A Comparison of the Effectiveness of Three Irrigation Methods in the Removal of Bacteria from Root Canals Following Instrumentation

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ABSTRACT

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(Under the direction of Dr. Eric M. Rivera)

The purpose of this *in vivo* study was to investigate the antimicrobial effectiveness of 6% sodium hypochlorite, when used with the conventional irrigation technique using a 30 gauge side-vented needle, the EndoVac™ System, and the ProUltra PiezoFlow™ Ultrasonic Irrigation Needle. It was hypothesized that both the EndoVac™ system and the PiezoFlow™ Ultrasonic Irrigation Needle would remove bacteria from root canals more effectively than irrigation using a 30 gauge side-vented needle. After obtaining informed consent from each subject, teeth were randomly divided into three treatment groups (n=30), and samples were collected following instrumentation and irrigation using one of the three irrigation techniques. The samples were cultured under aerobic and anaerobic conditions, and bacteria growth was measured by direct counting of colonies and grid specific calculations. **Conclusions:** The data indicated that the 30 gauge side-vented needle and the EndoVac™ System removed significantly more bacteria than the ProUltra PiezoFlow™ Ultrasonic Irrigation Needle.
Acknowledgements

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# Table of Contents

List of Tables..................................................................................................................vii

List of Figures..................................................................................................................viii

List of Abbreviations.......................................................................................................ix

Introduction.....................................................................................................................1

- Root Canal Disinfection..............................................................................................1
- Instrumentation and Irrigation.....................................................................................2
- Irrigation Concentration.............................................................................................3
- Irrigation Efficacy........................................................................................................3
- The EndoVac\textsuperscript{TM} System............................................................................4
- The ProUltra PiezoFlow\textsuperscript{TM} Ultrasonic Irrigation Needle.................................5

Materials and Methods.................................................................................................7

- Subject Recruitment and Qualification........................................................................7
- Inclusion criteria..........................................................................................................7
- Exclusion criteria.........................................................................................................8
- Treatment Group Assignment....................................................................................8
- Bacteria Sampling.......................................................................................................9
- Initial Sample (S1)......................................................................................................10
- Instrumentation..........................................................................................................10
- 0.04 Tapered Canal Preparation..................................................................................11
- Irrigation....................................................................................................................11
List of Tables

Table

1. Descriptive statistics for demographic characteristics and jaw location of molars included in the sample…………………………………………………………25

2. Mean and standard deviation of bacteria count log10 CFU by group………………..26

3. Type III Sum of Squares results for the main effects……………………………………27

4. Estimates of the marginal means for irrigation method and sampling time………..28
List of Figures

Figures

1. EndoVac™ System macrocannula……………………………………………………29

2. EndoVac™ System microcannula……………………………………………….30

3. 30-gauge side-vented needle and syringe…………………………………….31

4. ProUltra PiezoFlow™ Ultrasonic Irrigation Needle with syringe and ultrasonic attachment………………………………………………….32

5. Wire cutters………………………………………………………………………..33

6. Mean bacteria count log 10 CFU by group at each time point……………….34
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>ASA</td>
<td>American Society of Anesthesiologists</td>
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<td>CFU</td>
<td>Colony Forming Units</td>
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<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
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<tr>
<td>HIPPA</td>
<td>Health Insurance Portability and Accountability Act</td>
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<tr>
<td>IRM</td>
<td>Intermediate Restorative Material</td>
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<tr>
<td>ISO</td>
<td>International Standardization Organization</td>
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<td>LDT</td>
<td>Liquid Dental Transport Medium</td>
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INTRODUCTION

Successful endodontic therapy is based on the removal of all diseased pulp tissue, dentinal debris, bacteria and endotoxins from the root canal (1). Toxic metabolites and byproducts released from microorganisms present within the root canal system diffuse into the periapical tissues and elicit an inflammatory response, which is often accompanied with bone resorption (2-3). Miller (4) in 1890 was the first to observe the presence of bacteria in necrotic human pulps and in dentinal tubules of carious and noncarious dentin. Sjogren et al. (5) demonstrated that endodontic success was associated with a higher likelihood of obtaining negative bacterial cultures prior to root canal filling. In Sjogren’s study, periapical changes were observed for a 5-year period, and resulted in healing in 94% of those cases with negative cultures and in 68% of cases with a positive culture prior to root canal filling.

Root Canal Disinfection

Proper cleaning of the root canal system involves both chemical and mechanical methods. Mechanical instrumentation involves establishing a specific cavity form which permits instruments and irrigants easy access to the root canal space. This creates a tapered canal shape which facilitates both the final irrigation sequence and the subsequent obturation (6). Effective irrigants act as bactericidal agents, tissue solvents, lubricants, and remove organic and inorganic debris. Bystrom et al. (7) established that mechanical instrumentation of the root canal using saline irrigation alone frequently leaves cultivable bacteria in the canal system. Thus, disinfectants such as sodium hypochlorite are essential (8).
The ability of an irrigant to be distributed in the apical portion of a canal is dependent on canal anatomy, size and taper of mechanical instrumentation and the delivery system used. The presence of complicated anatomy, such as apical deltas and narrow isthmuses, makes the apical portion of the root canal difficult to clean with mechanical instrumentation methods alone (9,10,11). These areas can only be disinfected and reached by the irrigant entering the canal through either a notched or a side vented needle. The ability of the solution to debride the canal is dependent on the depth of needle placement and volume of irrigation used as demonstrated by Abou-Rass et al. (12). Traditionally, the tip of the needle is placed 2-3mm short of the apical end of the canal and the irrigant is saliently expressed for effective cleaning of the entire length of the canal (12,13). The risk of extruding the solution increases if the needle binds in the root canal or if the irrigant is forcibly expressed (14). Extrusion of an irrigant such as sodium hypochlorite can result in severe periapical tissue damage and post-operative pain (15-18). Since the goal of endodontic treatment is to clean, debride and disinfect the root canal system and dentinal tubules prior to canal obturation, establishing the optimal concentration, temperature, exposure time and volume of the irrigant is necessary to achieve disinfection and tissue dissolution while minimizing toxicity to the periapical tissues.

**Instrumentation and Irrigation**

The flushing action of the irrigant solution may be more important during the cleaning process than the ability of the irrigant solution to dissolve tissue (19). This flushing action is directly related to the shape and size of the root canal. Thus, narrow canals compromise the efficacy of irrigation and may need to be enlarged and their taper increased to allow effective irrigation (20-23). Van der Sluis et al. (24) showed that ultrasonic irrigation has a tendency to remove
more artificially placed dentin debris from a more tapered root canal. However, Zandbiglari et al. (25) reported that roots were significantly weakened when prepared with greater taper instruments. It is important to know the minimum diameter and/or taper that a root canal should be prepared to permit effective irrigation, and what diameter and/or taper reduces the effectiveness of the irrigation (26,27).

**Irrigation Concentration**

There is no true consensus as to the recommended concentration of sodium hypochlorite for endodontic use. Bystrom et al. (7,28) advocated the use of 5% sodium hypochlorite while Baumgartner et al. (29) claimed that 1% sodium hypochlorite completely removed pulpal remnants. Baumgartner et al. (29) used scanning electron microscopy to examine instrumented and non-instrumented surfaces of root canals irrigated with sodium hypochlorite at concentrations of 5.25%, 2.5%, 1%, and 0.5%. The 0.5% concentration resulted left more pulpal remnants and predentin debris than higher concentrations. The study also suggested that large volumes of irrigant, regardless of the concentration, will effectively remove superficial debris and replenish the chemical activity of sodium hypochlorite. After completion of in vitro cell studies and animal models, it was proposed by Pashley et al. (30) that higher concentrations of sodium hypochlorite must be used passively to prevent its extrusion into the periradicular tissues resulting in a severe inflammatory tissue reaction.

**Irrigation Efficacy**

Currently there are few studies which address other factors affecting irrigation efficacy such as temperature, volume and exposure time. It has been proposed by Cunningham et al. (31,32)
that increased temperature enhances the collagen-dissolving ability and bactericidal action of sodium hypochlorite. Thus, 2.6% sodium hypochlorite provided faster disinfection at body temperature (37°C) than at room temperature (22°C). The efficacy of apical irrigation is directly related to the depth of insertion of the needle, as demonstrated by Chow (14,33). The efficacy of irrigation is also dependent on the gauge of the needle and the degree of taper of the canal preparation (13).

The EndoVac™ System

The intracanal aspiration technique has been developed to avoid the effects of irrigant extrusion. Fukumoto et al. (34) showed that there are significant differences in the cleaning effect in the root canal system using the EndoVac™ (Figure 1 and 2) intracanal aspiration technique in comparison to conventional application of the irrigant. The use of intracanal aspiration has also been shown to diminish the ratio of apical extrusion of the irrigant (34). The EndoVac™ is an irrigation system that has been reported to have the ability to prevent extrusion of the irrigation solution as well as clean the entire root canal system. The irrigation needle is inserted to working length and connected to the EndoVac™ suction device, which creates a negative pressure to aspirate the irrigation solution at the apex. This creates a steady flow of irrigation solution through the entire root canal, which allows the irrigant to debride and disinfect especially in the last millimeter of the root canal without extrusion.

Previous EndoVac™ System studies have proven its effectiveness as an irrigation system for root canal therapy. Nielsen and Baumgartner (35) demonstrated that the EndoVac™ Irrigation system left the root canal complex with significantly less debris and delivered significantly
more irrigant at 1mm from the apex when compared to a 30 gauge irrigation needle (Figure 3). Siu and Baumgartner (36) reported in an in vivo study that irrigation using the EndoVac™ System resulted in significantly less debris at 1mm from the working length compared to the conventional needle irrigation. Mitchell et al. (37) reported a significantly less extrusion risk with irrigation using the EndoVac™ System compared with needle irrigation.

The **ProUltra PiezoFlow™ Ultrasonic Irrigation Needle**

Intracanal ultrasonic irrigation has been developed with the purpose of producing cleaner, bacteria free and debris free root canals and isthmuses. The ProUltra PiezoFlow™ Ultrasonic Irrigation Needle (Figure 4) has been designed to provide ultrasonic irrigation in the prepared root canal. Carver et al. (38) stated that the addition of ultrasonic irrigation after hand and rotary cleaning and shaping of the root canal significantly reduced CFU counts and was seven times more likely to yield a negative culture than hand and rotary instrumentation alone. Burleson et al. (39) indicated that a one minute use of ultrasonically activated irrigant following hand/rotary root canal cleaning and shaping has been shown to improve canal and isthmus cleanliness in terms of necrotic debris/biofilm removal.

Ultrasonic irrigation has proven to be an effective method of rendering the canal bacteria-free. In a study completed by Pafford et al. (40), it was stated that the addition of one minute of ultrasonic irrigation following hand/rotary instrumentation is a safe procedure when used in the root canals of teeth with vital and necrotic pulps. Gutarts et al. (41) stated that although the needle was not placed to the complete depth of the preparation, the high energy generated by
the ultrasound unit and use of sodium hypochlorite resulted in statistically cleaner canals and isthmuses.

The aim of this study was to investigate the effectiveness of three irrigation techniques in removing bacteria present in the root canal at various time points. Sodium hypochlorite was used as the irrigant in evaluating 1) the conventional irrigation technique using a 30 gauge side-vented needle, 2) irrigation using the EndoVac™ system, and 3) irrigation using the ProUltra PiezoFlow™ Ultrasonic Irrigation Needle. It was hypothesized that both the EndoVac™ system and the ProUltra PiezoFlow™ Ultrasonic Irrigation Needle would provide greater antimicrobial efficacy than the conventional irrigation technique with a 30 gauge side-vented needle. The null hypothesis was that there is no difference in the antimicrobial effectiveness of the conventional irrigation technique, the EndoVac™ system, and the ProUltra PiezoFlow™ Ultrasonic Irrigation Needle. Since it has been proven that the success of root canal therapy is related to the reduction of bacteria in the pulp canal space, then it is important to find the most effective method to eliminate these bacteria.
Materials and Methods

30 canals from teeth with a diagnosis of necrotic pulp with chronic apical periodontitis as verified radiographically were sampled for cultivable bacteria in this study. The teeth selected for sampling will be maxillary and mandibular molars. One tooth from each patient will be sampled, with 10 teeth assigned to each treatment group for a total sample size of 30. This sample size per group is based on previous studies completed in the Department of Endodontics at UNC School of Dentistry (26,27,42).

Subject Recruitment and Qualification

Thirty patients (older than 14 years old) presenting to the University of North Carolina School of Dentistry Graduate Endodontic Clinic for evaluation and treatment of necrotic pulps and apical periodontitis as verified radiographically were enrolled in this study. The primary investigator conducted all clinical and sampling procedures. The nature of the study, complications and associated risks were fully explained to the patients or patients’ guardians and written consent was obtained prior to treatment. Only the primary investigator had access to the linkage file that matched the subject study identification number to his or her clinical or HIPPA information. This study was approved by the UNC Institutional Review Board.

Inclusion criteria for this study were:

1. Radiographic evidence of a periapical radiolucent lesion associated with the tooth to be treated (any gender, ethnicity, race or age)
2. Necrotic pulp as indicated by thermal or electric pulp testing.
3. No history of previous endodontic treatment of the tooth.
4. Healthy patients (ASA I, ASA II)
5. Consent to participate in this study
6. Compliance with the treatment schedule

Exclusion criteria was:

1. Teeth with unfavorable conditions for rubber-dam application.
3. Immature teeth with open apices.
4. Patients younger than 14 years old.
5. Medically compromised patients with conditions that are contraindicated to the dental treatment (ASA III).
6. Severely curved canals in which apical instrumentation is not predictable.

Treatment Group Assignment
The thirty enrolled subjects were randomly assigned to group 1: 0.04 tapered preparation with conventional irrigation technique (control group), group 2: 0.04 tapered canal preparation technique with EndoVac™ irrigation system, or group 3: 0.04 tapered canal preparation with ProUltra PiezoFlow™ Irrigation Needle. Randomization was performed before the clinical examination using the “minimization method” as described by Pocock (43). Two randomization factors were considered: tooth jaw location and the size of the periapical lesion.
A total of 30 eligible teeth were evaluated with 10 teeth in each group. Upon the completion of root canal instrumentation at the end of the first appointment, all teeth were dressed with calcium hydroxide mixed with 2% chlorhexidine for at least one week.

**Bacteria Sampling**

Each tooth was isolated using a rubber dam and disinfected with 30% hydrogen peroxide (Professional Compounding Centers of America, Houston, TX) until no further bubbling of the hydrogen peroxide occurred. If difficulty occurred in attaining a bubble-free state, Oraseal Putty (Ultradent Products Inc., South Jordan, UT) was placed around the neck of the tooth and the process repeated. The entire tooth surface was then coated with 2% chlorhexidine solution (Central Compounding Center, Durham, NC) and allowed to dry. Gross caries removal and initial access form was accomplished with sterile high speed and low speed burs. The rubber dam and surrounding tooth structure was disinfected with 2% chlorhexidine (Central Compounding Center, Durham, NC) before completing the access with another sterile bur. After access was achieved, cases were randomly selected for sterility testing of the operating field. To assess the efficacy of the disinfection procedure, a sterile cotton pellet was moistened in 5% sodium thiosulfate solution (Central Compounding Center, Durham, NC) and used to swab the access cavity. It was then transferred to a vial containing 1mL of Liquid Dental Transport Medium (LDT) (Anaerobe Systems, Morgan Hill, CA) and sampled for bacterial growth. Sterile saline was used to flush debris from the chamber.
Initial Sample (S1)

Bacteria samples were collected from the canals. Sterile K3 G-pack files (SybronEndo, Orange, CA) orifice openers (sizes 25.12, 25.10, 25.08, 25.06, and 25.04) were used in sequence to open the orifice and initiate access into the canals. Sterile saline was again used to flush any debris from the chamber. The chamber was dried with sterile cotton pellets and/or paper points before placement of Liquid Dental Transport media (LDT) (Anaerobic Systems, Morgan Hill, CA) into the canals with a sterile tuberculin syringe (Becton Dickinson and Company, Franklin Lakes, NJ). The canals were instrumented to size ISO #20 with sterile stainless steel K-file (Kerr, Romulus, MI) to the estimated working length. Once each instrument was removed from the canal, the fluted part of the file was cut off with a sterile wire cutter (Figure 5) (Orthopli Corporation, Philadelphia, PA) and allowed to fall into the opened bottle of LDT media. The LDT media remaining in the canal was soaked up and transferred to the LDT bottle with sterile XX-fine paper points (Mynol, Block Drug Corp., Jersey City, NJ) that were measured and placed as close to working length as possible. This constituted the initial sample (S1). All samples were sent to the UNC School of Dentistry Dental Research Laboratory at Research Triangle Park, North Carolina within 24 hours of sampling.

Instrumentation

Working length was established to the root terminus using an apex locator (Root ZX, J. Morita, Irvine, CA) and confirmed radiographically. All canals were irrigated with 0.9% sterile saline solution (Baxter Healthcare, Deerfield, IL) using a 30-gauge side vented needle (Max-i-Probe,
Dentsply/Tulsa Dental, York, PA) and a syringe (BD, Luer-Lok™ Tip, Franklin Lakes, NJ) during instrumentation and RC-Prep (Block Drug Corp., Jersey City) was used as a lubricant.

0.04 Tapered Canal Preparation
Sterile Sterile K3 g-pack and 0.04 tapered rotary nickel-titanium files (SybronEndo, Orange, CA) were used to instrument all the canals with a crown-down technique to a predetermined final apical file size of ISO #40. Rotary instrumentation was used with an Aseptico ITR Electric Torque Control Motor (Dentsply/Tulsa Dental, Tulsa, Oklahoma) rotating at 600 rpm with a torque of 1:8. This instrumentation technique was performed in all treatment groups.

Irrigation
All groups were irrigated with 0.9% sterile saline solution (Baxter Healthcare, Deerfield, IL) during instrumentation using the conventional irrigation technique. Irrigation was performed after each rotary instrument with 2.0mL saline solution. A final rinse of 10mL 6.0% sodium hypochlorite with either the EndoVac™ irrigation system, the ProUltra PiezoFlow™ Ultrasonic Irrigation Needle, or the conventional irrigation technique was performed as follows:

Conventional irrigation technique (control):
Irrigation was performed with a 30-gauge side vented needle (Max-i-Probe, Dentsply/Tulsa Dental, York, PA) and a syringe (BD, Luer-Lok™ Tip, Franklin Lakes, NJ). This group served as the experimental control group. The syringe was filled with irrigation solution and the needle was introduced into the canal 2.0 mm short of working length without wedging and
evacuated with light hand pressure. This irrigation procedure was performed as a final rinse in treatment group 1.

**EndoVac™ Irrigation System:**

The EndoVac™ (Discus Dental, Smart™ Endodontics, Culver City, CA) system was used after completion of instrumentation by inserting the irrigation needle to the working length. The EndoVac™ micro-cannula is a 30-gauge, stainless steel irrigation needle with an array of twelve radial configured filtration holes, each hole measuring 0.1mm in diameter. The needle was connected to the 20mL EndoVac™ suction device to aspirate the irrigant solution in the canal while a steady flow of irrigation solution was maintained. This irrigation procedure was performed as a final rinse in treatment group 2.

**ProUltra PiezoFlow™ Ultrasonic Irrigation Needle:**

The ProUltra PeizoFlow™ Ultrasonic Irrigation Needle (Dentsply/Tulsa Dental, Tulsa, OK) was used after completion of instrumentation by inserting the irrigation needle to the middle third of the prepared root canal. The ProUltra PiezoFlow™ Ultrasonic Irrigation Needle cannula was a 25 gauge ultrasonic irrigation needle, which introduces a continuous ultrasonic irrigation flow into the root canal. The ProUltra PiezoFlow™ Ultrasonic Irrigation Needle is connected to an ultrasonic unit to introduce the ultrasonic irrigation. This irrigation procedure was performed as a final rinse in treatment group 3.
Post-instrumentation Sample (S2)

All canals were flushed with 2.0mL of 5% sodium thiosulfate (Sigma Corp, St. Louis, MO) to neutralize the sodium hypochlorite. The canals were then flushed with sterile saline and dried with sterile paper points. Using a new set of sterile instruments, the canals were then filled with LDT and dentinal shavings were produced with the final file size placed to the working length and pumped five times with minimal reaming motion. The entire canal contents were absorbed with sterile paper points and transferred to the LDT sample bottle. This constituted the post-instrumentation sample (S2). All samples were sent to the UNC School of Dentistry Dental Research Laboratory at Research Triangle Park, North Carolina within 24 hours of sampling.

Intracanal medicament

All canals were again irrigated with 6% sodium hypochlorite, followed by 0.6% saline (Braun Medical Inc., Irvine, CA) and then dried with paper points. A mixture of calcium hydroxide (Henry Schein, Inc., Melville, NY) and 2% chlorhexidine (Central Compounding Center, Durham, NC) was placed into all canals with a lentulo spiral filler (Caulk, Milford, DE) and the access cavity was sealed with IRM (Dentsply Int. Inc., York, PA). The intracanal medication (calcium hydroxide mixed with 2% chlorhexidine) was placed for a minimum of one week.

Post Intracanal Medicament Sample (S3)

At the second appointment, under rubber dam isolation, each tooth was accessed with the strict aseptic protocol as described above. Intracanal medicament was passively removed with a K-
file and sterile saline irrigation. Neutralization of the calcium hydroxide and 2% chlorhexidine (Central Compounding Center, Durham, NC) dressing was accomplished using 2.0mL 0.5% citric acid (Central Compounding Center, Durham, NC) followed by 2.0mL of 3% Tween 80/0.5% L-alpha-lecithin (Central Compounding Center, Durham, NC) introduced into each canal with a sterile tuberculin syringe. The canals were irrigated again with sterile saline and dried. As described above, LDT was introduced and collected, constituting the final sample (S3).

**Root canal filling**

If a patient was symptomatic, instrumentation was repeated and an intracanal dressing was placed for at least seven additional days. Once the patient was asymptomatic, the root canal filling was placed. All canals were filled at this appointment using cold lateral condensation or warm vertical condensation with Resilon™ with Epiphany™ sealer (Resilon Pentron, Wallingford, CT). Canals were rinsed with 10mL of 17% ethylenediaminetetraacetic acid (EDTA) (Benco Dental, Pittston, PA) to remove the smear layer (44), rinsed with 2% chlorhexidine (Central Compounding Center, Durham, NC) and dried prior to placing the root filling. The access cavities were temporized with Fuji IX dual cured glass ionomer (GC Corporation, Tokyo, Japan) until a permanent restoration could be placed.

**Microbial examination**

The laboratory procedures were performed at the University of North Carolina Dental Microbiology Laboratory (a CLIA certified laboratory). The vials with the paper point samples were agitated with a vortex (Fisher Scientific, Pittsburgh, PA) for 30 seconds at a
power setting of 4. A model D spiral plater (Microbiology International, Frederick, MD) delivered 49 uL of sample to each agar plate. The model D spiral plater delivered a 2.3 log dilution of the sample across each plate. Each sample was plated in duplicate on aerobic plates, anaerobic plates and chocolate plates. The anaerobic gas consisted of 5% carbon dioxide, 85% nitrogen, and 10% hydrogen. The chocolate plates were grown aerobically in a carbon dioxide enriched environment. This was to support the growth of Haemophilus and Neisseria species that normally will not grow on sheep blood agar. Bacteria growth was measured by direct counting of colonies and grid specific calculations. The spiral plater deposited a known volume of the sample to areas of the plate or grid. Once the colonies were counted in each grid, a dilution factor (determined by the manufacturer, Microbiology International, Frederick, MD) was used to translate the grid calculation to the original bacterial count in the sample. For statistically reliable counts any value less than 6.00x10^{2} CFU/mL was not considered accurate but an estimate. The upper limit for accurately determining counts with this assay was 1.00x10^{8} CFU/mL. Any value less than the lower limit was replaced with 6.00x10^{2} CFU/mL and any value greater than the upper limit was replaced with 1.00x10^{8} CFU/mL.

**Data Analysis**

Data was collected and entered into an Excel spreadsheet, then imported into SAS 9.2 (SAS Institute Inc., Cary, NC). A univariate analysis determined the distribution of the data and identified outliers. Scatter plots and other graphical displays were drawn to visualize the data. Bacteria colony-forming unit (CFU) count was quantified and recorded as a continuous variable. A log_{10}-transformation of each colony-forming unit (CFU) count was performed to
normalize the data before statistical evaluation. Any "0" value was replaced by "1" before log-transformation. A two-factor, repeated-measures mixed model was used where bacterial samples from each tooth over time was the within-subject factor, and irrigation type was the between-subject factor. Interaction between irrigation type and time effect of bacterial sampling was tested in the mixed model. The level of significance was set at 0.05 for all analyses.

**Resources and Environment**

All treatment and sample collections were completed at the University of North Carolina School of Dentistry Graduate Endodontic Department. The investigators had access to all supplies and equipment needed to complete the study. All sample analyses were completed at the University of North Carolina School of Dentistry Dental Research Laboratory at Research Triangle Park, North Carolina within 24 hours of sample collection.
Results

Individuals in the three irrigation groups were not statistically significantly different, on average, in age at the start of treatment (One-way ANOVA, P = 0.48) or in the proportion of females (Fisher’s Exact, P = 0.06) (Table 1). The proportion of mandibular molars were statistically different among the three groups (Fisher’s Exact, P = 0.04). A higher proportion of maxillary molars had been assigned to the ProUltra Piezo Flow™ Ultrasonic Irrigation group (Table 1).

Mean and standard deviation of bacteria count log10 CFU by group

Four readings of CFU count exceeded the upper limit of the assay and were replaced with the upper limit 1x 10^8 CFU/mL, and 120 readings were below the lower limit of the assay and were replaced with a count of 600 CFU/mL. The mean and standard deviation of log_{10} CFU counts for each irrigation method and time of sampling are reported in Table 2. For statistically reliable counts any value less than 6.00x10^2 CFU/mL was not considered accurate but an estimate.

General linear model

The interaction term between treatment groups and time points was not statistically significant (F = 0.77; P=0.55). The interaction term was removed from the model and a main effects only model was used (Table 3). On average, the bacterial log10 counts of the irrigation methods were significantly different (P=0.003) after adjusting for the effect of time. The control and EndoVac™ System irrigation methods were not significantly different (P = 0.66) while the
ProUltra PiezoFlow™ Ultrasonic Irrigation Needle method was significantly different, on average, from both the control (P=.002) and the EndoVac™ System (P=.006) methods (Table 4, Figure 6). The average bacterial count for the ProUltra PiezoFlow™ Ultrasonic Irrigation Needle method was the highest of the three methods. After adjusting for the effect of type of irrigation, the bacterial counts also were significantly different on average over time (Table 4). The mean log10 counts were significantly different at S1 than at S2 and S3 (P=<.0001 and P = .0004 respectively) with the mean count highest at S1. The mean log10 counts were not significantly different between S2 and S3 (P = .76).
Discussion

This \textit{in vivo} study was completed to evaluate the effectiveness of three irrigation techniques in removing bacteria present in the root canal at various time points during the procedure. Sodium hypochlorite was used as the irrigant in evaluating 1) the conventional irrigation technique using a 30 gauge side vented needle, 2) irrigation using the EndoVac\textsuperscript{TM} system, and 3) irrigation using the ProUltra PiezoFlow\textsuperscript{TM} Ultrasonic Irrigation Needle. Each irrigation technique resulted in a significant reduction of bacteria at the S1-S2 time point. Based on the results of this study, the EndoVac\textsuperscript{TM} system was significantly more effective in removing bacteria from root canals than the ProUltra PiezoFlow\textsuperscript{TM} Ultrasonic Irrigation Needle. The EndoVac\textsuperscript{TM} System was not significantly more effective than the experimental control group using a 30 gauge side-vented needle in removing bacteria from root canals. These findings confirmed the importance of mechanical instrumentation combined with the use of antibacterial irrigation in disinfecting root canals (5). Positive bacterial cultures revealed a higher number of aerobic species present and few anaerobic or enterococcus species for all samples taken at all time points.

Some samples in each of the experimental groups were observed to have an increase in bacteria CFU count at the S3 time point, which represents one week following calcium hydroxide placement. This increase in bacteria CFU counts could be due to an improper sterile technique by the operator at the time of sample collection or contamination during the culturing process. While previous studies have demonstrated the need for calcium hydroxide
to disinfect the tooth (26,42,45,46), more recent studies have revealed the limited effect that calcium hydroxide has on eliminating bacteria from root canals (47-49). These findings may also serve as a potential rationale for why some S3 samples had an increase in bacterial CFU counts. Still another rationale as to why some samples had an increase in bacteria CFU count at the S3 time point could be bacterial leakage through the temporary coronal restoration.

Previous studies have reported that the EndoVac™ System is safer and is more effective in cleaning the root canal especially in the apical third (35-37). Similarly, the ProUltra PiezoFlow™ Ultrasonic Irrigation Needle has been reported to significantly clean root canals (39-41). There are currently few studies that examine how well these devices remove bacteria from the root canal. One study by Hockett et al. (50) showed that the Endo Vac™ System had the ability to remove bacteria more effectively from root canals than traditional irrigation systems. This is not consistent with the findings of this study. Such differences may be due to differences in the experimental model. The study by Hockett et al. (50) was an in vitro study while the present study is an in vivo study. Furthermore, unlike the study by Hockett et al. (50) in which the volume of irrigant was not standardized, the volume of irrigant was standardized in this study. Probably the most important factor in achieving different results from the Hockett et al. (50) study was the fact that the present study was completed in association with instrumentation. The findings of the EndoVac™ System by Hockett et al. (50) were observed only after the canals were first instrumented and then contaminated with bacteria following instrumentation.
Based on the findings of this study, the ProUltra PiezoFlow™ Ultrasonic Irrigation Needle was not significantly more effective in removing bacteria from root canals. There currently is no study available to evaluate the effectiveness of bacterial removal using the ProUltra PiezoFlow™ Ultrasonic Irrigation Needle. However, one study completed by Carver et al. (38) found that one minute of ultrasonic irrigation resulted in significant reduction of CFU count and positive cultures when compared to samples collected from canals irrigated using sodium hypochlorite alone. However, in the study completed by Carver et al. (38) the apical instrumentation was much smaller than the apical instrumentation completed in this study.

Carver et al. (38) instrumented canals to a size 30.04 or 30.06 depending on the anatomy of the canal. All canals in this study were instrumented to an apical size of 40.04. Previous studies (20-23) have observed that narrow canals compromise the efficacy of irrigation and may need to be enlarged and their taper increased to allow effective irrigation. Additionally, the canals in this study which were irrigated using the conventional irrigation technique were irrigated using a 30-gauge side-vented needle. Carver et al. (38) did not indicate in their study the size of the needle used to irrigate those canals in which ultrasonic irrigation was not used. This is important because previous studies (12,13) have observed that the ability of the irrigation solution to debride the canal is dependent on the depth of needle placement as well as the volume of irrigation used. It definitely should be noted that the ProUltra PiezoFlow™ Ultrasonic Irrigation Needle treatment group contained significantly more maxillary molar teeth. The presence of a complicated root canal system for maxillary molar teeth may have proven challenging for the device to effectively remove bacteria (9).
One previous EndoVac™ System study (35) reported significant removal of debris from the apical part of the root canal (1mm short of the apex). In a similar manner, a previous ProUltra PiezoFlow™ Ultrasonic Irrigation Needle study (41) reported statistically significant debris free canals and isthmuses. The present study investigated the antibacterial effects in the entire canal, and the EndoVac™ System was significantly more effective than the ProUltra PiezoFlow™ Ultrasonic Irrigation Needle. It is not known if there would be a significant difference amongst the three irrigation techniques if the different portions of the root canal were cultured for the presence of bacteria following instrumentation and final irrigation.

Traditionally, the tip of the irrigation needle is placed 2-3mm short of the apical end of the canal and the irrigant is saliently expressed for effective cleaning of the entire length of the canal (12,13). The closer the irrigation needle is to the working length, the more effective the irrigant is in disinfecting the root canal (13,14). Studies have shown that the EndoVac™ System can be placed at the apical third of the canal with a limited risk of apical extrusion of sodium hypochlorite (37). In this study, the ProUltra PiezoFlow™ Ultrasonic Irrigation Needle and the conventional 30 gauge side-vented needle could not be placed as close to the apex as the EndoVac™ System. Regardless of the depth of needle penetration, there was no significant difference between the conventional 30 gauge side-vented needle and the EndoVac™ System in removing bacteria from root canals.

It has been proposed by Carver et al. (38) that the addition of ultrasonic irrigation after hand and rotary cleaning and shaping of the root canal significantly reduced CFU counts and was seven times more likely to yield a negative culture than hand and rotary instrumentation alone.
The ProUltra PiezoFlow™ Ultrasonic Irrigation Needle provides ultrasonic irrigation in the prepared root canal. Even though the EndoVac™ System and the conventional 30 gauge side-vented needle did not have the addition of ultrasonic irrigation, these two irrigation techniques significantly removed more bacteria from root canals in this study when compared to the ProUltra PiezoFlow™ Ultrasonic Irrigation Needle.

There are limitations to this study. The sample size for all treatment groups in this study is smaller than desired. Further studies with a larger sample size for each treatment group could better confirm the findings of the present study. Another limitation of this study is ensuring that the actual bacteria present could survive the culturing process. A study which combines PCR as well as bacterial CFU colony counts could possibly better confirm true bacteria populations.

The volume of irrigant used, the concentration of irrigant used, and the instrumentation completed were all constant during this study. The findings of this study further emphasize the importance of mechanical debridement in conjunction with chemical disinfection during root canal therapy as indicated in previous studies (7,8). As emphasized in previous studies (29), the findings of this study also demonstrate the need to use large volumes of irrigant to properly disinfect the root canal.

In conclusion, the results of this in vivo study indicated that the EndoVac™ System and the 30 gauge side-vented needle were significantly more effective in removing bacteria from root canals than the ProUltra PiezoFlow™ Ultrasonic Irrigation Needle. The EndoVac™ System
was not significantly more effective than the experimental control group using a 30 gauge side-vented needle in removing bacteria from root canals. Each irrigation technique resulted in a significant reduction of bacteria between the S1 and S2 time point. While the ProUltra PiezoFlow™ Ultrasonic Irrigation Needle may be more effective in debris removal, the 30 gauge side-vented needle and the EndoVac™ System both were significantly more effective in removing bacteria from root canals in this study.
Table 1. Descriptive Statistics for Demographic Characteristics and Jaw Location of Molars included in the Sample

<table>
<thead>
<tr>
<th></th>
<th>EndoVac&lt;sup&gt;TM&lt;/sup&gt;</th>
<th>PiezoFlow&lt;sup&gt;TM&lt;/sup&gt;</th>
<th>Control</th>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
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<tr>
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<td>Females</td>
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<td>%</td>
<td>N</td>
</tr>
<tr>
<td>Maxillary</td>
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<td>60</td>
<td>4</td>
</tr>
<tr>
<td>Molars</td>
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<td>30</td>
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Table 2. Mean and standard deviation of bacteria count log10 CFU by group

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<tbody>
<tr>
<td></td>
<td>Mean (CFU/mL)</td>
<td>SD</td>
<td>Mean (CFU/mL)</td>
<td>SD</td>
<td>Mean (CFU/mL)</td>
</tr>
<tr>
<td>S1</td>
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<td>5.33</td>
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<tr>
<td>S2</td>
<td>3.19</td>
<td>0.51</td>
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<tr>
<td>S3</td>
<td>3.38</td>
<td>0.74</td>
<td>3.13</td>
<td>0.60</td>
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Table 3. Type III Sum of Squares results for the main effects

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<th>Den DF</th>
<th>F Value</th>
<th>Pr &gt;F</th>
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<tr>
<td>Time Point</td>
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<td>27</td>
<td>16.78</td>
<td>&lt;0.0001</td>
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Table 4. Estimates of the marginal means for irrigation method and sampling time

<table>
<thead>
<tr>
<th>Effect</th>
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<th>Time Point</th>
<th>Group</th>
<th>Time Point</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>Adj P</th>
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<tbody>
<tr>
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<td>EndoVac™</td>
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<td>S3</td>
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Figure 1. EndoVac™ System macrocannula
Figure 2. EndoVac™ System microcannula
Figure 3. 30-gauge side-vented needle and syringe
Figure 4. ProUltra PiezoFlow™ Ultrasonic Irrigation Needle with syringe and ultrasonic attachment
Figure 5. Wire cutters
Figure 6. Mean bacteria count log 10 CFU by group at each time point
References Cited


