsumiya S, Kitamura M. Histo-pathological histo-bacteriological studies of the relationship between the condition of sterilization of the interior of the root canal the healing process of periapical tissues in experimentally infected root canal treatment. Bull Tokyo Dent Coll, 1 (1960), pp. 1–19.

270- Card SJ, Sigurdsson A, Ørstavik D, Trope M. The effectiveness of increased apical enlargement in reducing intracanal bacteria. J Endod. 2002; 28(11):779-83.

271- Ram Z. Effectiveness of root canal irrigation. Oral Surgery, Oral Medicine, and Oral Pathology, 1977;44, 306–12.

272- Salzgeber RM, Brilliant JD. An in vivo evaluation of the penetration of an irrigating solution in root canals. J Endodon 3(10):394-398, 1977.

273- Shuping GB, Orstavik D, Sigurdsson A, Trope M. Reduction of intracanal bacteria using nickel-titanium rotary instrumentation and various medications. J Endod. 2000 Dec; 26(12):751-5.

274- Khademi A, Yazdizadeh M, Feizianfard M. Determination of the minimum instrumentation size for penetration of irrigants to the apical third of root canal systems. J Endod. 2006 May; 32(5):417-20.

275- Mickel AK, Chogle S, Liddle J, Huffaker K, Jones JJ. The role of apical size determination and enlargement in the reduction of intracanal bacteria. J Endod. 2007 Jan; 33(1):21-3.

276- Spångberg L. The wonderful world of rotary root canal preparation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001; 92:479.

277- Boutsioukis C, Lambrianidis T, Verhaagen B, Versluis M, Kastrinakis E, Wesselink PR, van der Sluis LW. The Effect of Needle-insertion Depth on the Irrigant Flow in the Root Canal: Evaluation Using an Unsteady Computational Fluid Dynamics Model. J Endod. 2010; 36(10):1664-8.

278- Siu C, Baumgartner JC. Comparison of the debridement efficacy of the EndoVac irrigation system and conventional needle root canal irrigation in vivo. J Endod 2010; 36:1782–5.

279- Abou-Rass M, Piccinino MV. The effectiveness of four clinical irrigation methods on the removal of root canal debris. Oral Surg Oral Med Oral Pathol 1982; 54:323-328.

280- McDonnell G, Russell AD. Antiseptics and disinfectants: activity, action, and resistance. Clin Microbiol Rev. 1999; 12:147–79.

281- Chávez de Paz LE, Bergenholtz G, Svensäter G. The effects of antimicrobials on endodontic biofilm bacteria. J Endod. 2010 Jan; 36(1):70-7.

282- Liu H, Wei X, Ling J, Wang W, Huang X. Biofilm formation capability of Enterococcus faecalis cells in starvation phase and its susceptibility to sodium hypochlorite. J Endod. 2010 Apr; 36(4):630-5.

283- Ozdemir HO, Buzoglu HD, Calt S, Stabholz A, Steinberg D. Effect of ethylenediaminetetraacetic acid and sodium hypochlorite irrigation on Enterococcus faecalis biofilm colonization in young and old human root canal dentin: in vitro study. J Endod. 2010 May; 36(5):842-6.

284- Del Carpio-Perochena AE, Bramante CM, Duarte MA, Cavenago BC, Villas-Boas MH, Graeff MS, Bernardineli N, de Andrade FB, Ordinola-Zapata R. Biofilm dissolution and cleaning ability of different irrigant solutions on intraorally infected dentin. J Endod. 2011 Aug; 37(8):1134-8.

285- Seet AN, Zilm PS, Gully NJ, Cathro PR. Qualitative comparison of sonic or laser energisation of 4% sodium hypochlorite on an Enterococcus faecalis biofilm grown in vitro. Aust Endod J. 2012 Dec; 38(3):100-6.

286- Louis I. Grossman, Benjamin W. Meiman. Solution of pulp tissue by chemical agents. JADA 28:223 Feb 1941.

287- Trepagnier, C.M.; Madden, R.M.; and Lazarri, E.P. Quantitative study of sodium hypochlorite as an in vitro endodontic irrigant. J Endod. 1977 May;3(5):194-6.

288- Clegg MS, Vertucci FJ, Walker C, Belanger M, Britto LR. The effect of exposure to irrigant solutions on apical dentin biofilms in vitro. J Endod. 2006 May; 32(5):434-7.

289- Ballal NV, Yegneswaran PP, Mala K, Bhat KS. In vitro antimicrobial activity of maleic acid and ethylenediaminetetraacetic acid on endodontic pathogens. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011; 112:696‑700.

290- Ordinola‑Zapata R, Bramante CM, Cavenago B, Graeff MS, Gomes de Moraes I, Marciano M, Duarte MA. Antimicrobial effect of endodontic

solutions used as final irrigants on a dentine biofilm model. Int Endod J 2012; 45:162‐8.

291- Arias-Moliz MT, Ferrer-Luque CM, Espigares-Garcia M, Baca P (2009) Enterococcus faecalis biofilms eradication by root canal irrigants. Journal of Endodontics **35**, 711–4.

292- Stewart GG, Kapsimal SP, Rappaport H. EDTA and urea peroxide for root canal preparation. J Dent Res 1969; 78:335–8.

293- Johnson WT, Noblett WC. Cleaning and shaping in Endodontics: Principles and Practice. 4th ed. Saunders, Philadelphia, PA, 2009.

294- Kuah HG, Lui JN, Tseng PS, Chen NN. The effect of EDTA with and without ultrasonics on removal of the smear layer. J Endod 2009; 35:393‐6.

295- Calt S, Serper A, Time-dependent effects of EDTA on dentin structures J Endod. 2002:28,1:17-19.

296- Erdemir A, Eldeniz AU, Belli S, Pashley DH. Effect of solvents on bonding to root canal dentin. J Endod 2004;30(8): 589-92.

297- Lai SC, Mak YF, Cheung GS, Osorio R, Toledano M, Carvalho RM, Tay FR, Pashley DH. J. Reversal of compromised bonding to oxidized etched dentin. Dent Res. 2001 Oct; 80(10):1919-24.

298- Zehnder M, Schicht O, Sener B, Schmidlin P. Reducing surface tension in endodontic chelator solutions has no effect on their ability to remove calcium from instrumented root canals. J Endod. 2005 Aug;31(8):590-2.

299- Krell KV, Johnson RJ, Madison S. Irrigation patterns during ultrasonic canal instrumentation. Part I. K-type files. J Endod. 1988;14(2):65-8.

300- Cunningham WT, Martin H. A scanning electron microscope evaluation of root canal debridement with the endosonic ultrasonic synergistic system. Oral Surg Oral Med Oral Pathol 53(5):527-31, 1982.

301- Zou L, Shen Y, Li W, Haapasalo M. Penetration of sodium hypochlorite into dentin. J Endod. 2010;36(5):793–6.

302- Levy G, Rizoiu L, Friedman S, Lam H. Pressure waves in root canals induced by Nd:YAG laser. J Endod 1996; 22:81–4.

303- Baysan A, Lynch E. The use of ozone in dentistry and medicine. Prim Dent Care 2005; 12:47–52.

304- Lee SJ, Wu MK, Wesselink PR. The effectiveness of syringe irrigation and ultrasonics to remove debris from simulated irregularities within prepared root canal walls. Int Endod J 2004; 37:672–8.

305- Cameron JA. The synergistic relationship between ultrasound and sodium hypochlorite: a scanning electron microscope evaluation. J Endod 1987; 13:541–5.

306- Walker TL, del Rio CE. Histological evaluation of ultrasonic and sonic instrumentation of curved root canals. J Endod. 1989 Feb; 15(2):49-59.

307- Goldberg F, Snares I, Massone EJ, Scares IM. Comparative debridement study between hand and sonic instrumentation of the root canal. Dent Traumatol. 1988;4(5):229–34.

308- Baker MC, Ashrafi SH, Van Cura JE, Remeikis NA. Ultrasonic compared with hand instrumentation: a scanning electron microscope study. J Endod. 1988;14(9):435–40.

309- Gutarts R, Nusstein J, Reader A, Beck M. in vivo debridement efficacy of ultrasonic irrigation following hand-rotary instrumentation in human mandibular molars. J Endod. 2005 Mar;31(3):166-70.

310- Burleson A, Nusstein J, Reader A, Beck M. The in vivo evaluation of hand/rotary/ultrasound instrumentation in necrotic, human mandibular molars. J Endod. 2007 Jul;33(7):782-7.

311- Blank-Gonçalves LM, Nabeshima CK, Martins GH, Machado ME. Qualitative analysis of the removal of the smear layer in the apical third of curved roots: conventional irrigation versus activation systems. J Endod. 2011;37(9):1268–71.

312- Jensen SA, Walker TL, Hutter JW, Nicoll BK. Comparison of cleaning efficacy of passive sonic activation and passive ultrasonic activation after hand instrumentation in molar root canals. J Endod 1999; 25:735– 8.

313- Siqueira JF, Machado AG, Silveira RM, Lopes HP, De Uzeda M. Evaluation of the effectiveness of sodium hypochlorite used with three irrigation methods in the elimination of Enterococcus faecalis from the root canal, in vitro. Int Endod J. 1997 Jul;30(4):279-82.

314- Boutsioukis C, Lambrianidis T, Kastrinakis E. Irrigant flow within a prepared root canal using various flow rates: a computational fluid dynamics study. Int Endod J 2009; 42:144–55.

315- Yost RA, Bergeron BE, Kirkpatrick TC, Roberts MD, Roberts HW, Himel VT, Sabey KA. Evaluation of 4 Different Irrigating Systems for Apical Extrusion of Sodium Hypochlorite. J Endod. 2015 Sep;41(9):1530-4.

316- Hockett JL, Dommisch JK, Johnson JD, Cohenca N. Antimicrobial efficacy of two irrigation techniques in tapered and non-tapered canal preparations: an in vitro study J Endod 2008 34(11):1374-77.

317- De Miranda RG, Gusman HD, Colombo AP. Antimicrobial efficacy of the EndoVac system plus PDT against intracanal Candida albicans: an ex vivo study. Braz Oral Res. 2015;29(1).

318- Brito PR, Souza LC, Machado de Oliveira JC, Alves FR, De-Deus G, Lopes HP, Siqueira JF Jr. Comparison of the effectiveness of three irrigation techniques in reducing intracanal Enterococcus faecalis populations: an in vitro study J Endod. 2009 Oct;35(10):1422-27.

319- Versiani MA, Alves FR, Andrade-Junior CV, Marceliano-Alves MF, Provenzano JC, Rôças IN, Sousa-Neto MD, Siqueira JF Jr. Micro-CT evaluation of the efficacy of hard-tissue removal from the root canal and isthmus area by positive and negative pressure irrigation systems. Int Endod J. 2016 Nov;49(11):1079-1087.

320- Uroz-Torres D, Gonzales-Rodriguez MP, Ferre-Luque CM. Effectiveness of the Endoactivator system in removing the smear layer after root canal instrumentation. J Endod. 2010 Feb;36(2):308–11.

321- Caron G, Nhan K, Bronnec F, Machtou P. Effectiveness of different final irritant activation protocols on smear layer removal in curved canals. J Endod. 2010 Aug;36(8):1361–6.

322- Catuna MC. Ultrasonic energy: a possible dental application. Preliminary report of an ultrasonic cutting method. Ann Dent 1953; 12:256 – 60.