Antimicrobial Efficacy of Two Irrigation Techniques in Tapered and Nontapered Canal Preparations: An *In Vitro* Study

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Abstract

The aim of this *in vitro* study was to determine whether irrigation with apical negative pressure was more effective than traditional positive-pressure irrigation in eradicating *Enterococcus faecalis* from preshaped root canals. Fifty-four extracted mandibular molars were instrumented to produce either a non-tapered or tapered preparation, sterilized, inoculated with *E. faecalis* for 30 days, and then randomly assigned into the following groups: group 1—non-tapered preparation and negative-pressure irrigation, group 2—non-tapered preparation and positive-pressure irrigation, group 3—tapered preparation and positive-pressure irrigation, and group 4—tapered preparation and negative-pressure irrigation. Mesial canals were sampled before and after final irrigation and samples incubated aerobically for 48 hours at 37°C. Scanning electron microscopic analysis confirmed dense bacterial colonies in the positive control, consistent with biofilm formation. A statistically significant difference was evident when comparing apical negative-pressure irrigation to positive-pressure irrigation ($p=0.004$). There was no statistically significant difference in colony-forming units (CFUs) between sizes #35 and #45, nor between tapered and non-tapered preparation. The results of this *in vitro* study showed that apical negative-pressure irrigation has the potential to achieve better microbial control than traditional irrigation delivery systems. *(J Endod 2008;34: 1374–1377)*

Key Words

Canal preparation, disinfection, irrigation, nontapered, tapered

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Toxic metabolites and byproducts released from microorganisms within the canal diffuse into periapical tissues and elicit inflammatory responses and bone resorption (1, 2). Since a correlation exists between healing and negative cultures obtained before root filling, endodontic treatment aims to eliminate microorganisms and their endotoxins from the canal system (3–7). Bacterial reduction to the extent of a negative culture should be considered the desired "clinical outcome" of endodontic treatment.

Cleaning the canal system entails mechanical and chemical removal of the canal contents. Mechanical instrumentation is the establishment of a specific cavity form to permit easy access of instruments and irrigants into the canal space and for optimal obturation (8). Irrigation acts as a flush to remove organic and inorganic debris as well as a bactericidal agent, tissue solvent, and lubricant. Bystrom and Sundqvist (9) established that mechanical instrumentation of the root canal followed by saline irrigation alone leaves bacteria in the canal system. Therefore, disinfectants such as sodium hypochlorite (NaOCl) are necessary (10, 11).

The ability of an irrigant to be distributed to the apical portion of a canal is dependent on canal anatomy, size of mechanical instrumentation, and delivery system (12–14). Apical areas can only be disinfected if reached by the irrigant (15). Irrigation is traditionally achieved by expressing the irrigant into the canal through a notched end or side-vented needle. Recently, Vinothkumar et al. (16) showed that safety-ended needles with a single side port are more effective than a double side port and hypodermic needles when used up to 1 mm short of the working length. The effectiveness of the solution is also dependent on the depth of needle placement and the volume of irrigation used (17). Traditionally, the tip of the needle is placed 2 to 3 mm short of the apical end of the canal, and the irrigant is passively expressed. However, if the needle is placed too close to the apical foramen or the irrigant is forcibly expressed, the chance of extrusion of the solution increases (18). Extrusion of an irrigant, such as NaOCl, may result in severe periapical tissue damage and postoperative pain (19).

The apical negative-pressure technique has been shown to avoid the adverse side effects related to apical extrusion of irrigating solutions. Even in teeth with open apices, negative-pressure irrigation was shown to greatly reduce the amount of extruded irrigant (20). Nielsen and Baumgartner (21) showed significantly cleaner canals at the apical 1-mm level using a new device, EndoVac (Discus Dental, Culver City, CA), which uses apical negative-pressure irrigation, compared with traditional syringe irrigation. The EndoVac irrigation needle is inserted to the working length and connected to the EndoVac suction device, which is connected to the HiVac, creating a negative pressure to aspirate the irrigating solution in the apical portion of the canal. The microcannula has an array of 12 radial configured filtration holes and creates a steady flow of irrigating solution through the entire root canal, allowing the irrigant to debride and disinfect the last millimeters of the canal without extrusion.

The flushing action of the irrigant may be more important during the cleaning process than the ability of the irrigant to dissolve tissue (22). It was shown that narrow canals compromise the efficacy of irrigation and may need to be enlarged and their taper increased to allow for effective irrigation (23, 24). It is not known whether the negative-pressure irrigation technique is more effective at microbial disinfection than positive-pressure irrigation or whether it is more effective in tapered- or nontapered-shaped canals. Therefore, the aim of this study was to compare the antimicrobial

efficacy of traditional positive-pressure irrigation and apical negative-pressure irrigation in root canals with either a tapered or nontapered preparation.

Materials and Methods

Fifty-four caries-free extracted mandibular molars were stored in diluted 0.5% NaOCl. Teeth were sectioned 2 mm above the cemento-enamel junction and accessed. Orifice openers (Dentsply/Tulsa Dental, Tulsa, OK) and Gates-Glidden burs (Dentsply Maillefer. Tulsa, OK) were used to open the orifice of the mesiobuccal (MB) and mesiolingual (ML) canals. Teeth were randomly divided into four experimental groups of 12 teeth as follows: (1) group 1 (LE): Lightspeed LSX (Discus Dental, Culver City, CA) nontapered preparation with the EndoVac irrigation system, (2) group 2 (LT): LSX nontapered preparation with traditional irrigation technique, (3) group 3 (PT): Pro-Taper (Dentsply/Tulsa Dental)-tapered preparation with traditional irrigation technique, and (4) group 4 (PE): Pro-Taper–tapered preparation with the EndoVac irrigation system.

LSX (Nontapered) Instrumentation

LightSpeed LSX instruments were used to instrument the MB/ML canals of mandibular molars according to the manufacturer's recommendations. The apical part of the canals was prepared to the final apical size International Organization for Standardization (ISO) #45 rotating at 2,000 rpm. Finally, a .02 tapered nickel titanium hand file, corresponding to the final apical size (ISO #45), was used in a clockwise rotation to ensure the removal of any debris at the apical third.

Tapered Instrumentation

Pro-Taper nickel-titanium files (Dentsply/Tulsa Dental) were used to instrument the canals with a crown-down technique to a size F3 at 300 rpm. The apical portion of canals was enlarged with .02 tapered nickel-titanium hand files to an ISO #35 to ensure the removal of any debris at the apical third.

Postinstrumentation Irrigation

Irrigation was performed in all groups using 6% NaOCl and 17% EDTA with positive-pressure irrigation. First, 3 mL 6% NaOCl was used for irrigation followed by 1.5 mL 17% EDTA and then 3 mL 6% NaOCl. All teeth were then placed in an ultrasonic bath of EDTA and then NaOCl for 5 minutes to ensure the removal of debris and smear layer.

Specimen Sterilization

All prepared teeth were kept in sterile saline until sterilized. Moisture was removed from the canals with sterile paper points, and the teeth were air dried for 8 hours before sterilization. Sterilization was accomplished with a standard STERRAD (Advanced Sterilization Products, Ontario, Canada) cycle that uses a combination of hydrogen peroxide vapor and low-temperature gas plasma to rapidly sterilize items without leaving toxic residues. Teeth were then immediately placed into sterile plastic vials containing 15 mL sterile soy broth medium and kept in an anaerobic incubator at 37°C for 48 hours to check the efficacy of the sterilization procedure.

The Cultivation of *Enterococcus faecalis* and Specimen Inoculation

Pure isolated 24-hour colonies of *E. faecalis* (American Type Culture Collection 19433) grown on sheep blood agar plates were suspended in 15 mL soy broth for 8 hours. Five drops of this bacteria medium were inoculated in a new 15 mL soy broth for 4 hours. These bacteria suspensions were adjusted to match the turbidity of 1.5×10^8 colony-forming units (CFUs)/mL (equivalent to 0.5 McFarland standard). The glass tubes containing the specimens were opened under laminar flow, and sterile pipettes were used to add 15 mL of the bacterial inoculums to the vials containing the teeth suspended in sterile medium. The tubes were then closed and kept at 37°C for 30 days, with replacement of half the inoculums broth with 15 mL fresh sterile medium every 2 days to avoid medium saturation. The turbidity of the medium during the incubation indicated bacterial growth. The purity of the cultures was confirmed by Gram staining after 30 days and later verified in the positive control sample under scanning electron microscopy (SEM). Microbial growth was conducted under anaerobic conditions.

Controls

Two control groups of three teeth each were also evaluated. Negative control (sterile) teeth were prepared with Pro-Taper instrumentation, debris and smear layer removed, and then sterilized. Teeth in this group were not inoculated with bacteria. The final irrigation sequence was accomplished with the positive-pressure technique using 6% NaOCl and 17% EDTA. The positive control teeth were instrumented with Pro-Taper, debris and smear layered removed, and sterilized. Then, the teeth in this group were inoculated with *E. faecalis*. The final irrigation was completed with positive-pressure irrigation by using sterile water only for 5 minutes.

Preparation for Bacteria Sampling

After 30 days of inoculation with *E. faecalis,* teeth were taken out of the bacteria medium, rinsed with sterile saline, and the outside of each tooth wiped with alcohol. A sterile cotton pellet was placed into the chamber and the access cavity sealed with Cavit (3M ESPE, St Paul, MN) for the external disinfection procedure. The apical foramens were sealed with hot glue. A rubber dam was applied and sealed with hot glue. Each tooth was disinfected with 30% hydrogen peroxide (H_2O_2) until no further bubbling of the peroxide occurred. All surfaces were then coated with 10% tincture of iodine and allowed to dry. The tooth surface was swabbed with a 5% sodium thiosulfate solution (Sigma Corp, St Louis, MO) to inactivate the iodine tincture. The Cavit and cotton pellet were removed, the chamber was flushed with 2 mL sterile saline, and it was dried with sterile cotton pellets and paper points. The orifice of the distal canal was then sealed with Cavit until all samples were collected. The MB/ML canals of mandibular molars were left unsealed for sampling.

Initial Sample (S1)

Bacteria samples were collected from the MB and ML canals. With a sterile tuberculin syringe, 0.5 mL Liquid Dental Transport Media (LDTM) (Anaerobic Systems, Morgan Hill, CA) was inserted into the canals. Excess LDTM in the chamber was removed so that only the root canals remained filled. In order to collect dentin shavings, a #30 size SS K-file (Kerr, Romulus, MI) was used to instrument a wall of the MB canal at WL for 5 seconds. The file was removed and the fluted part cut off with a sterile wire cutter and allowed to fall into the opened bottle of LDTM. LDTM remaining in the canal was removed with a sterile x-fine paper point (Mynol; Block Drug Corp, Jersey City, NJ) by placing it at WL and then transferring it to the LDTM bottle. This constituted the initial sample (S1). All samples reached the laboratory within 6 hours.

Positive-pressure Irrigation Technique

For groups 2 and 3, irrigation was performed with a 30-G side-vented needle (Max-i-Probe; Dentsply/Tulsa Dental, York, PA) and a 10-mL syringe. The syringe was filled with irrigating solution, and the needle was introduced into the canal 1.5 mm short of the working length without wedging and the irrigant delivered with moderate pressure. Irrigation was performed with 6% NaOCl for 2 minutes followed by

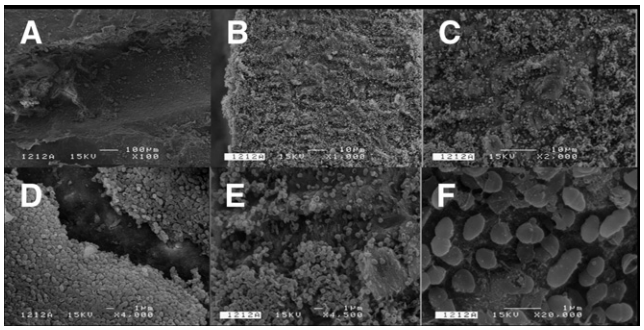

Figure 1. Positive control. SEM analysis. (*A*) At ×100, the presence of bacteria over the root canal surface is shown. (*B–D*) At ×1,000, ×2,000 and ×4,000 respectively, the bacterial arrangement as biofilms is observed. (*E*) At ×4,500, note the cell aggregations of bacteria covering the dentinal tubules. (*F*) *E. faecalis* colonization of the root canal and dentinal tubules is confirmed at ×20,000.

17% EDTA for 1 minute and 6% NaOCl for 2 minutes. The total final irrigation time was 5 minutes for the traditional group.

Apical Negative-pressure Irrigation Technique

For groups 1 and 4, the EndoVac system was used according to the manufacturer's recommendations (23). Macroirrigation of each canal was accomplished with 6% NaOCl over a period of 30 seconds. The open-ended macrocannula was moved up and down in the canal from a point where it started to bind to a point just below the orifice. The macrocannula was then removed quickly so that no air was drawn into the canal space and the canal was left full of irrigant. Microirrigation began immediately after macroirrigation with three cycles of irrigation. During a cycle of microirrigation, the pulp chamber was maintained full with irrigant, whereas the microcannula was taken to the full working length for 6 seconds, then lifted 2 mm coronally for 6 seconds, and then moved back to the working length for 6 seconds. This up-down motion was continued for 30 seconds, thus ensuring 18 seconds of active irrigation directly at the working length. After 30 seconds of irrigation, the microcannula was withdrawn from the canal in the presence of sufficient irrigant in the pulp chamber to ensure that the canal remains totally filled with irrigant and no air is drawn into the canal space. The canals were left filled with irrigant from apex to pulp chamber undisturbed for 30 seconds. The second cycle of irrigation used 17% EDTA, and the third used 6% NaOCl again. The total final irrigation time was 3 minutes 30 seconds for the EndoVac groups. At the end of the third microirrigation cycle, the microcannula was left at the working length without replenishment to remove excess fluid.

Final Irrigation Sample (S2)

Canals were flushed with 2 mL 5% sodium thiosulfate to neutralize the NaOCl and then 2 mL sterile saline. Canals were dried, and the samples were taken using the same procedure as the S1 sample, except that dentin shavings were obtained by filing all canal walls circumferentially for 5 seconds with a size #30 SS K-file.

Microbial Examination

The sampling vials containing 1 mL LDTM, the file, and paper points were agitated in a mechanical mixer for 1 minute, and 10-fold serial dilutions to 10^{-2} were made in dilution banks. The dilution 10^{-2} aliquots of 10 μL were inoculated onto plates of trypticase soy agar with 5% sheep blood (Remel, Lenexa, KS). The plates were incubated aerobically for 48 hours at 37°C. CFUs were counted, and the purity of the cultures was confirmed by Gram staining and colony morphology.

SEM Examination

Samples from the positive control group were prepared for SEM. Teeth were longitudinally grooved and split. Samples were rinsed in phosphate-buffered saline, soaked in glutaraldehyde for 1 hour, and post fixed in 1% OsO_4 for 30 minutes. Samples were then dehydrated, mounted on SEM discs, and spatter coated with palladium gold. The analysis of the samples focused on the apical third, and photographs were taken within 2 mm of the working length.

Statistical Analysis

The data were statistically analyzed using the Fischer exact test with the two-sided p value and level of significance established at $p < 0.05$.

Results

S1 Culture

The modified contamination protocol used for this investigation achieved well established bacterial biofilms attached to the dentinal wall as exhibited by the high CFU counts in all samples (except negative controls) and confirmed with SEM in two positive control samples (Fig. 1).

S2 Culture

All the specimens irrigated with the apical negative-pressure irrigation technique rendered negative cultures obtained after 48 hours. Eight specimens of the positive-pressure irrigation groups rendered a positive culture at the end of the incubation period. A statistically significant difference was evident when comparing the apical negative-pressure irrigation to the traditional positive-pressure irrigation for negative culture (24/24 versus 16/24 with a two-sided p value of 0.004) (Fig. 2). A nonparametric analysis of variance revealed no differences between groups 1 and 4 (negative-pressure irrigation in tapered versus nontapered canals) and between groups 2 and 3 (positive-pressure irrigation in tapered versus nontapered) (Fig. 3). All teeth from the negative control rendered negative cultures, and all teeth in the positive control group rendered positive cultures.

Discussion

In this study, teeth were incubated with *E. faecalis* for 30 days to ensure adequate penetration of bacteria into dentinal tubules (25). The positive cultures, Gram staining, and colony morphology obtained in all S1 samples, and the SEM images confirmed the presence of *E. faecalis* within the root canal system and dentinal tubules. Another important factor of the design of this study was that the apices of all sampled teeth

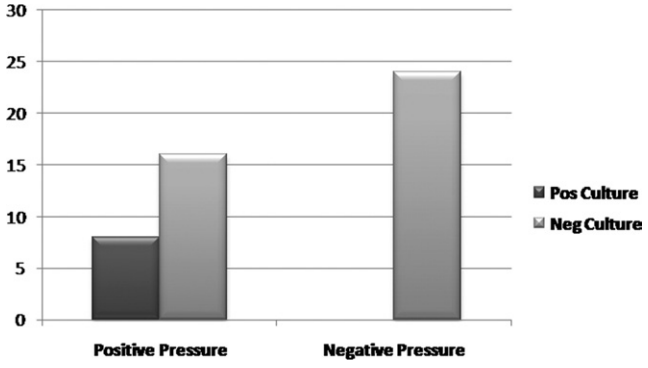

Figure 2. Eight specimens of the positive pressure irrigation groups rendered a positive culture at the end of the incubation period. A significant statistical difference was evident when comparing the apical negative pressure irrigation versus positive pressure irrigation ($p = 0.004$).

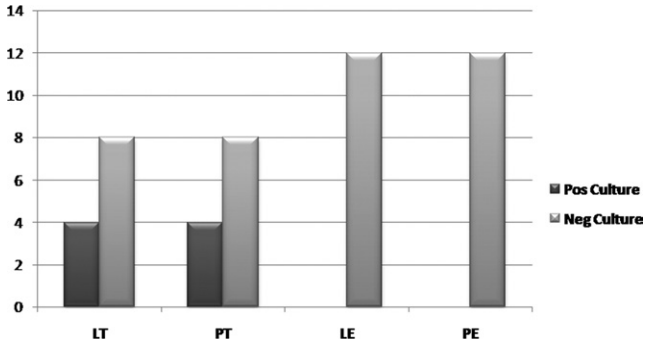

Figure 3. A nonparametric analysis of variance revealed no differences between tapered versus nontapered canals.

were sealed to prevent any contamination from the outer tooth surface during the sampling procedure and also to more closely resemble the clinical situation of the canal acting as a closed-end tube rather than an open-ended cylinder. The fluid dynamics work very differently in the two models.

Despite all the advances in endodontic therapy, outcomes remain unchanged. The main reason to explain this dissonance is related to the fact that none of the new advances and techniques eliminates bacterial contamination. The limitations of current cleaning, shaping, and irrigation techniques were confirmed by Nair et al. (26) in an *in vivo* study in which the anatomic complexity of the root canal system of mandibular molar roots were shown. They concluded that the organization of the flora as biofilms in inaccessible areas of the canal system cannot be completely removed by instruments and traditional irrigation alone.

In order to remain clinically relevant using the most common instrumentation techniques, tapered canals were prepared to ISO #35, which is the minimal size to allow efficient irrigation with either the negative-pressure microcannula (gauge 0.32) or positive-pressure irrigation (27, 28). Apical instrumentation in nontapered canals was further increased to ISO #45 based on recent literature recommendations (24, 29). The hypothesis was that increasing apical enlargement will mechanically remove more contaminated dentin without weakening the coronal/cervical third, thus rendering more negative cultures. Our results showed that if an efficient chemical disinfection is predictably achievable, perhaps the apical enlargement could be limited to ISO #35. Clinically, this might be very important, especially for curved canals.

Although the consequences of microbes remaining in the dentinal tubules after root canal treatment are not clear (27), the main goal of root canal treatment is still the elimination of microorganisms, thus improving the long-term outcome of the treatment. Within the parameters of this *in vitro* study, apical negative-pressure irrigation has the potential to achieve significantly better infection control than current irrigation-delivery systems. Clinical randomized controlled trials are warranted to corroborate the impact of negative cultures obtained with apical negative-pressure irrigation and the outcome of the treatment. Additionally, future studies comparing negative-pressure irrigation with passive ultrasonic irrigation are warranted.

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