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Comparison Of Root Canal Irrigation Systems In Reducing Intracanal Microorganisms Using Saline-An In Vitro Study

Pranav Desai
Virginia Commonwealth University

Comparison Of Root Canal Irrigation Systems In Reducing Intracanal Microorganisms Using Saline-An *In Vitro* Study

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Dentistry at Virginia Commonwealth University.

by

Pranav Desai, BDS, DDS
BDS, VS Dental College, Bangalore, India 2001
DDS, University of Colorado, College of Dentistry, 2006

Director: Karan J. Replogle, DDS, MS,
Department Chair, Department of Endodontics,
Virginia Commonwealth University School of Dentistry

Virginia Commonwealth University
Richmond, Virginia
May 9, 2012

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Abstract

COMPARISON OF ROOT CANAL IRRIGATION SYSTEMS IN REDUCING INTRACANAL MICROORGANISMS USING SALINE-AN *IN VITRO* STUDY

By Pranav Desai, BDS, DDS

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Mechanical and chemical debridement plays an important role in reducing intracanal microorganisms. Effective root canal irrigation depends on both the root canal irrigant and irrigation system. The objective of this study was to evaluate the debridement efficiency of four root canal irrigation systems, Endovac®, PiezoFlow™, EndoActivator® and traditional needle irrigation using saline as an irrigant. Seventy-five, single canal, extracted, mature teeth were selected. Teeth were standardized to canal lengths of 15 mm and instrumented to Master Apical File size #40 with 4% taper. Teeth were mounted in a centrifuge tube using PVS impression material. Teeth were randomly divided into four experimental groups (n=15) and one control group (n=15). The root canals were inoculated with 24-hour culture of *Streptococcus mutans* and incubated for 72 hours. Saline was delivered via each of the irrigation systems at the rate of 7 ml/min using a precision syringe pump. Immediately following the treatment, samples were collected from the untreated control and the experimental groups and plated on agar

plates. Results were analyzed using repeated measures ANOVA and Tukey's HSD multiple comparison tests. All the experimental groups were significantly better than the control group ($p<0.0001$). Among the experimental groups, Endovac® and PiezoFlow™ were significantly better in reducing microorganisms compared to needle and EndoActivator® groups ($p<0.05$). There was no statistical difference between Endovac® and PiezoFlow™. Debridement efficiency of Endovac® and PiezoFlow™ is better than needle and EndoActivator® irrigation systems using saline as an irrigant. Funded by Alexander Fellowship, VCU School of Dentistry.

Introduction

Apical periodontitis is an inflammatory disease of microbial origin primarily caused by infection of the root canal system. It was in 1894, with a milestone study by WD Miller that the association of bacteria and apical periodontitis was hypothesized. This hypothesis was confirmed by Kakehasi (1) who investigated the response of the dental pulps of conventional and germ free rats exposed to saliva. He demonstrated that pulp necrosis developed in conventional rats when bacteria were present in the root canal system whereas germ free rats did not develop pulp necrosis. The role of bacteria in apical periodontitis was further confirmed by Moller (2) and Sundqvist (3). Later studies demonstrated that root canal infections are polymicrobial in nature with a variety of bacterial species depending upon whether it is primary or refractory in nature (4, 5).

Current molecular studies show that bacteria form biofilms in the root canal system to survive. Ricucci et al evaluated the presence of biofilms in primary and refractory cases in the presence of apical periodontitis. Seventy-seven percent of the overall teeth evaluated had biofilms in the apical third region of the root canal space. Biofilms covered the dentinal walls as well as canal isthmuses and ramifications. Furthermore, they noted that teeth with large periapical lesions had a higher prevalence of intraradicular biofilms. They therefore, concluded that apical periodontitis is a biofilm induced disease (6). Several other investigations have confirmed that bacterial biofilms are more prone to cause persistent infection than any individual bacterial species (7, 8). Microbiota that infect the root canal spaces are not only present in the main root canal, but also reside in apical ramifications and dentinal tubules (9, 10, 11). These anatomical

complexities as a result, render disinfection of the root canal system a true challenge (12, 13).

It becomes apparent that maximum reduction of the microbial load and their associated biofilm are the most crucial step in influencing the success of endodontic therapy. Studies have demonstrated that when a positive microbial culture is obtained after debridement of root canal systems, healing rate diminishes significantly. Fabricius et al investigated the influence of residual bacteria on periapical tissue healing in *Macaca Fascicularis* monkeys after the debridement of the root canal system. Results demonstrated that when bacteria remained in the root canal system after the endodontic treatment, 79% of periapical lesions did not heal (14). Similarly, Sjogren et al performed a study where 55 single canal teeth were treated and followed for 5 years. Cultures were obtained at the end of instrumentation and prior to obturation. The authors noted that complete periapical healing occurred 94% of the time when a negative culture was achieved; however when a positive culture was obtained, only 68% of the teeth healed (15).

Currently, the best available methods to reduce the microbial load in a root canal system are thorough mechanical and chemical debridement (chemomechanical debridement). Mechanical debridement involves debridement of the root canal space using various hand and rotary, stainless steel and nickel-titanium instruments. Peters et al conducted a study investigating the effect of nickel-titanium (NiTi) hand and rotary instruments on canal geometry utilizing micro-computed tomography (CT). They concluded that hand and rotary instruments left 35% or more surface area of the canal unchanged. It is therefore impossible for the instruments to thoroughly clean canal

intricacies (16). Dalton performed an in-vivo study comparing NiTi instrumentation to stainless steel instrumentation in reducing microbial load. He concluded that neither instrument could clean the canals completely free of microorganisms (17). Shuping et al investigated the reduction in microbial load at different stages of instrumentation and after the use of sodium hypochlorite as a final rinse. He observed that when an antimicrobial irrigant was used, significantly more canals were bacteria free compared to initial instrumentation (18). Conclusively, these studies are all in agreement that mechanical debridement alone is insufficient to render canals free of microorganisms.

Chemical debridement involves the use of antimicrobial irrigants and irrigation systems, which either transport and/or activate the irrigants into the root canal system (19, 20, 21). The desired properties of a root canal irrigant are removal of organic and inorganic tissues, microbes and their biofilms and removal of debris while at the same time not irritating vital periapical tissues. Various types of irrigants, irrigation devices and protocols have been advocated for successful debridement of root canals. No single irrigating solution or device has been found to be ideal in adequately cleaning the root canal system.

Sodium hypochlorite (NaOCl) is the most widely used primary irrigating solution due to its ability to dissolve both vital (22, 23) and necrotic tissues (24) as well as its broad-spectrum antimicrobial property (25, 26, 27). It also removes the organic portion of the smear layer created during instrumentation of the root canal space. However, the main disadvantage of sodium hypochlorite is its severe cytotoxic effects on periapical tissues (28, 29).

Sodium hypochlorite is unable to remove the inorganic portion of the smear layer from the root canal system. To overcome this, ethylene diamine tetra acetic acid (EDTA), has been advocated to primarily be used as a chelating agent. EDTA removes inorganic content from the root canal (30, 31) along with the smear layer (32).

Chlorhexidine (CHX) has also been used as a root canal irrigant due to its antimicrobial property and substantivity. Several studies have confirmed that 2% CHX possesses antimicrobial activity that is comparable to sodium hypochlorite (26, 33, 34, 35). Consequently, combinations of irrigating solutions like sodium hypochlorite, EDTA and CHX in a specific sequence have been recommended to predictably obtain the goals of safe and effective irrigation (30, 32, 36, 37, 38).

Effective root canal irrigation depends on both root canal irrigant and its delivery system. To achieve the above-mentioned desired properties, the root canal irrigant has to reach the root canal complexities in effective depth and volume. Baker et al performed an *in vitro* study using scanning electron microscope comparing efficacy of various irrigating solutions like sodium hypochlorite, hydrogen peroxide, saline, RC prep etc. He concluded that successful debridement of a root canal system is the function of quantity of root canal irrigants rather than the type of solution used. Flushing of the root canal is more important than the type of irrigant used (39). According to Chow, for the solutions to be mechanically effective it has to a) reach the apex, b) create a current and c) carry the particles away (40). The effectiveness of any root canal irrigation system depends on its ability to carry an adequate flow and volume of the irrigant to the working length without forcing the irrigant into the periapical tissues (28, 29, 40).

Collectively, it can be concluded that chemical and mechanical debridement, together called 'chemomechanical debridement' is an essential part of the successful root canal treatment. The primary goal of the chemomechanical debridement is to eliminate microrganisms and their biofilm as well as removal of organic and inorganic debris from the root canal system (25, 26).

Recently, many irrigation devices with different mechanisms of flushing action have been introduced into the market. The Endovac® system is an apical negative pressure irrigation system. It is composed of three components: a master delivery tip (MDT), macro cannula and micro cannula. MDT delivers and evacuates the irrigant simultaneously within the root canal system. The macro cannula is made of plastic with an open end measuring 0.55 mm in diameter and 0.02 taper. It is used to suction irrigants from the coronal and middle third of the root canal. The micro cannula is made up of stainless steel with a closed end. The external diameter of the tip is 0.32mm. The micro cannula contains 12 microscopic holes of 0.1mm diameter. Unlike the macro cannula, the micro cannula is taken to the working length (WL) to aspirate irrigants and debris.

In vitro studies have demonstrated that Endovac® system provides better cleaning efficiency and smear layer removal compared to needle irrigation (19, 41). *In vivo* studies performed by Chris Siu et al demonstrated that Endovac® provides better debris removal compare to conventional needle irrigation (42). Munoz et al demonstrated that Endovac® can carry the irrigant to the working length very efficiently compared to conventional needle irrigation (43). Gondim et al compared the postoperative pain level between Endovac® and needle irrigation and concluded that the use of Endovac® resulted in a

significant reduction in postoperative pain level (44). Because of the apical negative pressure system Endovac® also reduced the risk of apical extrusion of irrigants (45, 46).

The EndoActivator® is a battery operated, cordless sonic handpiece that activates the non-cutting polymer tips to agitate the irrigating solution. Sonic devices operate at a frequency of 2-3 KHz, compared to ultrasonic devices which operate at 25-40 KHz. The activator tips are available in three sizes a) Yellow 15/02, b) Red 25/04, c) Blue 35/04. The handpiece has three activation speeds: 2000, 6000 and 10,000 cycles/min. The manufacturer recommends this device be used after the completion of the chemomechanical debridement of the root canal system. On placing irrigant into the canal and chamber, passively fitting tips are activated at 10,000 cycles/min for 30–60 seconds.

It has been reported that sonic irrigation is capable of producing clean canals (47, 48). Kanter et al compared EndoActivator® to an ultrasonic irrigation in an *in vitro* study and noticed that EndoActivator® produced significantly cleaner canals free of debris, which resulted in better obturation of lateral canals (49). An *in vitro* study by Pasqualini also reported that EndoActivator® was significantly better in reducing bacterial load compared to needle irrigation (50).

Ultrasonic irrigation of the root canal can be performed with or without simultaneous instrumentation. When ultrasonic irrigation is performed without instrumentation it is called Passive Ultrasonic Irrigation (PUI). Passive ultrasonic irrigation can be performed with a small file or smooth wire (size 10–20) oscillating freely in the root canal to induce powerful acoustic streaming (51). Weller first described PUI. Later Ahmad et al described that acoustic streaming is the mechanism of action

where ultrasonic waves are transmitted from the oscillating files to the irrigant in the root canal (52, 53). Gutarts et al histologically compared the *in vivo* debridement efficacy of hand/rotary canal preparation with that of a hand/rotary/ultrasound technique using an ultrasonic needle in a MiniEndo (Spartan EIE Inc., San Diego, CA) unit in the mesial root canals of vital mandibular molars. The authors concluded that the 1-minute use of the ultrasonic needle after hand/rotary instrumentation resulted in significantly cleaner canals and isthmi in the mesial roots of mandibular molars (54). Burleson et al confirmed that biofilm/necrotic debridement efficiency was significantly increased in the mesial roots of mandibular molars after 1 minute of ultrasonic irrigation through an irrigation needle directly connected to a MiniEndo ultrasonic unit (55). Using the same ultrasonic device, Carver et al showed that the addition of 1 minute of ultrasonic irrigation significantly reduced positive bacterial cultures (56). On the basis of these positive results, PiezoFlow™ (Dentsply Tulsa Dental Specialties, Tulsa, OK) has been introduced. PiezoFlow™ is an ultrasonic irrigation needle, which simultaneously irrigates and agitates irrigant within the root canal. The needle is connected to the ultrasonic unit. The needle also has a connecting tube, which carries the irrigant from the syringe to the needle.

Needle irrigation with a side-ported needle (ProRinse®; Dentsply International, York, PA) using positive pressure within 1–3 mm of working length is the most commonly used endodontic irrigation system (57, 58). Chow investigated the influence of the size, the depth of the insertion of the needle and the pressure of irrigation on the effectiveness of the apical portion of the root canals. He concluded that smaller diameter needles were more effective than larger diameter needles. Depth of displacement of the

irrigant from the needle tip was not great. The apical extent of the effectiveness of the irrigation is the result of depth of needle insertion (40). Instances of expressing irrigants into periapical tissues causing significant tissue damage and postoperative pain have been reported with the use of positive pressure irrigation (28, 29).

Various *in vitro* studies have been performed using the aforementioned antimicrobial irrigants and irrigation systems. These *in vitro* studies have proven efficient in removing intracanal microorganisms as well as smear layer and debris removal (59, 60, 61, 62). These studies prove that it is the synergistic action of antimicrobial irrigant as well as irrigation systems that produces cleaner, bacteria free canals. They do not answer the question; "Is the mechanism of action of an irrigation device alone effective in reducing the microbial flora of the root canal system?"

The objective of this study was to compare the flushing ability of different root canal irrigation systems, Endovac® (apical negative pressure irrigation system), EndoActivator® (sonic irrigation system), needle irrigation (positive pressure irrigation system) and PiezoFlow™ (ultrasonic irrigation system) in reducing intracanal microbial flora using saline as a root canal irrigant.

Materials and Methods

Canal Preparation

Seventy-five (n=75) extracted, single canal, maxillary and mandibular teeth with mature apices were selected. Teeth were decoronated and the root length of 15mm was kept constant. Teeth were accessed and working length (WL) was recorded. WL was determined as the point where #10 size K-file was 0.5mm short of the major diameter of the tooth. WL was confirmed with periapical radiographs. Canals were shaped using a crown-down technique with Endo Sequence, rotary nickel titanium instruments (Brasseler USA Dental Instrumentation, Savannah, GA) to a master apical file (MAF) size of #40/04. To ensure patency recapitulation was completed using #10 stainless steel hand file to WL. Final irrigation was completed using 5.25% NaOCl and 17% EDTA.

Test Specimen Preparation

Prepared teeth were mounted in micro centrifuge tubes (Seal-Rite, USA Scientific, Ocala, FL) using polyvinyl siloxane (PVS) impression material. Teeth were mounted at a consistent height in the micro centrifuge tube to create an artificial pulp chamber. Saline was used to keep the teeth hydrated and to identify leakage around the PVS impression material in the test specimen. Test specimens were kept in a mounting rack and wrapped with aluminum foil before sterilization. They were sterilized at 121°C for 35 minutes and cooled for 30 minutes in a heat sterilizer (Sterilmatic, Market Forge Industries Inc., New York). Canals were filled with physiologic saline to prevent drying during sterilization.

Culture Preparation

Streptococcus mutans was used for the study. It is a facultative Gram positive bacteria. *Streptococcus* species is a normal inhabitant of the oral cavity and endodontic infection. It is commonly found in early carious lesions as well as later stages of root canal infection. Once the strain was obtained for the experiment, it was grown on agar plates for 48 hours to evaluate any contamination. Freezer aliquots of uncontaminated *S. mutans* strain were made. Freezer aliquots were made for future use of the strain for the experiment. Multiple freezer aliquots measuring 300 microliters were prepared adding 30% glycerol and Brain Heart Infusion (BHI) media in micro centrifuge tubes. Freezer aliquots were stored in a cold storage room at -70°C.

Freezer aliquots were used to prepare an overnight culture. The aliquots were placed in an ice bucket for 10 minutes to return to a liquid state. Once in a liquid state 40 µl of bacterial culture was added into 5 ml of prepared BHI. Prepared culture suspension was kept in a 6% Oxygen jar (Anoxomat, Advanced Intruments Inc., Norwood, MA). The jar was stored in an incubator at 37°C for 24 hours.

Inoculation culture was prepared using BHI and 0.5% Sucrose. To create 0.5% concentration, 9 ml of BHI was added to 1 ml of 5% sucrose. Sucrose was added to the BHI to create a biofilm. Pilot study was performed where *S. Mutans* V403 was added to the BHI + 0.5% Sucrose in a glass test tube. Overnight growth showed a biofilm creation on walls of test tube. 30µl of overnight culture suspension was added to 3 ml of BHI + 0.5% Sucrose solution making 1:100 dilutions. Ten microliters of this inoculation culture was inoculated into each of the sterilized teeth. Inoculation culture was added only up to the canal orifice. Test specimens were not flooded with the inoculation culture. Lids of

the test specimen were closed and then placed in a 6% O2 jar. Test specimens were incubated for 72 hours in an incubator at 37°C. Every 24 hours inoculation culture was replaced with new 10µl of culture. Entire process of inoculating the test specimen was always performed under the biosafety cabinet (Purifier Class 2 Biosafety Cabinet, Labconco Corporation, Kansas City, MO).

On the day of the experiment micro centrifuge tubes which are used to make serial dilutions were sterilized at 121°C for 35 minutes. After a few pilot studies, it was decided to make four serial dilutions 1:1, 1:10, 1:100, 1:1000. Serial dilutions were made using 10% phosphate buffered solution (PBS). In the pilot studies, BHI was used to make serial dilution but due to the multiple events of contamination BHI was replaced by PBS, which did not cause contamination. The process of making serial dilutions was also performed under the biosafety cabinet.

Testing Procedures

Seventy-five (n=75) Teeth were divided in five groups. Group 1 (Endovac®)-15 teeth, Group 2 (EndoActivator®)-15 teeth, Group 3 (ProRinse® Needle)-15 teeth, Group 4 (PiezoFlow™)-15 teeth and Group 5 (Control)-15 teeth. Saline was used as an irrigant which was sterilized at 121°C for 35 minutes on the day of the experiment. To maintain irrigation consistency, a programmable precision syringe pump (PSP) (Alladin, AL 1000 - World Precision Instruments, Inc. 175 Serasota Center Blvd, Sarasota, FL) was used to deliver 7.0 ml at the precise rate of 7.0 ml/min. A portable suction pump was used for suctioning of irrigant.

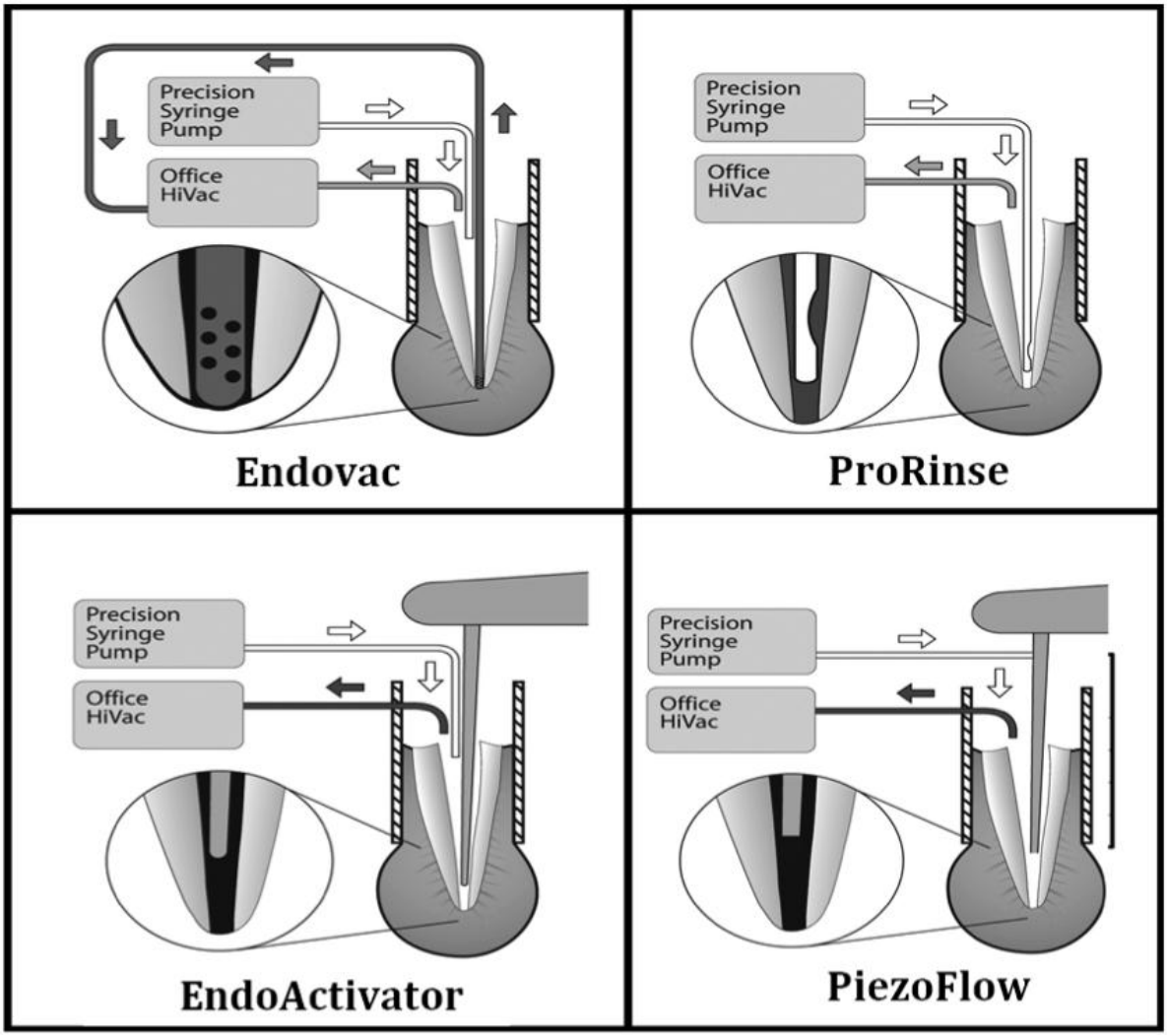

Figure 1: Testing procedure

1) Endovac® irrigation: (15 teeth)

The MDT was attached to the PSP to deliver irrigant into the artificial pulp chamber. The Macro cannula was attached to the Endovac® handpiece. Macro cannula was used according to manufacturer's instruction. Its apical advancement ended wherever the intracanal diameter prevented its further apical extension. Three and a half milliliter of saline was delivered using PSP for 30 seconds.

Micro cannula was attached to the Endovac® fingerpiece. It was taken to WL and used according to manufacturer's instructions. Three and half milliliter of saline was

delivered using PSP for 30 seconds.

2) EndoActivator® irrigation: (15 teeth)

The PSP was attached to 30-gauge irrigation needle (ProRinse®) that delivered irrigant into the pulp chamber. The EndoActivator® tip (25/04, Red) was placed within 2 mm of WL and activated at 10,000 cycles/min while moving in an up and down motion for 1 minute, according to manufacturer's instructions.

3) Needle irrigation using Prorinse®: (15 teeth)

The 30-gauge ProRinse® needle attached to the PSP was placed 2 mm short of WL, without binding and moved in an up and down motion during irrigation. 7 ml of saline was delivered for 1 minute.

4) PiezoFlow™ irrigation needle: (15 teeth)

Ultrasonic unit used was Suprasson P5 Booster (Satelec, Acteon North America, Mount Laurel, NJ, USA). PiezoFlow™ irrigation needle was secured in an ultrasonic unit and placed in the canal short of the binding point, which was mostly 4-5 mm short of the working length. PiezoFlow needle was activated for 1 minute with simultaneous continuous flow of irrigant.

5) Control: (15 teeth)

Teeth in the control group did not receive any irrigation.

After the completion of irrigation protocol, size #40/02 stainless steel K-file was used to scrap the dentin walls to remove any bacteria adhered to the canal walls. It was moved in an up and down motion for 10 times. Microbial specimen was then collected using micropipette tips (ART Gel 20P, Fisher Scientific, Houston, TX) and delivered into the prepared 1:1 serial dilution tube made for all experimental and control groups. This

process of collecting microbial specimens from tooth was performed in close proximity to the flame to avoid contamination. 1:1 dilution tubes for each experimental and control group test specimen were sonicated for 1 minute in an ultrasonic homogenizer (Ultrasonic Homogenizer 150V/T, Biologics Inc., Manassas, VA).

Agar plates (USA Scientifics, Ocala, FL) were used to study colony-forming units (CFU). Agar plates were stored in cold storage and were allowed to return to normal temperature by keeping them in an incubator at 37°C for 30 minutes. Each agar plate was divided in four sections, one for each dilution 1:1, 1:10, 1:100, and 1:1000. Twenty microliters of PBS dilutions were plated on an agar plates in triplicates (3 spots). All serial dilutions 1:1, 1:10, 1:100, 1:1000 were plated the same way on agar plates. Agar plates were kept in 6% O2 jar and were incubated for 48 hours in an incubator at 37°C. At the end of the 48 hours CFU's were counted.

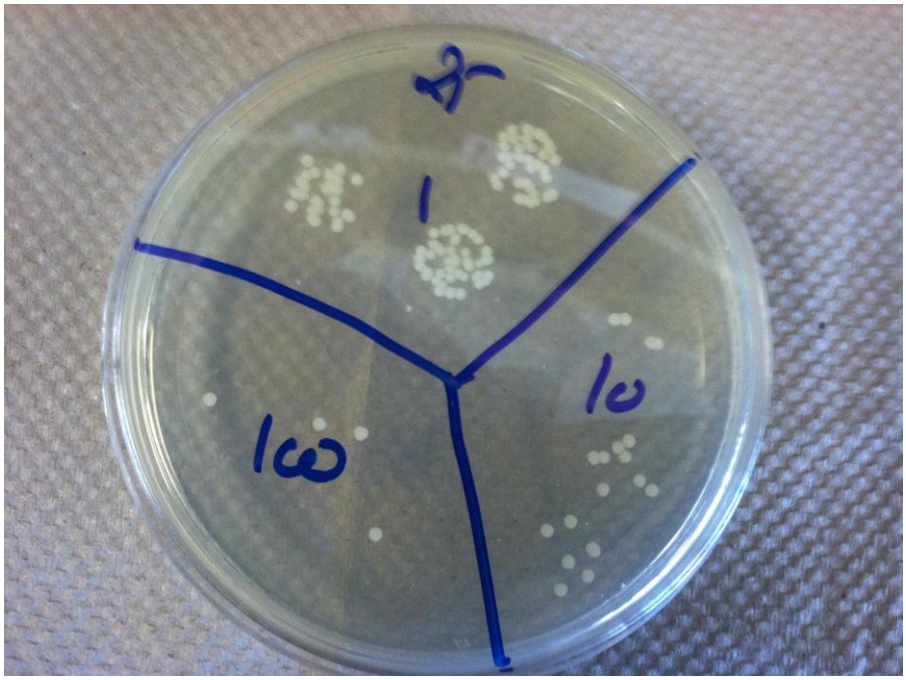

Figure 2: Plating on agar plates in triplicates

Data Collection and Analysis

A repeated-measures mixed-model ANOVA on the log-transformed values was used to compare the five groups. Tukey's HSD (honest significant difference) multiple comparison procedure was used to identify group differences ($p < 0.05$).

Results

The four experimental groups and the control were run in 15 trials, each with three replicates. The five test groups were compared using a repeated-measures analysis that took into account the relationship between the 3 spots within the 15 independent trials. Since CFU/ml is strongly skewed, the log-transformed values were used and the results back-transformed to geometric means for display. Counts of zero were analyzed as 0.5 so that the log transformation would be defined. The calculated CFU/ml values are summarized.

	CFU/ml			
Group	**N**	**Median**	**Min.**	**Max.**
Control	45	22000	11500	100000
Needle	42	4500	1000	10000
EndoActivator®	41	2500	0	5000
Endovac®	45	50	0	300
PiezoFlow™	45	50	0	200
N = 15 trials done in triplicate N < 45 due to contamination				

Table 1: CFU/ml^2 in each of the five groups

		CFU/ml			
Group	**/Group**	**Ratio**	**95% CI**		***p*-Value**
Control	**/PiezoFlow™**	600.42	350.63	1028.17	<.0001*
Control	**/Endovac®**	496.49	289.94	850.19	<.0001*
Needle	**/Piezoflow™**	93.52	54.43	160.70	<.0001*
Needle	**/Endovac®**	77.33	45.00	132.88	<.0001*
EndoActivator®	**/PiezoFlow™**	49.73	28.92	85.52	<.0001*
EndoActivator®	**/Endovac®**	41.12	23.91	70.72	<.0001*
Control	**/EndoActivator®**	12.07	7.02	20.76	<.0001*
Control	**/Needle**	6.42	3.74	11.03	<.0001*
Needle	**/EndoActivator®**	1.88	1.09	3.25	0.0152*
Endovac®	**/PiezoFlow™**	1.21	0.71	2.07	0.8588

Table 2: Tukey's HSD comparison test used to identify group differences at *p*<0.05.

Table 2 shows the ratios of the group means for statistical comparison. Since the analysis used the log-transformed values, the differences on the log-scale are back-transformed to the original scale and ratios are the result. For instance, the ratio of the geometric mean CFU/ml for the control group to the geometric mean CFU/ml for the PiezoFlow™ groups is 26781/45 = 600.42). Thus compared to the control, the PiezoFlow™ method results in a 600-fold reduction in CFU/ml (95% CI between 351

fold and 1028 fold, $p < 0.0001$). The simultaneous 95% confidence intervals on the ratios are also shown, as are the *p*-values for the statistical comparison of groups.

Table 2 also identifies that each of the four experimental groups were different from control groups except that Endovac® and PiezoFlow™ were not significantly different from one another. This is further illustrated in Table 3.

	CFU/ml			
Group	**Mean**	**95% CI**		*
Control	26780.7	20426.7	35111.2	(A)
Needle	4171.3	3170.3	5488.3	(B)
EndoActivator®	2218.1	1684.4	2921.0	(C)
Endovac®	53.9	41.1	70.7	(D)
PiezoFlow™	44.6	34.0	58.5	(D)

Table 3: ANOVA results comparing the Groups ($p<0.0001$)

Table 3 shows the geometric mean value for each of the groups. In addition to being different from the control, there was a significant difference between the four test conditions. Groups not sharing the same letters are significantly different from each other. Groups with letter A, B and C are significantly different compared to groups with letter D. Thus, PiezoFlow™ and Endovac® are significantly different compared to the control, needle and EndoActivator® groups; but there is no difference between Endovac® and PiezoFlow™ groups.

The repeated-measures mixed-model ANOVA indicated that there was a significant difference between the five groups ($p<0.0001$) and that the control group was significantly different from each of the test groups ($p<0.0001$).

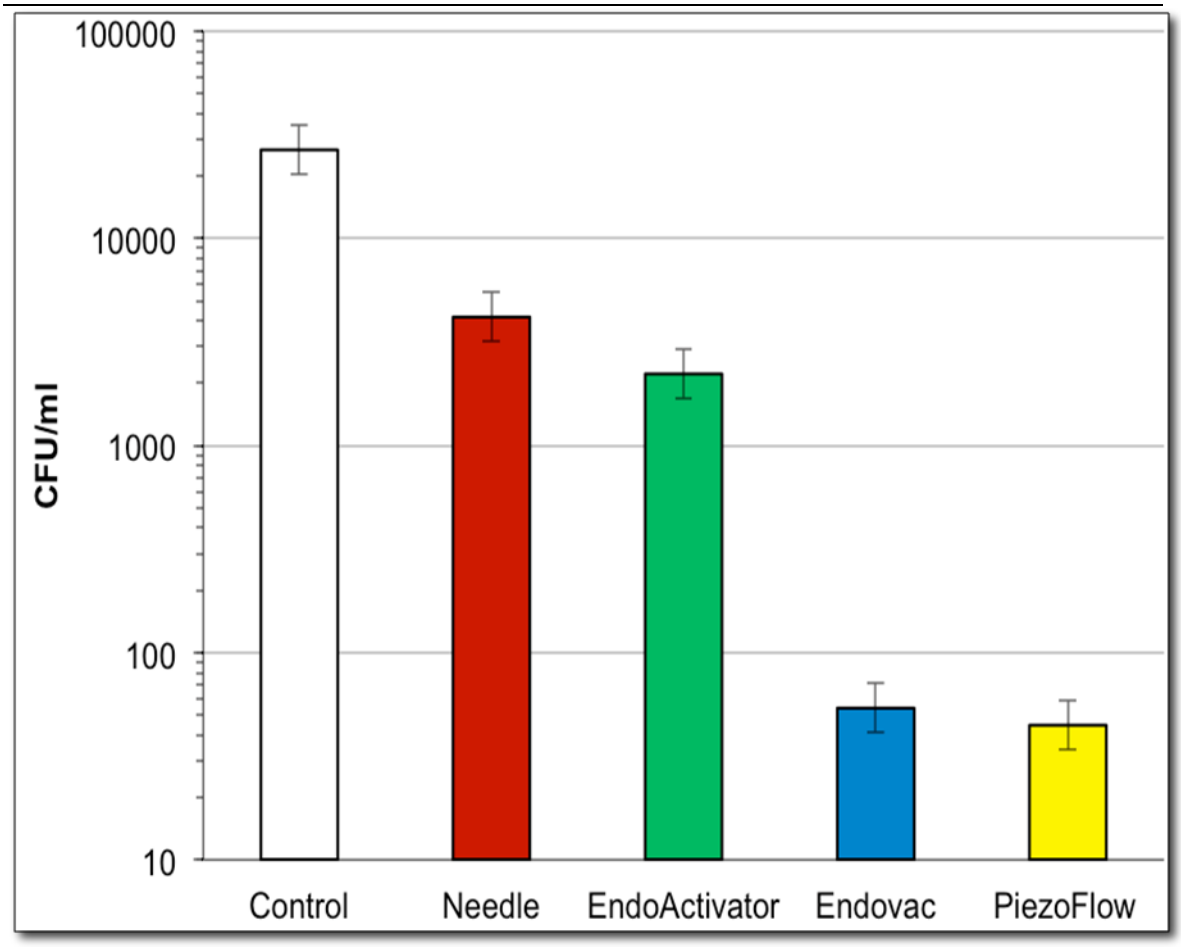

Figure 3: Geometric Mean CFU/ml per Group

There was a statistical significant difference between experimental and control groups ($p< 0.0001$). PiezoFlow™ and Endovac® reduced colony forming units significantly better compared to needle and Endoactivator® groups ($p< 0.0001$). EndoActivator® reduced significantly more microbial load compared to needle irrigation ($p= 0.0152$). PiezoFlow™ was not significantly better than Endovac® in reducing bacterial load ($p= 0.8588$).

Discussion

Various *in vitro* and *in vivo* microbial reduction studies have been performed using multiple irrigation systems along with antimicrobial solution showing excellent results. It becomes difficult to assess if the microbial reduction is the result of antimicrobial solution and/or action of irrigation device (59, 60, 61). Therefore, the purpose of this study was to evaluate the debridement efficacy of irrigation devices using saline as an irrigation solution in an effort to determine if one irrigation device was better than another in reducing microbial load.

This *in vitro* study evaluated the flushing ability of four irrigation devices Endovac®, EndoActivator®, PiezoFlow™ and traditional needle irrigation using saline as an irrigant. All techniques showed significant reduction in microbial load compared to the control group. These findings confirm the important role irrigation devices have in the elimination of microorganisms within the root canal system.

In this study canals were enlarged to size #40. According to Ram Z (64) and Chow (40) canals should be enlarged to size #40 for better penetration of irrigant into the apical third area. Shuping et al also confirmed that larger file sizes are needed for better penetration of irrigant and cleaning of canals (18). A conventional 27-gauge irrigation needle was used in this study. Enlarging the canal to size #40 allowed the penetration of 27-gauge ProRinse® needle to 2-3 mm from the working length. Moreover, apical diameter of size #40 also allowed the placement of micro cannula of the Endovac® to working length because its tip diameter is 0.32 mm.

A syringe pump was used in this study to eliminate a variable of manual irrigation. It is techniqually difficult to irrigate manually with the same rate of delivery

and pressure using syringe and needle, which can ultimately affect the debridement efficiency of an irrigation device. In this study, all the irrigation devices were used according to the manufacturer's instruction and the variables like irrigant volume and irrigation time were kept consistent.

During the pilot study the author noticed that teeth were dehydrating after initial sterilization. Dehydration ultimately caused evaporation of inoculation culture, which in turn lead to no microbial growth. To overcome this hurdle, root canals were filled with saline during sterilization. Twenty-four hours before the inoculation, saline in the root canal was replaced with BHI media.

BHI was used to make the dilutions in the pilot study. BHI unexpectedly allowed growth of airborne microorganisms contaminating the specimen. Later, BHI was replaced by phosphate buffered solution (PBS), which did not show contamination. Inoculations, dilutions and plating on agar plates were performed under the biosafety cabinet thus reducing the contamination significantly.

In this study conventional side-port needle irrigation did not remove the bacteria efficiently. Chow concluded in his study that there was not much fluid displacement beyond the tip of the needle (40). Munoz et al also studied the delivery of irrigant to the working length of the canal using contrast media comparing Endovac®, passive ultrasonic and needle irrigation. It was observed that fluid displacement occurred only up to 1.1 mm beyond the tip of the conventional needle. Endovac® and passive ultrasonic irrigation devices were able to deliver the irrigant to the working length of the root (43).

The actual volume of the irrigant reaching to the working length appears to be a major factor in canal cleanliness. Sedgley et al compared the effectiveness of irrigation

comparing the depth of needle at 1 mm and 5 mm from working length using bioluminescent bacteria. The author concluded that 6 ml of irrigant at 1 mm from working length reduced more bacteria compared to 3 ml (68). Baker et al also investigated the removal of microbial load and debris from the root canal using various root canal irrigants such as physiologic saline, sodium hypochlorite, EDTA, RC Prep, etc. The author found that there was no significant difference among any irrigant in removal of microorganisms and debris from the root canal. It was concluded that root canal irrigation depends on quantity of irrigant rather than type of irrigant used (39).

This study showed that passive ultrasonic irrigation using PiezoFlow™ reduced the number of bacteria significantly more compared to needle irrigation. This is in agreement with Ahmad, Burleson and Carver who concluded that ultrasonic debridement can produce cleaner canals compared to traditional needle irrigation (53, 55, 56). Gutarts et al also concluded that 1-minute of ultrasonic irrigation after hand and rotary instrumentation produced cleaner canals (54). Carver et al in his *in vivo* study concluded that passive ultrasonic irrigation could produce negative culture seven times more often compared to needle irrigation.

PiezoFlow™ was significantly better than EndoActivator® in this study, which was in contrast to the study by Townsend et al, who demonstrated that ultrasonic irrigation was not significantly different compared to EndoActivator®. The difference of the two studies may be attributed to the fact that the study by Townsend et al was performed on plastic blocks and not on extracted teeth. Plastic block with simulated curved canals, unlike teeth do not have tubules where bacteria can penetrate and hide. It is also difficult for bacteria to form biofilm in the plastic block. In the study herein

passive ultrasonic irrigation with simultaneous flow of irrigant into the canal was used while Townsend used a passive ultrasonic device without simultaneous irrigation. Continuous replenishment of irrigant into the canal might have reduced more microorganisms. De Gregorio et al studied the effect of ultrasonic irrigation on displacement of the irrigants into the lateral canals in an *in vitro* model. The author concluded that ultrasonic irrigation resulted in better irrigation of lateral canals in the apical third of the root canal system compared to traditional needle irrigation (67).

Sonic activation of irrigant using EndoActivator® reduced more bacteria compared to control and needle irrigation groups but significantly less compared to passive ultrasonic irrigation by PiezoFlow™ devices. This is in agreement with the study by Sabins et al who compared passive sonic and passive ultrasonic irrigation using sodium hypochlorite as an irrigant for 30 and 60 second time periods. He concluded that passive ultrasonic irrigation reduced significantly more debris compared to passive sonic irrigation at different time periods (47).

In contrast to the findings in this study, a study by Jensen et al found no difference between sonic and passive ultrasonic irrigation. Jensen et al studied the effect of passive sonic and passive ultrasonic irrigation in curved roots of molar teeth and compared it to hand instrumentation only. Jensen concluded that there was no significant difference between passive sonic and passive ultrasonic irrigation (48). Brito et al in an *in vitro* study also compared EndoActivator® to Endovac® in reducing intracanal microorganism. No significant difference was found when the two devices were compared. Both devices reduced bacterial load more than 99%. They were used along

with antimicrobial solution sodium hypochlorite in contrast to the herein study where saline was used as an irrigant.

Sonic activation reduced significantly less microbial load compared to ultrasonic activation. This may be due to the fact that the amount of energy created by sonic activation (1-8 kHz) is less compared to ultrasonic activation (25-30 kHz). Unlike a sonic device, ultrasonic energy produces acoustic streaming which in turn produces hydrodynamic stresses, which helps in disruption of bacteria and their biofilms. PiezoFlow™ also has an ability to carry the irrigant to the working length using ultrasonic activation and positive pressure irrigation. Furthermore, PiezoFlow™ ultrasonically activates the irrigant with continuous exchange of fluids in the canal unlike EndoActivator®. Therefore, more irrigation solution is reaching to the apical third of the canal. According to Sabins et al, sonic handpiece causes greater horizontal amplitude at the tip of the file compared to an ultrasonic tip, which can produce dampening effect of sonic energy hence producing less hydrodynamic forces. This may be another drawback of sonic devices and may have accounted for the significant difference in the microbial load reduction (47).

In this study sonic activation produced significantly cleaner canals compared to needle irrigation, which is in agreement with the study by Sabins et al (47). According to de Gregorio sonic activation using EndoActivator® causes more irrigant displacement into lateral canals compared to traditional needle irrigation.

There have been multiple studies performed comparing Endovac® to other irrigation systems with conflicting results. Brito et al performed an *in vitro* study comparing three irrigation techniques on the reduction of *Enterococcus faecalis* and

found that there was no statistical difference among Endovac®, EndoActivator® and needle irrigation (59). This is in contrast to the study herein.

Townsend et al performed a study similar to this study using sterile water as an irrigant and concluded that ultrasonic irrigation was better in reducing intracanal bacterial load compared to Endovac® and needle irrigation (63). The present study produced similar results i.e. Endovac® was better than needle irrigation in reducing microbial load but differed in the result when Endovac® was compared to PiezoFlow™. These differences may be due to Townsend's use of plastic blocks as test specimens instead of extracted teeth and that ultrasonic irrigation was performed only for 30 seconds, half the time used in this study.

Nielsen and Baumgartner also concluded that Endovac® was able to clean apical third and isthmus areas better than conventional needle irrigation (65). Hockett et al investigated the effect of Endovac® in tapered and non-tapered canals of molars in an *in vivo* study and concluded that it was significantly better than needle irrigation in reducing microbial load. Findings herein are in agreement with these studies.

PiezoFlow™ and Endovac® reduced significantly more CFUs compared to other groups. Both devices carry the solution to the working length, and provide continuous exchange of fluids, which may explain their superior ability to clean the canals compared to other groups. The author assumes that these devices reduced bacterial load due to physical disruption of bacteria rather than killing, since saline is a non-antimicrobial solution. Testing using Polymerase Chain Reaction (PCR) would have been beneficial to prove whether the reduction in bacterial load was just a decrease in number of live bacteria or whether bacteria were killed.

There was no significant difference between the Endovac® and PiezoFlow™. Ultrasonic energy seems not to have been a significant factor in the reduction of microbial load in these teeth with straight canals. Whether this would prove to be the same in curved canals is yet to be determined.

In this study the formation of biofilm in the canal was not controlled. Biofilm was created in the test tube when 0.5% sucrose was added to the culture of *Streptococcus mutans.* This was visually noted on inspection of culture tubes.

One of the greatest advantages of using Endovac® is that it is an apical negative pressure system, which does not cause extrusion of irrigant into the periapical area making it safe to use in canals with open apices (45). Desai and Himel collected the aspirated irrigant in a fluid recovery device and noticed that 100% of irrigant was collected when macro cannula was used and 50% was collected when micro cannula was used. They concluded that macro cannula can carry 100% of irrigant to the apical third area and micro cannula can carry 50% of irrigant to the working length without causing any extrusion (45). One of the disadvantages is that it is cumbersome to use requiring tubes and components that must be connected prior to using it. In contrast, PiezoFlow™ may be more easily assembled, but it irrigates the canal with positive pressure which increases the chances of periapical extrusion (45).

On the basis of the results of present study it can be concluded that Endovac® and PiezoFlow™ have better debridement efficiency compared to EndoActivator® and needle irrigation systems when saline is used as an irrigant. Apical negative pressure and passive ultrasonic irrigation provides better flushing ability compared to sonic and positive pressure irrigation techniques thus significantly reducing the microbial load.

References

1. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol 1965;20(3):340–349.

2. Moller AJR, Fabricius L, Dahlen G. Influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys. Scandinavian Journal of Dental Research 1981;89(6):475–480.

3. Sundqvist G. Bacteriological studies of necrotic dental pulps. University of Umea Odontology Dissertation no.7. Umea, Sweden. 1976;1-94.

4. Sundqvist G. Taxonomy, ecology, and pathogenicity of the root canal flora. Oral Surg Oral Med Oral Pathol 1994;78:522-530.

5. Figdor D, Sundqvist G. A big role for the very small understanding the endodontic microbial flora. Aust Dent J. 2007 Mar;52(1 Suppl):S38-51.

6. Ricucci D, Sequeira JF Jr. Biofilms and apical periodontitis: study of prevalence and association with clinical and histopathologic findings. J Endod 2010;36(8):1277-88.

7. Chavez de Paz LE. Redefining the persistent infection in root canals: possible role of biofilm communities. J Endod 2007;33:652–62.

8. Ricucci D, Siqueira JF Jr, Bate AL et al. Histologic investigation of root canal treated teeth with apical periodontitis: a retrospective study from twenty-four patients. J Endod 2009;35:493–502.

9. Vertucci FJ. Root canal anatomy of the human permanent teeth. Oral Surg Oral Med Oral Pathol 1984;58(5):589–599.

10. Vertucci FJ. Root canal anatomy of the mandibular anterior teeth. J Am Dent Assoc 1974;89(2):369-71.

11. Wu MK, Dummer PM, Wesselink PR. Consequences of and strategies to deal with residual post-treatment root canal infection. Int Endod J 2006 9(5):343-56.

12. Peters LB, Wesselink PR, Buijs JF, Van Winkelhoff AJ. Viable bacteria in root dentinal tubules of teeth with apical periodontitis. J Endod 2001;27(2):76-81

13. Ricucci D, Siqueira JF Jr, Bate AL. Histological investigation of root canal treated teeth with apical periodontitis: a retrospective study from twenty-four patients. J Endod 2009;35:493–502.

14. Fabricius L, Dahlen G, Sundqvist G, Happonen R-P, Moller A Jr. Influence of residual bacteria on periapical tissue healing after chemomechanical treatment and root filling of experimentally infected monkey teeth. Eur J Oral Sci 2006; 114: 278–285.

15. Sjögren U, Figdor D, Persson S, Sundqvist G. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. Int Endod J 1997;30:297–306.

16. Peters OA, Schφnenberg K, Laib A. Effects of four Ni-Ti preparation techniques on root canal geometry assessed by micro computed tomography. Int Endod J 2001;34:221-30.

17. Dalton BC, Ørstavik D, Phillips C, Pettiette M, Trope M. Bacterial reduction with nickel-titanium rotary instrumentation. J Endod 1998;24: 763–7.

18. Shuping G, Ostravik D, Sigurdsson A, Trope M. Reduction of intracanal bacteria using nickel-titanium rotary instrumentation and various medications. J Endod 2000;26:751-5.

19. Nielsen BA, Baumgartner J. Comparison of the EndoVac system to needle irrigation of root canals. J Endod 2007;33:611–5.

20. Ruddle C. Endodontic disinfection-tsunami irrigation. Endodontic Practice 2008;5:8–17.

21. 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;33:782–7

22. Senia E, Marshall F, Rosen S. The solvent action of sodium hypochlorite on pulp tissue of extracted teeth. Oral Surg 1971;31:96.

23. Rosenfeld EF, James AC, Burch BS. Vital pulp response to sodium hypochlorite. J Endod 1978;4:140-6.

24. Hand RE, Smith MI, Harrison JW. Analysis of the effect of dilution on the necrotic tissue dissolution property of sodium hypochlorite. J Endod 1978;4:60-4.

25. 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;32:434–7.

26. Dunavant TR, Regan JD, Glickman GN, Solomon ES, Honeyman AL. Comparative evaluation of endodontic irrigants against *Enterococcus faecalis* biofilms. J Endod 2006;32:527–31.

27. Rôcas IN, Siqueira JE. Comparison of the *in vivo* antimicrobial effectiveness of sodium hypochlorite and chlorhexidine used as a root canal irrigants. A molecular microbial study. J Endod 2011,37:143-150.

28. Kleier DJ, Averbach RE, Mehdipour O. The sodium hypochlorite accident: experience of diplomates of the American Board of Endodontics. J Endod 2008;34(11):1346-50.

29. Mehdipour O, Kleier DJ, Averbach RE. Anatomy of sodium hypochlorite accidents. Compend Contin Educ Dent 2007;28:544–6,548,550.

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

31. 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;21:592–593.

32. 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.

33. Jeansonne M, White RR. A comparison of 2.0% chlorhexidine gluconate and 5.25% sodium hypochlorite as antimicrobial endodontic irrigants. J Endod 1994;20:276–8.

34. Ercan E, Ozekinci T, Atakul F, Gul K. Antibacterial activity of 2% chlorhexidine gluconate and 5.25% sodium hypochlorite in infected root canal: *in vivo* study. J Endod 2004;30:84–87

35. White RR, Hays GL, Janer LR. Residual antimicrobial activity after canal irrigation with chlorhexidine. J Endod 1997;23:229–31.

36. Haapasalo M, Shen Y, Qien W, Gao Y. Irrigation in Endodontics. Dent Clin North Am. 2010;54(2):291-312.

37. Khademi AA, Mohammadi Z, Havaee A. Evaluation of the antibacterial substantivity of several intra-canal agents. Australian Endodontic Journal 2006;32:112–115

38. Torabinejad M, Khademi AA, Babagoli J et al. A new solution for the removal of the smear layer. J Endod 2003; 29:170–5.

39. Baker NA, Eleazer PD, Averbach RE, Seltzer S. Scanning electron microscopic study of the efficacy of various irrigation solutions. J Endod 1975;4:127-35.

40. Chow TW. Mechanical effectiveness of root canal irrigation. J Endod 1983;9:475–479.

41. Abarajithan M, Dham S, Velmurugan M, Valerian-Albuquerque D, Ballal S, Senthikumar H. Comparison of Endovac irrigation system with conventional irrigation for removal of intracanal smear layer: an *in vitro* study. Oral Surg Oral Med Pral Pthol Oral Radiol Endod 2011;112(3):407-11

42. 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.

43. Munoz HR, Camacho-Caudra K. *In vivo* efficacy of three different endodontic irrigation systems for irrigant delivery to working length of mesial canals of mandibular molars. J Endod 2012 Article in press.

44. Gondim E Jr, Setzer FC, Dos Carmo CB, Kim S. Postoperative pain after the application of two different irrigation devices in a prospective randomized clinical trial. J Endod 2010;36:1295-301.

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

46. Mitchell RP, Yang SE, Baumgartner JC. Comparison of apical extrusion of NaOCl using the Endovac or needle irrigation of root canals. J Endod 2010;36:338–41.

47. 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.

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

49. Kanter V, Weldon E, Nair U, Varella C, Kanter K, Anusavice K, Pileggi R. A quantitative and qualitative analysis of ultrasonic versus sonic endodontic systems on canal cleanliness and obturation. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;112(6):809-13.

50. Pasqualini D, Cuffini AM, Scotti N, Mandras N, Scalas D, Pera F, Berutti E. Comparative evaluation of the antimicrobial efficacy of a 5% sodium hypochlorite subsonic-activated solution. J Endod 2010;36(8):1358-60.

51. Van der Sluis LW, Versluis M, Wu MK, Wesselink PR. Passive ultrasonic irrigation of the root canal: a review of the literature. Int Endod J 2007;40(6):415-26.

52. Weller RN, Brady JM, Bernier WE. Efficacy of ultrasonic cleaning. J Endod 1980;6(9):740-3.

53. Ahmad M, Pitt Ford TJ, Crum LA. Ultrasonic debridement of root canals: acoustic streaming and its possible role. J Endod 1987;13(10):490-9.

54. 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(3):166-70.

55. 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;33(7):782-7.

56. Carver K, Nusstein J, Reader A, Beck M. *In vivo* antibacterial efficacy of ultrasound after hand and rotary instrumentation in human mandibular molars. J Endod 2007;33(9):1038-43.

57. 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(3):323-8

58. Vinothkumar TS, Kavitha S, Lakshminarayanan L, Gomathi NS, Kumar V. Influence of irrigating needle-tip designs in removing bacteria inoculated into instrumented root canals measured using single-tube luminometer. J Endod 2007;33(6):746-8.

59. 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;35(10):1422-7.

60. Howard RK, Kirkpatrick TC, Rutledge RE, Yaccino JM. Comparison of debris removal with three different irrigation techniques. J Endod 2011;37(9):1301-5.

61. 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(12):1983-7.

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

63. 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;35:1040–1043.

64. Ram Z. Effectiveness of root canal irrigation. Oral Surg Oral Med Oral Pathol 1977;44(2):306-12.

65. Nielsen BA, Baumgartner JC. Comparison of the Endovac system to needle irrigation of root canals. J Endod 2007;33:611–5.

66. HockettJL, DommischJK, JohnsonJD, CohencaN. Antimicrobial efficacy of two irrigation techniques in tapered and nontapered canal preparations: an *in vitro* study. J Endod 2008;34:1374–7.

67. De Gregorio C, Estevez R, Cisneros R, Heilborn C, Cohenca N. 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.

68. Sedgley CM, Nagel AC, Hall D, Applegate B. Influence of irrigant needle depth in removing bioluminescent bacteria inoculated into instrumented root canals using real-time imaging in vitro. Int Endod J 2005;38:97–104.

Appendix

Table 4: Raw Data Collection

Sample	Diln	Spot 1	Spot 2	Spot 3	GeoMean	Diln	Cfu/ml (= total CFU recovered)
Control							
1	1x 10-fold 100-fold 1000-fold	30	36	45	3.65E+01	2.00E-02 2.00E-03 2.00E-04 2.00E-05	**1.82E+04**
2	1x 10-fold 100-fold 1000-fold	24	40	34	3.20E+01	2.00E-02 2.00E-03 2.00E-04 2.00E-05	**1.60E+04**
3	1x 10-fold 100-fold 1000-fold	9	8	7	7.96E+00	2.00E-02 2.00E-03 2.00E-04 2.00E-05	**3.98E+04**
4	1x 10-fold 100-fold 1000-fold	48	36	44	4.24E+01	2.00E-02 2.00E-03 2.00E-04 2.00E-05	**2.12E+04**
5	1x 10-fold 100-fold 1000-fold	10	9	7	8.57E+00	2.00E-02 2.00E-03 2.00E-04 2.00E-05	**4.29E+04**
6	1x 10-fold 100-fold 1000-fold	7	6	4	5.52E+00	2.00E-02 2.00E-03 2.00E-04 2.00E-05	**2.76E+04**
7	1x 10-fold 100-fold 1000-fold	8	8	9	8.32E+00	2.00E-02 2.00E-03 2.00E-04 2.00E-05	**4.16E+04**
8							

	1x					2.00E-02	
	10-fold					2.00E-03	
	100-fold	16	12	10	1.24E+01	2.00E-04	**6.21E+04**
	1000-fold					2.00E-05	
9							
	1x					2.00E-02	
	10-fold	28	23	40	2.95E+01	2.00E-03	**1.48E+04**
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
10							
	1x					2.00E-02	
	10-fold					2.00E-03	
	100-fold	10	8	7	8.24E+00	2.00E-04	**4.12E+04**
	1000-fold					2.00E-05	
11							
	1x					2.00E-02	
	10-fold	32	41	35	3.58E+01	2.00E-03	**1.79E+04**
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
12							
	1x					2.00E-02	
	10-fold	42	30	33	3.46E+01	2.00E-03	**1.73E+04**
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
13							
	1x					2.00E-02	
	10-fold	29	38	33	3.31E+01	2.00E-03	**1.66E+04**
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
14							
	1x					2.00E-02	
	10-fold					2.00E-03	
	100-fold	12	20	11	1.38E+01	2.00E-04	**6.91E+04**
	1000-fold					2.00E-05	
15							
	1x					2.00E-02	
	10-fold	29	32	36	3.22E+01	2.00E-03	**1.61E+04**
	100-fold					2.00E-04	
	1000-fold					2.00E-05	

Needle

1	1x 10-fold 100-fold 1000-fold	8	6	3	5.24E+00	2.00E-02 2.00E-03 2.00E-04 2.00E-05	**2.62E+03**
2	1x 10-fold 100-fold 1000-fold	4	5	7	5.19E+00	2.00E-02 2.00E-03 2.00E-04 2.00E-05	**2.60E+03**
3	1x 10-fold 100-fold 1000-fold	10	4	C	6.32E+00	2.00E-02 2.00E-03 2.00E-04 2.00E-05	**3.16E+03**
4	1x 10-fold 100-fold 1000-fold	14	11	13	1.26E+01	2.00E-02 2.00E-03 2.00E-04 2.00E-05	**6.30E+03**
5	1x 10-fold 100-fold 1000-fold	C	C	20	2.00E+01	2.00E-02 2.00E-03 2.00E-04 2.00E-05	**1.00E+04**
6	1x 10-fold 100-fold 1000-fold	16	18	11	1.47E+01	2.00E-02 2.00E-03 2.00E-04 2.00E-05	**7.34E+03**
7	1x 10-fold 100-fold 1000-fold	1	1	1	1.00E+00	2.00E-02 2.00E-03 2.00E-04 2.00E-05	**5.00E+03**
8	1x 10-fold 100-fold 1000-fold	12	12	11	1.17E+01	2.00E-02 2.00E-03 2.00E-04 2.00E-05	**5.83E+03**
9	1x 10-fold	11	13	8	1.05E+01	2.00E-02 2.00E-03	**5.23E+03**

	100-fold					2.00E-04	
	1000-fold					2.00E-05	
10							
	1x					2.00E-02	
	10-fold	9	8	7	7.96E+00	2.00E-03	**3.98E+03**
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
11							
	1x					2.00E-02	
	10-fold	15	11	15	1.35E+01	2.00E-03	**6.76E+03**
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
12							
	1x					2.00E-02	
	10-fold	8	9	4	6.60E+00	2.00E-03	**3.30E+03**
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
13							
	1x					2.00E-02	
	10-fold	6	4	6	5.24E+00	2.00E-03	**2.62E+03**
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
14							
	1x					2.00E-02	
	10-fold	7	6	8	6.95E+00	2.00E-03	**3.48E+03**
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
15							
	1x					2.00E-02	
	10-fold	9	4	2	4.16E+00	2.00E-03	**2.08E+03**
	100-fold					2.00E-04	
	1000-fold					2.00E-05	

Endovac

1							
	1x	3	2	1	1.82E+00	2.00E-02	**9.09E+01**
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
2							
	1x	1	2	1	1.26E+00	2.00E-02	**6.30E+01**
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	

3							
	1x	4	3	6	4.16E+00	2.00E-02	**2.08E+02**
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
4							
	1x	2	1	1	1.26E+00	2.00E-02	**6.30E+01**
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
5							
	1x	1	1		1.00E+00	2.00E-02	**5.00E+01**
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
6							
	1x	2	2		2.00E+00	2.00E-02	**1.00E+02**
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
7							
	1x	2	3		2.45E+00	2.00E-02	**1.22E+02**
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
8							
	1x	1	1		1.00E+00	2.00E-02	**5.00E+01**
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
9							
	1x	1			1.00E+00	2.00E-02	**5.00E+01**
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
10							
	1x					2.00E-02	
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
11							
	1x					2.00E-02	
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	

12							
	1x	2	1		1.41E+00	2.00E-02	**7.07E+01**
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
13							
	1x	1	3		1.73E+00	2.00E-02	**8.66E+01**
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
14							
	1x	1	1		1.00E+00	2.00E-02	**5.00E+01**
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
15							
	1x	2	2	1	1.59E+00	2.00E-02	**7.94E+01**
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	

EndoActivator

1							
	1x					2.00E-02	
	10-fold	3	4	6	4.16E+00	2.00E-03	**2.08E+03**
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
2							
	1x	22	18	12	1.68E+01	2.00E-02	**8.41E+02**
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
3							
	1x					2.00E-02	
	10-fold	8	9	9	8.65E+00	2.00E-03	**4.33E+03**
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
4							
	1x					2.00E-02	
	10-fold	3	9	8	6.00E+00	2.00E-03	**3.00E+03**
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
5							
	1x	18	15	18	1.69E+01	2.00E-02	**8.47E+02**
	10-fold					2.00E-03	

	100-fold					2.00E-04	
	1000-fold					2.00E-05	
6							
	1x					2.00E-02	
	10-fold	8	3	3	4.16E+00	2.00E-03	**2.08E+03**
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
7							
	1x					2.00E-02	
	10-fold	5	9	C	6.71E+00	2.00E-03	**3.35E+03**
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
8							
	1x					2.00E-02	
	10-fold	4	4		4.00E+00	2.00E-03	**2.00E+03**
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
9							
	1x					2.00E-02	
	10-fold	7	7	8	7.32E+00	2.00E-03	**3.66E+03**
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
10							
	1x					2.00E-02	
	10-fold	8	6	9	7.56E+00	2.00E-03	**3.78E+03**
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
11							
	1x					2.00E-02	
	10-fold	9	9	4	6.87E+00	2.00E-03	**3.43E+03**
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
12							
	1x					2.00E-02	
	10-fold	4	4	8	5.04E+00	2.00E-03	**2.52E+03**
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
13							
	1x					2.00E-02	
	10-fold	C	C	10	1.00E+01	2.00E-03	**5.00E+03**
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
14							
	1x	12	14	18	1.45E+01	2.00E-02	**7.23E+02**
	10-fold					2.00E-03	

	100-fold					2.00E-04	
	1000-fold					2.00E-05	
15							
	1x					2.00E-02	
	10-fold	C	8	7	7.48E+00	2.00E-03	**3.74E+03**
	100-fold					2.00E-04	
	1000-fold					2.00E-05	

PiezoFlow

1	1x	1	1		1.00E+00	2.00E-02	**5.00E+01**
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
2							
	1x	2	2	2	2.00E+00	2.00E-02	**1.00E+02**
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
3							
	1x	1	2		1.41E+00	2.00E-02	**7.07E+01**
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
4							
	1x	1			1.00E+00	2.00E-02	**5.00E+01**
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
5							
	1x					2.00E-02	
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
6							
	1x	1	2		1.41E+00	2.00E-02	**7.07E+01**
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
7							
	1x	4	1	1	1.59E+00	2.00E-02	**7.94E+01**
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
8							

	1x	1			1.00E+00	2.00E-02	**5.00E+01**
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
9							
	1x					2.00E-02	
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
10							
	1x	2	1	1	1.26E+00	2.00E-02	**6.30E+01**
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
11							
	1x	1		1	1.00E+00	2.00E-02	**5.00E+01**
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
12							
	1x	1	1	2	1.26E+00	2.00E-02	**6.30E+01**
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
13							
	1x	1	1	1	1.00E+00	2.00E-02	**5.00E+01**
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
14							
	1x	1		3	1.73E+00	2.00E-02	**8.66E+01**
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	
15							
	1x					2.00E-02	
	10-fold					2.00E-03	
	100-fold					2.00E-04	
	1000-fold					2.00E-05	

Vita

Dr. Pranav Desai was born on November 25, 1978 in India. He is currently a citizen of the United States of America. Dr. Desai received a Bachelor of Dental Surgery from VS Dental College in India in 2002 followed by a Doctor of Dental Surgery from the University of Colorado, College of Dentistry in 2006. Dr. Desai practiced general dentistry for three years prior to enrolling in the Advanced Specialty Program in Endodontics at Virginia Commonwealth University School of Dentistry. Dr. Desai is a member of the AAE, ADA, and Virginia Dental Association. Dr. Desai will enter private practice upon graduation. He will graduate from VCU with a Master of Science in Dentistry and a Certificate in Endodontics.