In Vivo Antibacterial Efficacy of Ultrasound after Hand and Rotary Instrumentation in Human Mandibular Molars

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Abstract

The purpose of this prospective, randomized, single-blind study was to compare the in vivo antibacterial efficacy of a hand/rotary technique versus a hand/rotary/ultrasound technique in mesial root canals of necrotic mandibular molars. The hand/rotary group consisted of 16 mesial roots prepared with a hand/rotary technique. The hand/rotary/ultrasound group consisted of 15 mesial roots prepared similarly, followed by 1 minute of ultrasonic irrigation per canal with an ultrasonic needle in a MiniEndo unit and 15 mL/canal of 6.0% sodium hypochlorite. Canals were sampled before and after instrumentation and after 1 minute of ultrasonic irrigation. Samples were incubated anaerobically on reduced blood agar for 7 days at 37°C, and colony-forming units (CFUs) were counted. The addition of 1 minute of ultrasonic irrigation resulted in significant (p = .0006) reduction in CFU count and positive cultures (p = .0047). Logistic regression analysis indicated the addition of ultrasonic irrigation was 7 times more likely to yield a negative culture. (*J Endod 2007;33:1038–1043*)

Key Words

Bacterial reduction, ultrasonic irrigation, ultrasound

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The goal of endodontic instrumentation is the removal of all vital or necrotic tissue, microorganisms, and microbial by-products from the root canal system. The intricate nature of root canal anatomy complicates complete debridement of all areas of the root canal (1–10). Isthmuses, fins, webs, anastomoses, and other irregularities within the root canal often harbor tissue and debris after instrumentation (1–12).

In vivo research has failed to demonstrate complete elimination of the microbial population within infected molar root canals after instrumentation and irrigation procedures (13–18). After rotary instrumentation and irrigation with sterile saline, Dalton et al (13) rendered only 28% of canals bacteria-free. Shuping et al (14) achieved negative cultures in only 62% of teeth after nickel-titanium rotary instrumentation and irrigation with 1.25% sodium hypochlorite. Therefore, it would be advantageous to improve the antibacterial efficacy of current instrumentation procedures.

Various instrumentation techniques including increased apical instrumentation size, calcium hydroxide as an interappointment medication, and the use of alternative irrigants have been suggested as a means of further reducing viable bacterial counts within infected molar root canals (14, 18–22). In molar samples, Card et al (19) achieved 93% negative cultures after instrumentation to size #60 and 1% sodium hypochlorite irrigation. In an in vitro study, Rollison et al (21) found that instrumentation of the mesiobuccal canals of mandibular molars to an apical size #50 with saline irrigation significantly reduced more intracanal bacteria than canals instrumented to an apical size #35. Although these techniques improved debridement in the mesial roots of mandibular molars, the larger apical instrumentation sizes used in these studies might predispose a canal to transportation or ledging. Shuping et al (14) increased the percentage of negative cultures in mesial roots of mandibular molars from 62% to 93% after the placement of calcium hydroxide for 1 week. Ørstavik et al (20) decreased the percentage of positive cultures from 61% after instrumentation to 35% after placement of calcium hydroxide for 1 week. In an in vitro study, Shabahang et al (22) achieved negative cultures in 100% of samples inoculated with *Enterococcus faecalis* after a 5-minute exposure to Biopure MTAD cleanser (Dentsply Tulsa Dental, Tulsa, OK). Although some studies showed an enhanced ability to reduce viable bacteria by using new irrigants or calcium hydroxide, these systems required extended treatment times, and many have not been tested in vivo, where conditions are ecologically diverse.

The use of ultrasonics has been proposed as a possible solution to the problem of debriding and disinfecting the root canal system. The use of ultrasound after completion of hand or rotary instrumentation has been shown to reduce the number of bacteria (23–27).

No study to date has examined the antibacterial efficacy of an ultrasonic irrigating needle as an adjunct to hand and rotary instrumentation. When connected to a MiniEndo (Spartan EIE Inc, San Diego, CA) piezoelectric ultrasonic system, the needle was reported to have high ultrasonic output and produce cavitation in an instrumented canal (12). This system was shown to remove vital tissue from canals and isthmuses significantly better than hand and rotary instrumentation alone (12).

The purpose of this in vivo, prospective, randomized, single-blinded study was to compare the antibacterial efficacy of a hand/rotary instrumentation technique with a hand/rotary instrumentation plus 1-minute ultrasound technique in the mesial roots of infected, necrotic mandibular molars.

Materials and Methods

Thirty-one adult subjects participated in our study. The Human Subjects Review Committee of The Ohio State University approved the study, and we obtained written informed consent from each subject. The subjects were in good health as determined by written and oral questioning.

Clinical examination by thermal (Green Endo-Ice; Hygenic Corp, Akron, OH), electric pulp (Kerr Vitality Scanner; Kerr Dental, West Collins Orange, CA), and percussion testing indicated all mandibular molars had an initial diagnosis of pulpal necrosis with acute or chronic periapical periodontitis. If vital tissue was encountered on access of the pulp, the tooth was excluded from the study. Radiographs of the test teeth confirmed the presence of an associated 2×2 mm (minimum) radiolucency on the mesial root periapex. Radiographic examination also allowed for evaluation of the number of canals present and whether there was sclerosis of the canals. Teeth with sclerosis were excluded.

Before initiation of the study, random 6-digit numbers were recorded on a master code list corresponding to the experimental groups. The master code list was used to randomly assign subjects to each group before hand/rotary instrumentation was completed. The master code list was consulted only after hand/rotary instrumentation. Therefore, operator bias was eliminated because it was not known which tooth would receive ultrasonic irrigation until after the hand/rotary cleaning and shaping were completed. Both canals of the mesial root of the experimental mandibular molars received the same treatment.

The 31 experimental teeth were randomly divided into 2 groups. Group 1 consisted of 16 teeth prepared with a manual hand-file/rotary instrumentation cleaning and shaping technique but with no ultrasonic irrigation. Group 2 consisted of 15 experimental teeth prepared with the same manual hand-file/rotary instrumentation cleaning and shaping technique, followed by 1 minute of ultrasonic irrigation per canal. Initial radiographs of the experimental teeth were taken with a parallel film holder and were analyzed with the method of Schneider (28) to determine the curvature of the mesial roots. The size of the apical radiolucency was recorded by measuring the greatest diameters along the vertical and horizontal axes with an endodontic ruler, and these values were multiplied to give an estimated area (mm²).

After achieving adequate anesthesia and application of the rubber dam, the operative field was disinfected with 30% hydrogen peroxide until no further effervescence of the peroxide occurred. If bubbling was incessant, Cavit (3M ESPE, Seefeld, Germany) was placed around the rubber dam clamp and tooth, and the process was repeated. Povidone-iodine 10% swab sticks (Medline Industries, Mundelein, IL) were then used to complete the disinfection procedure, and the tooth was accessed with a sterile #4 round bur.

Initial bacterial sampling was taken after initial access into the pulp chamber of all experimental teeth. The distal canal(s) or canals were occluded with Cavit to prevent cross-contamination into the mesial canals. The coronal portion of each mesial canal was enlarged with ProFile GT orifice shapers (DentsplyTulsa Dental, Tulsa, OK), and 2 mL of 0.9% sodium chloride (Baxter Healthcare Corp, Deerfield, IL) was used to flush debris from the pulp chamber. The pulp chamber was then dried with sterile cotton pellets, and 0.02 mL of liquid dental transport fluid (LDT; Anaerobe Systems, Morgan Hill, CA) was added to each canal via a sterile tuberculin syringe. Sterile #10-20 K-type hand files (Dentsply Maillefer, Tulsa, OK) were placed to within 1 mm of estimated working length and pumped 5 times with minimal reaming motion to disperse the LDT fluid. Sterile paper points were used to absorb the canal contents and then transferred to a vial containing 1 mL of LDT fluid. This constituted the initial bacterial sample (S1).

Canal Preparation of Groups 1 and 2

K-type hand files and rotary ProFile GT were used for canal preparation of each tooth. The technique for canal cleaning and shaping followed the technique previously described by Gutarts et al (12) and used 6.0% sodium hypochlorite (The Clorox Co, Oakland, CA). Final apical preparation was to a size 30 hand file. In addition, either a 0.04 or 0.06 30-tip ProFile GT was used apically as dictated clinically by the canal curvature and initial size. After completion of hand/rotary cleaning and shaping, an additional 15 mL of 6.0% sodium hypochlorite was used to irrigate each mesial canal.

After hand/rotary instrumentation of the mesial canals, a second bacterial sample was collected. Canals were dried with sterile paper points, flushed with 2 mL of 5.0% sodium thiosulfate (Red Bird Service, Osgood, IN) for 1 minute to neutralize the NaOCl, and then rinsed with 2 mL of sterile 0.9% sodium chloride. The canals were dried again with sterile paper points, and 0.02 mL of LDT fluid was placed in both mesial canals with a sterile tuberculin syringe. A sterile K-type file equal in size to the master apical file was placed to working length and pumped 5 times with a minimal reaming motion to disperse the canal contents into the LDT fluid. Sterile paper points were then used to absorb the canal contents, and the paper points were placed into a vial containing 1.0 mL of LDT fluid. This constituted sample S2.

Postinstrumentation Irrigation of Groups 1 and 2

After hand/rotary cleaning and shaping and collection of sample S2, Group 2 received 1 minute of ultrasonic irrigation. The ultrasonic unit used was a MiniEndo. The power adjustment for the unit was set at the maximum power setting. A new 1.5-inch, 25-gauge, sterile, beveled irrigating needle (Becton Dickinson & Co, Franklin Lakes, NJ) was used for each tooth. Each needle was inserted through the rear aperture of the shaft of the ultrasonic tip device and connected to the MiniEndo handpiece (Figs. 1 and 2). The needle was at a 45-degree angle to the long axis of the ultrasonic handpiece. The needle was directed through the bore of the shaft and out the end, where it was tightened in place by a screw-on hub so that 15–20 mm of the needle was exposed. Luer-Lok (Becton Dickinson & Co) intravenous tubing connected the needle to a 20-mL syringe containing 20 mL of 6.0% sodium hypochlorite.

Each canal in Group 2 was filled with 1 mL of 6.0% sodium hypochlorite before ultrasonic irrigation. Before activation of the ultrasonic unit, a sterile silicon stopper was placed on the irrigating needle, and the needle was inserted into the canal to a point just short of binding. The silicon stopper was adjusted to this length and then measured with

Figure 1. Perspective view of ultrasonic device.
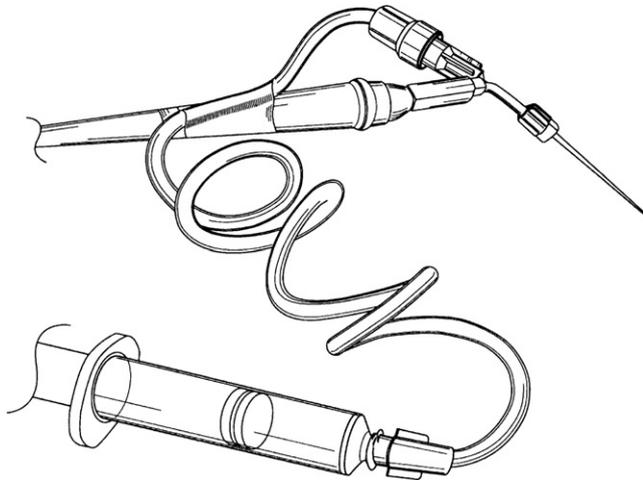
Figure 2. Diagrammatic representation of ultrasonic irrigating device used in this study.

a millimeter ruler to determine the depth of penetration of the ultrasonic irrigating needle. High-speed suction, by using a surgical aspirating tip, was placed at the distal aspect of the access opening and maintained in position during irrigation. The ultrasonic needle was placed to the premeasured depth and, on activation, moved passively in an up-and-down motion to ensure it did not bind within the root canal. The energized ultrasonic needle was used continuously for 1 minute per mesial canal, while the sodium hypochlorite was delivered at a rate of 15 mL per minute. The same ultrasonic technique and needle were used for each canal.

After irrigation, bacterial samples (S3) were taken from the mesial roots of all Group 2 experimental teeth in the same manner as used for sample S2. On completion of collection of all S3 samples, the distal canals of the experimental teeth were cleaned and shaped, and a temporary restorative material was placed in the teeth. The patients were then scheduled to have the root canal therapy completed at a later date.

Microbiologic Preparation and Evaluation

All samples (S1–S3) were immediately transferred to a microbiology laboratory for quantification of colony-forming units (CFUs). Each sample was vortexed for 30 seconds and serially diluted (10-,

100-, and 1000-fold), and 0.25-mL aliquots of each dilution were plated on Brucella blood agar (Anaerobe Systems, Morgan Hill, CA) by using a cell spreader. The plates were labeled by using the random experimental numbers, sample number, dilution factor, and date of sampling and then incubated at 37°C for 7 days in an anaerobic chamber containing 5% carbon dioxide, 10% hydrogen, and 85% nitrogen.

CFUs were counted after a 7-day anaerobic incubation with the aid of an operating microscope at $10\times$ magnification. The number of CFUs per sample was calculated by using the formula (14): (#CFU $\times$ 4) $\times$ $10^{y+1} \times$ 1 mL LDT/sample vial = #CFU/sample. In this formula, y is equivalent to the dilution factor used in the specimen. A log transformation of each recorded S1, S2, and S3 CFU count was used to normalize the data before statistical analysis.

Between-group differences for age, size of periapical lesion, canal curvature, working length, and initial CFU count were analyzed with the independent t test. Between-group differences for tooth type, canal type, and final taper were analyzed with the χ^2 test. Differences in CFU counts were assessed with an analysis of variance. Post hoc testing was done with the Tukey-Kramer procedure. A logistic regression modeling the presence of bacteria after treatment was computed with the following independent variables: tooth type, canal type, instrument taper, and method of instrumentation.

Results

All experimental teeth tested positive for bacterial growth before instrumentation (S1). Tables 1 and 2 show the comparison of the variables between Groups 1 and 2. There were no significant differences between Group 1 and Group 2. Table 3 shows the log transformation of each S1 and S2 CFU count. Hand/rotary instrumentation in Group 1 and Group 2 significantly reduced the number of bacteria from initial counts (p < .0001). Table 3 also shows the mean log CFU count taken from Group 2 at the post-ultrasonic sampling time (S3). A significant (p = .0006) reduction in log CFU count was noted when ultrasonic irrigation was added to the cleaning procedure. Table 4 shows differences for positive cultures at S2 and S3 for Group 2. There was a significant difference between S2 and S3 values for Group 2 (p = .0047).

Table 5 summarizes the logistic regression analysis for instrument taper, tooth type, canal type, and method of instrumentation. Gender, tooth type, and canal type were not significant referents in the analysis. Method of instrumentation was significant (p = .002), indicating that

TABLE 1. Between-group Differences for Age, Lesion Area, Canal Curvature, Working Length, Initial CFU Count (S1), and Associated p Values for Group 1 (hand and rotary instrumentation without ultrasound) and Group 2 (hand and rotary instrumentation with ultrasound)

Variable	N	Mean	SD	Minimum	Maximum	p Value*
Patient age (y)						
Group 1	16	34.8	13.4	20	64	.2679
Group 2	15	40.3	14.8	19	66	
Lesion area (mm²)						
Group 1	16	33.6	32.6	6	132	.5266
Group 2	15	36.1	25.2	5	90	
Curvature (degrees)						
Group 1	16	20.4	14.1	3	56	.4057
Group 2	15	23.1	13.7	7	61	
Working length (mm)						
Group 1	16	20.8	1.8	18	24	.9260
Group 2	15	20.9	2.7	17	23	
S1 (log CFU counts)						
Group 1	16	12.1	2.6	5.5	15.5	.2199
Group 2	15	13.0	1.6	10.3	15.2	

SD, standard deviation.

There were no significant differences between Groups 1 and 2.

*Independent t test.

TABLE 2. Between-group Differences for Tooth Type, Canal Type, Final Taper, and Associated p Values for Group 1 (hand and rotary instrumentation without ultrasound) and Group 2 (hand and rotary instrumentation with ultrasound)

| Variable | N | | p Value* |
	Group 1 (N = 16)	Group 2 (N = 15)	
Tooth type			
First molar	11 (69%)	12 (80%)	.4744
Second molar	5 (31%)	3 (20%)	
Canal type			
II	8 (50%)	8 (53%)	.8528
III	8 (50%)	7 (47%)	
Final taper			
0.04	9 (56%)	7 (47%)	.5936
0.06	7 (44%)	8 (53%)	

There were no significant differences between the groups.

*χ^2 test.

TABLE 4. Group 2 Differences for the Number of Positive Cultures at S2 and S3

| Variable | Group 2 (N = 15) | | p Value |
	S2	S3	
Positive culture			
Yes	11 (73%)	3 (20%)	
No	4 (27%)	12 (80%)	.0047

There was a significant difference between S2 and S3 (McNemar test).

ultrasound was 7 times more likely to yield a negative culture than no ultrasound.

Discussion

The patients' age, lesion size, root curvature, working length, initial CFU counts, tooth type, canal type, and final canal taper were not significantly different between the hand/rotary group and hand/rotary/ultrasound group (Tables 1 and 2). Therefore, the effects of these variables would be minimized between the 2 groups. Clinically, the in vivo nature of this study makes isolation, access, working length determination, and cleaning and shaping more relevant than in in vitro studies. In vitro studies are able to control for poor access, determine working lengths accurately by visualizing files at the apical terminus, and instrument teeth without regard to tooth accessibility or clinical time constraints. Therefore, in vivo studies might be more clinically relevant than in vitro studies. Logistic regression analysis indicated that the use of ultrasonic irrigation for 1 minute per canal after hand/rotary instrumentation was 7 times more likely to yield a negative culture than hand/rotary cleaning and shaping with conventional irrigation alone (Table 5). No other variable, ie, final canal taper, tooth type, or canal type, affected the outcome.

Root canal cleaning and shaping with hand and rotary instrumentation and irrigation with 6.0% sodium hypochlorite were shown with the dependent t test to significantly reduce ($p < .0001$) the log CFU counts in both experimental groups (Table 3). However, bacteria still remained in the canals. The use of ultrasonic irrigation produced a significantly greater reduction ($p = .0006$) in CFU counts (Table 3). In addition, a significantly higher ($p = .0047$) percentage of canals cultured no bacteria after the addition of ultrasonic irrigation (80%) than

after hand/rotary instrumentation alone (27%) as seen in Table 4. Recent research by Farbicius et al (29) indicates that bacteria-free canals are more apt to heal periapically (both radiographically and histologically) as compared with teeth in which bacteria remained in canals in 2- to 2.5-year follow-up study in primates.

Several authors have investigated the mechanisms responsible for ultrasonic cleaning. Stumpf et al (30) implicated cavitation as the primary mechanism responsible for the destruction of bacteria after exposure to ultrasonics. Cavitation is the radical oscillation and subsequent collapse of gas bubbles in the acoustic field, which results in the generation of high temperatures and free radicals. Yumita et al (31) showed that transient cavitation could damage cell walls and cell membranes through the production of high temperatures and pressures and from the production of hydrogen and hydroxyl radicals. However, Pitt et al (32) demonstrated this cell membrane disruption was quickly repaired, indicating that ultrasound alone was not lethal to bacteria. Qian et al (33) also showed that the effects of transient cavitation are not lethal to cells in a bacterial biofilm. Ahmad (34) proposed that cavitation had little practical importance in the disruption of root canal bacteria. Therefore, even though the ultrasonic instrument used in our study produced cavitation (12), the effects of transient cavitation are not the only explanation for the trend toward greater reduction of intracanal bacteria observed with ultrasonic irrigation.

Joyce et al (35) showed that low-frequency ultrasound caused de-agglomeration of bacterial biofilms through the action of cavitation. This declumping of bacterial cells within a root canal might make individual bacteria more susceptible to attack by sodium hypochlorite. These authors also demonstrated that high power ultrasound in small volumes of bacterial suspension resulted in a continuous reduction of bacterial cell numbers. The cavitation produced might also cause temporary weakening of the cell membrane, making the bacteria more permeable to sodium hypochlorite. The deaggregation of clusters of bacteria within the bacterial biofilm in the root canal resulting from the high power ultrasound, in combination with the biocidal activity of a constantly replenished supply of sodium hypochlorite, is the most likely reason for the trend toward greater reduction of intracanal bacteria observed in our study.

TABLE 3. Within-group Differences for Log Initial CFU Count (S1), Log Post Hand and Rotary Instrumentation CFU Count (S2), and Post Ultrasonic Irrigation (S3)

Variable	N	Mean	SD	Min	Max	p Value
Group 1						
S1	16	12.1	2.6	5.5	15.5	
S2	16	3.4	3.7	0.0	12.1	<.0001*
Group 2						
S1	15	13.0	1.6	10.2	15.2	
S2	15	5.6	4.0	0.0	11.4	<.0001*
S3	15	1.2	2.6	0.0	7.3	.0006†

Min, minimum; Max, maximum.

There were significant differences among the values for S1 and S2.

*Significant differences found when compared with respective S1 values.

†Significant difference found when comparing Group 2 S2 and S3 values.

TABLE 5. Logistic Regression Indicating the Likelihood of Bacterial Presence at S3 Associated with Instrument Taper, Tooth Type, Canal Type, and Method of Instrumentation with Standard Error (SE), Adjusted Odds Ratios, and 95% Confidence Intervals

Parameter	Estimate	SE	Adjusted Odds Ratio	Confidence Interval		Wald χ^2	p Value
				Lower.95	Upper.95		
Intercept	−1.825	0.659				7.663	.006
Taper (0.06 vs 0.04)	0.702	0.647	2.018	0.576	7.544	1.178	.278
Tooth type (second vs first molar)	−1.084	0.724	0.338	0.073	1.313	2.238	.135
Canal type (III vs II)	0.376	0.629	1.456	0.419	5.090	0.356	.551
Method of (ultrasound vs instrumentation no ultrasound)	1.943	0.633	6.979	2.148	26.385	9.427	.002

The MiniEndo system is a piezoelectric unit that does not require an external cooling source and is more powerful than magnetorestrictive units. Ahmad et al (36) reported that cleaning within a canal, via cavitation, occurs at the tip of the ultrasonic file. They also reported that high energy is required to allow cavitation to occur. In this study, cavitation was achieved by using the MiniEndo system at full power capacity. Apical preparations within this study were never larger than a size #30 file. The 25-gauge ultrasonic needle had an outside diameter of 0.50 mm (size #50 file), and the ultrasonic needle depth was 6−7 mm short of the apical preparation. The ultrasonic needle, on average, was used at 64% (13.3 mm) of working length in Group 2 experimental teeth. In this study, the action of the ultrasonically activated irrigation included both cavitation and acoustic streaming. Although the needle was not placed to the complete depth of the preparation, the high energy generated by the ultrasound unit and the use of sodium hypochlorite resulted in less bacterial growth. No needle breakage occurred during the study.

In conclusion, the addition of ultrasonic irrigation after hand and rotary cleaning and shaping significantly reduced CFU counts and was 7 times more likely to yield a negative culture than hand and rotary instrumentation alone. Reducing bacterial levels within infected molar root canals should, empirically, improve the success rate of endodontic therapy on these teeth. The ultrasonic irrigating device used in our study might help to achieve this goal.

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