

The In Vivo Evaluation of Hand/Rotary/Ultrasound Instrumentation in Necrotic, Human Mandibular Molars

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Abstract

This in vivo, prospective, randomized, single-blinded study histologically compared biofilm/necrotic debridement efficiency of a hand/rotary technique versus a hand/rotary/1 min ultrasound technique in the mesial roots of necrotic, human mandibular molars. The hand/rotary group consisted of 20 mesial roots. The hand/rotary/ultrasound group consisted of 20 mesial roots prepared with the same hand/rotary technique followed by 1 min of ultrasonic irrigation, per canal, utilizing an ultrasonic needle in a MiniEndo™ unit. Following extraction, histologic preparation and staining, 0.2 μm cross-sections from the 1- to 3-mm apical levels were evaluated for percentage of biofilm/necrotic debris removal. Cleanliness results at the 1-, 2- and 3-mm levels for the hand/rotary and hand/rotary/ultrasound techniques, respectively, were: Canals, 80% versus 95%, 92% versus 99%, and 95% versus 100%; Isthmuses, 33% versus 83%, 31% versus 86%, 45% versus 91%. Statistical analysis revealed mean percent canal and isthmus cleanliness values to be significantly higher for hand/rotary/ultrasound technique at all levels evaluated. (*J Endod* 2007;33:782–787)

Key Words

Biofilm, necrotic debris, ultrasonic irrigation, ultrasound

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The goal of endodontic therapy 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 has complicated the 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, microbes and debris following instrumentation (1–12).

Biofilm has been described as an aggregation of bacteria associated with a surface and embedded in an extra-cellular matrix of polysaccharide. Millward and Wilson (13) reported that biofilm bacteria differ greatly in phenotype when compared to their planktonic counterparts and are far less susceptible to antimicrobial killing. Mah et al. (14) and Stewart et al. (15) described potential mechanisms of biofilm resistance to antimicrobial agents. This includes resistance to penetration of the antimicrobial agent through the biofilm matrix; bacteria entering a non-growing, protective state due to decreasing concentrations of nutritive substrate or increases in inhibitive waste product accumulation; and formation of bacteria in a unique, highly protected phenotypic state similar to a spore. Nair et al. (16) looked at the microbial status of the apical root canal system of necrotic, mandibular first molars with apical periodontitis after single-visit endodontic therapy. After removal of the apical root segments and evaluation using correlative light and transmission electron microscopy, they found that 14 of 16 teeth had residual intracanal infection. They noted that the microbes were located in inaccessible recesses, isthmuses and accessory canals mostly as biofilm.

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 following completion of hand or rotary instrumentation has been shown to reduce the number of bacteria (17–22). Carver et al. (22) found that the use of ultrasonic irrigation following hand/rotary instrumentation in vivo produced a significantly greater reduction in CFU counts in infected necrotic human molars. Additionally, a significantly higher percentage of canals cultured no bacteria following the addition of ultrasonic irrigation (80%) than following hand/rotary instrumentation alone (27%).

No study to date has examined the efficacy of an ultrasonic irrigating needle as an adjunct to hand/rotary instrumentation in the debridement of bacterial biofilm/necrotic debris in necrotic human molars. The needle, when connected to a MiniEndo™ piezoelectric ultrasonic system, has been reported to have high ultrasonic output and produce cavitation in an instrumented canal (12). This system has been 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 histologically compare biofilm/necrotic debris debridement efficacy of a hand/rotary instrumentation technique to a hand/rotary instrumentation plus one minute ultrasound technique in the mesial roots of human, necrotic mandibular molars.

Materials and Methods

Forty-eight adult subjects participated in this study. The Human Subjects Review Committee of The Ohio State University approved the study, and written informed consent was obtained 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 percus-

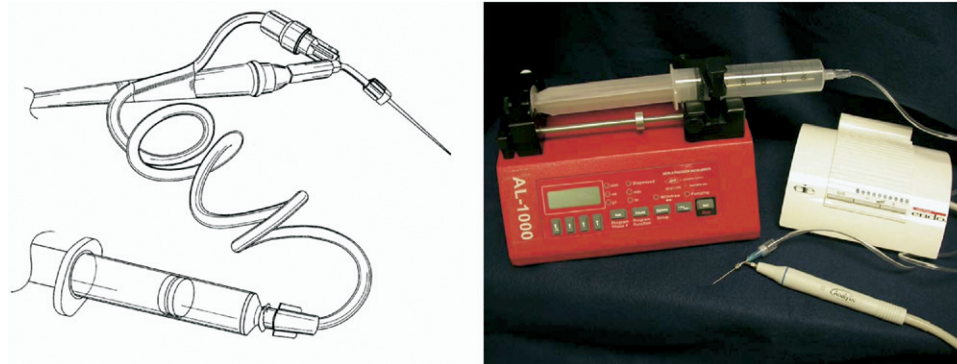


Figure 1. Diagrammatic representation and perspective view of the ultrasonic irrigating device utilized in this study.

sion testing indicated all mandibular molars had an initial diagnosis of pulpal necrosis with acute or chronic periapical periodontitis. If, upon access of the pulp chamber, vital tissue was encountered, 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.

Prior to initiation of the study, random six-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 prior to hand/rotary instrumentation being 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 was completed. Both canals of the mesial root of the experimental mandibular molars received the same treatment.

The 48 experimental teeth were randomly divided into three groups. Group 1 consisted of 20 teeth prepared using a manual hand-file/rotary instrumentation cleaning and shaping technique but with no ultrasonic irrigation. Group 2 consisted of 20 experimental teeth prepared with the same manual hand-file/rotary instrumentation cleaning and shaping technique followed by 1 min of ultrasonic irrigation per canal. Initial radiographs of the experimental teeth were taken with a parallel film holder and were analyzed using Schneider's (23) method 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 multiplied to give an estimated area (mm^2). Group 3 consisted of 8 freshly extracted teeth that received no endodontic treatment. These teeth served as histologic controls and were also determined to have necrotic pulps and apical periodontitis.

After achieving adequate anesthesia and application of the rubber dam a standard access opening was made with a #4 round bur in a high-speed handpiece. The presence/absence of pulpal hemorrhage was noted as confirmation of pulpal necrosis.

Group 1 and 2 Canal Preparation:

K-type hand files (Dentsply Maillefer, Tulsa, OK) and rotary ProFile® GT® (Dentsply TulsaDental, Tulsa OK) 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 utilized 6.0% sodium hypochlorite (The Clorox Co., Oakland, CA). Final apical preparation was to a size 30 hand file.

Group 1 and 2 Post-instrumentation irrigation

Following completion of hand/rotary cleaning and shaping, Group 1 teeth received an additional 15 ml of 6.0% sodium hypochlorite to irrigate each mesial canal delivered via a 30 ml syringe placed in an Aladdin mechanical pump (World Precision Instruments, Sarasota, FL) set at 15 ml/min. and delivered through IV tubing to a 25-gauge, 1.5 inch sterile needle (Becton Dickinson & Co., Franklin Lakes, NJ).

Group 2 received 1 min of ultrasonic irrigation. The ultrasonic unit used was a MiniEndo™ (Spartan EIE Inc., San Diego, CA). 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 (Fig. 1). The needle was at a 45° 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 intravenous tubing connected the needle to a 30 ml syringe containing 30 ml of 6.0% sodium hypochlorite. The syringe was placed in an Aladdin mechanical pump (World Precision Instruments, Sarasota, FL) to dispense the irrigating solution at a precise and constant flow.

Each canal in Group 2 was filled with 1 ml of 6.0% sodium hypochlorite prior to ultrasonic irrigation. Prior to activation of the ultrasonic unit, a sterile silicon stopper was placed on the irrigating needle and the needle inserted into the canal to a point just short of binding. The silicon stopper was adjusted to this length and then measured with a millimeter ruler to determine the depth of penetration of the ultrasonic irrigating needle. High-speed suction, 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 pre-measured depth and, upon 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 min per mesial canal while the sodium hypochlorite was delivered at a rate of 15 ml per minute. The same ultrasonic technique and needle was used for each canal. After completion of the hand/rotary/ultrasonic preparation, the canals were dried with coarse and medium paper points.

In both groups, a sterile cotton pellet was then placed in the pulp chamber of each tooth and the access sealed with Cavit™ (3M ESPE AG, Seefeld, Germany). The teeth were immediately extracted using a root resection surgical technique. Each canal was irrigated with 1 ml of 6.0% sodium hypochlorite to remove any possible pieces of loose Cavit™ or blood that had entered the canal during the extraction process. Each

TABLE 1. Summary of Mean Percentage Cleanliness (\pm SE)

Level (mm)	Canal Cleanliness						Isthmus Cleanliness							
	n	Group 1 Hand/Rotary No Ultrasound	n	Group 2 Hand/Rotary Ultrasound	p Value*	n	Group 3 Control	n	Group 1 Hand/Rotary No Ultrasound	n	Group 2 Hand/Rotary Ultrasound	p Value*	n	Group 3 Control
1.0	39	80.1 \pm 24.8	36	94.7 \pm 7.6	0.0022	13	37.6 \pm 28.0	12	33.3 \pm 29.7	11	82.8 \pm 17.8	0.0002	2	0.00 \pm 0.0
1.2	40	80.7 \pm 25.1	38	97.8 \pm 4.2	< 0.0001	13	35.1 \pm 25.7	15	31.5 \pm 32.2	17	88.6 \pm 12.8	< 0.0001	3	21.6 \pm 8.6
1.4	40	81.8 \pm 24.0	38	97.7 \pm 4.8	< 0.0001	13	35.0 \pm 21.8	16	36.0 \pm 31.1	17	87.9 \pm 14.1	< 0.0001	2	8.7 \pm 12.3
1.6	40	87.0 \pm 22.5	38	98.3 \pm 4.1	0.0016	13	34.1 \pm 21.8	17	29.8 \pm 30.7	17	86.0 \pm 16.9	< 0.0001	2	6.8 \pm 9.6
1.8	41	89.0 \pm 19.1	38	98.9 \pm 3.4	0.0014	13	30.9 \pm 23.1	16	35.7 \pm 31.6	18	85.8 \pm 17.8	< 0.0001	2	9.3 \pm 13.2
2.0	41	91.6 \pm 16.0	38	99.0 \pm 3.0	0.0070	13	32.2 \pm 21.9	15	31.4 \pm 28.9	18	86.1 \pm 17.0	< 0.0001	3	22.6 \pm 20.7
2.2	41	88.4 \pm 22.7	38	97.0 \pm 14.7	0.0486	14	35.2 \pm 23.8	16	36.0 \pm 30.0	18	88.1 \pm 14.3	< 0.0001	3	28.8 \pm 25.0
2.4	41	92.2 \pm 17.5	38	99.7 \pm 1.5	0.0070	14	35.4 \pm 26.1	15	31.5 \pm 26.4	18	87.2 \pm 14.9	< 0.0001	2	17.7 \pm 25.0
2.6	41	90.9 \pm 19.9	38	99.6 \pm 1.5	0.0037	14	34.1 \pm 25.9	16	33.8 \pm 25.8	17	89.9 \pm 14.3	< 0.0001	2	16.6 \pm 23.5
2.8	41	94.0 \pm 13.5	38	99.6 \pm 1.4	0.0096	14	35.3 \pm 26.8	17	35.2 \pm 31.1	16	89.5 \pm 14.3	< 0.0001	2	9.8 \pm 13.9
3.0	41	95.1 \pm 13.0	38	99.6 \pm 1.3	0.0288	14	38.7 \pm 26.0	16	44.6 \pm 28.4	16	91.1 \pm 14.9	< 0.0001	2	11.2 \pm 15.9

*Bonferroni adjusted p value.

canal was then irrigated with 1 ml of 10% formalin to allow for fixation of any remaining pulpal tissue. A 0.5 mm vertical groove was placed in the buccal surface of the mesial root with a #1/2 round bur to help maintain root orientation and aid in canal identification. Following groove placement, each tooth was immediately placed into a 20 ml vial of 10% formalin and labeled with the random six-digit number. Teeth remained in these vials until histologic processing was started.

Histologic Preparation

Following fixation for 1 month, all teeth were decalcified in an aqueous solution of equal parts 50% formic acid and 20% sodium citrate for 10 days. Teeth were then dehydrated and infiltrated using Skinner’s method (24). Following infiltration, the teeth were placed in an embedding boat containing Paraplast (Sherwood Medical Ind. Inc., St Louis, MO). The specimens were oriented in two planes to produce perpendicular sections from the same level in both root canals.

After the Paraplast had set, 5 μ m sections were obtained using an American Optical Model 820 microtome (American Optical Co., Buffalo, NY). Sections were collected until the apical foramen was located. Starting at the 1 mm level from the apical foramen, slides were prepared of 4 sections every 0.2 mm until the 3 mm level was reached. Slides were stained using Brown and Brenn stain for bacteria (Poly Scientific R&D Corp., Bay Shore, NY). The best, technically error-free section was chosen from each stained slide for evaluation. All slides were labeled with the specimen’s six-digit random number and sequentially numbered according to the order in which they were taken and evaluated blindly as to the method of canal preparation.

Method of Evaluation

Blinded and mounted sections from all three groups were evaluated by the primary investigator (AB) using a computer (Dell, Intel Pentium IV, Windows 2005) attached to a Nikon Eclipse e600 microscope (Nikon, Melville, NY). NeuroLucida Image Analysis Program version 5.0 (MicroBrightField, Inc., Colchester, VT) was used to measure the area of the root canals and isthmuses and all biofilm/necrotic debris contained within them. The full-color section was projected onto the monitor screen at 40x, 100x and 200x magnification. The magnification selected to evaluate cleanliness was determined by the largest magnification in which the entire canal/isthmus could be viewed in its entirety.

Since the Brown and Brenn stain for bacteria was utilized, the following structures were identified by the following colors: gram negative organisms-pink to red; gram positive organisms-blue; nuclei-red; filaments of Nocardia and Actinomyces- blue; and tissue elements-yellow (25). These colors helped in identifying bacteria from tissue, but the type of bacteria present in each section were not recorded. A com-

puter mouse was used to trace the outline of the root canal. The area of the canal space was calculated (μ m²) by the software. The measurement was performed three times and the average of the three measurements was used to get the final area that was recorded to control for potential evaluator error and reliability. Anything in the canal system other than dentin was considered bacterial biofilm/necrotic debris. The area of remaining biofilm/necrotic debris was calculated in the same manner just described. If more than one area of remaining bacterial biofilm/necrotic debris was present within each section being analyzed, the cursor was moved to each separate area and identified to the computer and the area calculated. The total area of bacterial biofilm/necrotic debris was calculated by adding together all the separate “remaining bacterial biofilm/necrotic debris” areas using a calculator. Each of these measurements was performed three times taking the average of the three to determine the final area recorded.

The isthmuses between canals were traced separately from the primary root canals, and their total area and the area of their remaining bacterial biofilm/necrotic debris recorded as described above. Tracing both the mesiobuccal and mesiolingual canals differentiated the boundaries between the canals and the isthmuses. When tracing canals, the area connected to the isthmus was traced last. In this area the canal outline was closed by drawing a connecting line which followed the arc formed by the circular shape of the canal. After completion of this step, the remaining canal area was identified as the isthmus.

In order to calculate canal cleanliness, the area of remaining bacterial biofilm/necrotic debris was divided by the total area of the canal or isthmus to yield the percentage of remaining debris. The percentage of the debris removed, or cleanliness, was determined by subtracting the percentage of remaining debris from 100 percent.

The Randomization test was used to statistically analyze mean cleanliness values for both canals and isthmuses (Table 1). The raw P values were then adjusted by the step-down Bonferroni method of Holm. Fischer’s Exact Tests were used to analyze differences in tooth and canal type. Curvature was analyzed for differences between the groups using χ^2 test. Comparisons were considered significant at p < 0.05. Statistical analysis was run using the SAS program (version 9.1).

Results

A comparison of canal and isthmus cleanliness is shown in the Table 1. Fig. 2 shows examples of canal preparation for the hand/rotary group and hand/rotary/ultrasound group.

For canal cleanliness, there were significant differences at all eleven apical levels between the two techniques (Table 1). Isthmus

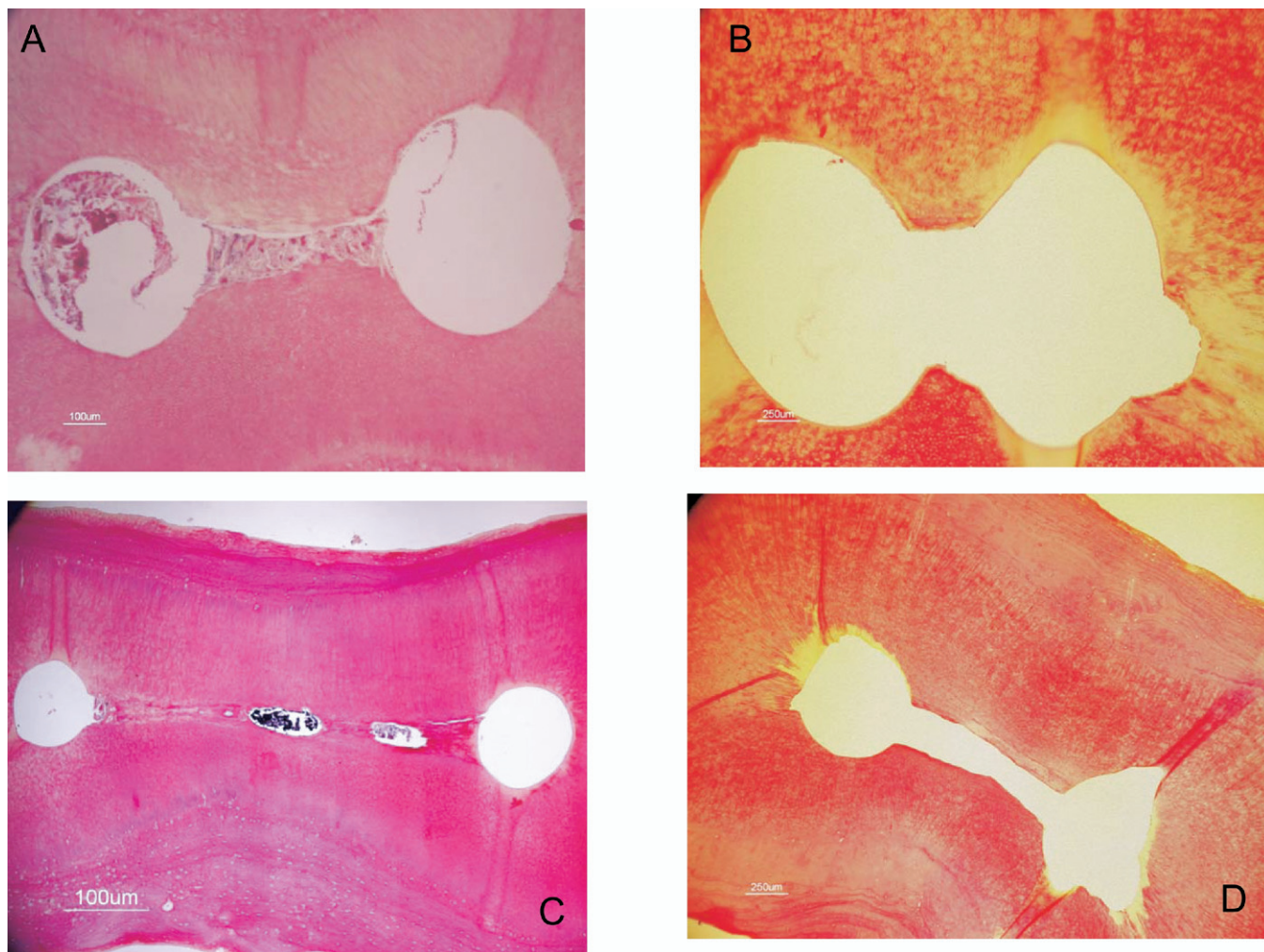


Figure 2. A. Photomicrograph of representative cross-sections at: (A) the 1.0 mm level: hand/rotary group (1) – 94% cleanliness in mesiolingual canal (right), 58% cleanliness in mesiobuccal canal (left) and 14% cleanliness in isthmus, original magnification 40x; (B) the 1.2 mm level: hand/rotary/ultrasound group (2) - 100% canal and isthmus cleanliness, original magnification 40x; (C) the 1.8 mm level: hand/rotary group (1) - 93% cleanliness in mesiolingual canal (left), 100% cleanliness in mesiobuccal canal (right) and 10% cleanliness in isthmus, original magnification 40x; (D) the 2.6 mm level: hand/rotary/ultrasound group (2) - 100% canal and isthmus cleanliness, original magnification 40x. All utilized the Brown and Brenn stain.

cleanliness values were significantly different between the two treatment groups at all eleven levels examined (Table).

The uninstrumented control teeth (Group 3) showed mean canal cleanliness values ranging from 30.9% at the 1.8 mm level to 38.7% at the 3.0 mm level (Table). Mean isthmus cleanliness values ranged from 0% at the 1.0 mm level to 28.8% at the 2.2 mm level.

The distribution of teeth was as follows: first molars – 70% (14/20) Group 1, 70% (14/20) Group 2; second molars – 25% (5/20) Group 1, 25% (5/20) Group 2; third molars – 5% (1/20) Group 1, 5% (1/20) Group 2. There were no significant differences between the groups ($p = 1.000$, Fisher's Exact test).

Clinical and histologic typing of the mesial roots was as follows: type III – 95% (19/20) Group 1; 85% (17/20) Group 2; type II – 5% (1/20) Group 1, 15% (3/20) Group 2. There were no significant differences between the groups ($p = 1.000$, Fisher's Exact test).

Curvature of the canals, as determined by Schneider's method (23), were as follows: moderate (less than 25 degrees) – 85% (17/20) Group 1, 65% (13/20) Group 2; severe (26 to 52 degrees) – 15% (3/20) Group 1, 35% (7/20) Group 2. There were no significant differences between the groups ($p = 0.1441$, χ^2 test).

Discussion

The mean canal cleanliness values (Table 1) for the hand/rotary instrumentation group are better as compared to those reported by other authors utilizing step-back preparation method (2–4) and similar to those reported by Gutarts et al. (12). These studies all utilized vital teeth and therefore more tissue had to be removed as compared to the necrotic root canals (Group 3 – Table) used in this study. The addition of 1-minute of ultrasonically activated NaOCl significantly improved the overall mean canal cleanliness values at all eleven apical canal levels. The addition of ultrasonic irrigation also produced more consistent cleaning of the canals as demonstrated by the low standard deviations seen at all of levels in Group 2 as compared to Group 1 (Table).

Isthmus cleanliness values remained constant from the 1-mm to 3-mm level within the hand/rotary group (Group 1) with cleanliness values only ranging from 30% to 45%. These results are similar to those reported by Lev et al. (3), Goodman et al. (2), Haidet et al. (4), Metzler et al. (5), Archer et al. (11), and Gutarts et al. (12). The values are very similar to those found in the control group (Group 3) and may confirm

that complete tissue/biofilm/debris removal from isthmuses of teeth is impossible with hand or hand/rotary instrumentation alone. The addition of 1-minute of ultrasonically activated irrigation following hand/rotary instrumentation significantly increased the cleanliness values (83% to 91%) at all levels evaluated (Table). While increasing cleanliness significantly, complete debridement of the isthmuses was not always attained. We did observe some biofilm/necrotic debris remaining in very narrow isthmuses; however, ultrasonic irrigation was significantly better than hand/rotary instrumentation alone.

Carver et al. (22) found that the use of ultrasonic irrigation following hand/rotary instrumentation in vivo produced a significantly greater reduction in CFU counts in infected necrotic human molars. Additionally, a significantly higher ($p = 0.0047$) percentage of canals cultured no bacteria following the addition of ultrasonic irrigation (80%) than following hand/rotary instrumentation alone (27%). Fabricius et al. (26) have indicated that bacteria-free canals are more apt to heal periapically (both radiographically and histologically) as compared to teeth where bacteria remained in canals in 2-2.5 yr follow-up study in primates.

Joyce et al. (27) reported that low-frequency ultrasound causes de-agglomeration of bacterial biofilms via the action of cavitation. This de-clumping of bacterial cells within a root canal may 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 continuous reduction of bacterial cell numbers. The cavitation produced may also cause temporary weakening of the cell membrane making the bacteria more permeable to sodium hypochlorite. The de-aggregation 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 6% sodium hypochlorite, is the most likely reason for the trend toward greater reduction of intracanal biofilm observed in our study.

The finding of no significant differences between the two groups for tooth distribution, mesial root morphology and root curvature suggests that the groups were of equal difficulty. The in vivo nature of this study makes isolation, access, working length determination and instrumentation more clinically relevant than in vitro studies. That is, in vitro studies can control for poor access, can determine working lengths accurately by visualizing files at the apical foramen and instrument teeth without regard to difficult access or clinical time constraints. Therefore, in vivo studies may be more clinically relevant than in vitro studies.

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. (28) 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 have cavitation occur. In this study, cavitation was achieved by utilizing the MiniEndo™ system at full power capacity. Acoustic streaming, on the other hand, occurs on the sides of the ultrasonic file and can occur with either high or low energy (28–30). Apical preparations within this study were never larger than a size #30 file and the 25-gauge ultrasonic needle had an outside diameter of 0.50-mm (size #50 file). Therefore, the ultrasonic needle reached only to within 4-5 mm of the apical preparation. 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 canal preparation, the high energy generated by the ultrasound unit, and use of sodium hypochlorite, resulted in statistically cleaner canals and isthmuses. Improved canal and isthmus cleanliness may be possible if longer ultrasound times are utilized. Further studies could evaluate additional ultrasonic time. However, increasing the time of treatment may become inconvenient in clinical practice.

The use of an ultrasonic irrigating needle allowed for the continual deposition and renewal of irrigating solution within the canal. This differed from previous studies (2–5, 11) where irrigant was delivered only into the coronal access opening. The delivery of fresh irrigating solution within the root canal may also have contributed to the improved cleanliness values. However, canal and isthmus mean cleanliness values never reached 100%.

In conclusion, the 1-minute use of ultrasonically activated irrigation, following hand/rotary root canal cleaning and shaping, has been shown to improve canal and isthmus cleanliness in terms of necrotic debris/biofilm removal. Empirically this should improve the clinical outcomes of these types of cases. However, further research is needed.

Acknowledgment

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The ultrasonic irrigating device has been patented and is owned by The Ohio State University. Dr. Nusstein is the inventor with potential financial interest in this device.

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