The Influence of Canal Curvature on the Mechanical Efficacy of Root Canal Irrigation In Vitro Using Real-Time Imaging of Bioluminescent Bacteria

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Abstract

There are no quantitative data on the mechanical efficacy of irrigation in the removal of bacteria from curved canals. This study quantitatively analyzed the effects of root canal curvature and preparation size on the mechanical efficacy of irrigation using 33 mandibular single-rooted bicuspids allocated to groups according to root canal curvatures, group 1 (straight) 4 to 8 degrees, group 2 (intermediate curvature) 15 to 19 degrees, and group 3 (greatest curvature) 24 to 28 degrees. Teeth were sequentially instrumented to sizes 27/.04, 36/.04, and 46/.04 using a crown-down technique. Suspensions of the bioluminescent reporter strain *Pseudomonas fluorescens* 5RL (1.5×10^6 cells) were inoculated into canals of sterilized teeth after each sequential instrumentation. Canals were irrigated with 6 ml of irrigant delivered 1 mm from working length using a 30-gauge needle. Remaining bacteria were quantified using real-time bioluminescent imaging. Irrigation was significantly less effective in 24 to 28 degrees curvature canals prepared to size 27/.04 compared to 46/.04 ($p < 0.007$, repeated-measures ANOVA). *(J Endod 2006;32: 1077–1080)*

Key Words

Bacteria, bioluminescence, curved root canals, endodontic, irrigation, preparation size

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Microorganisms have been shown to induce pathological changes in the dental pulp and inflammatory lesions in the periradicular tissues of teeth (1, 2). Therefore, an important objective of endodontic therapy is the elimination of infected pulp tissue and intracanal bacteria via biomechanical instrumentation and intracanal irrigation (3–8). Many clinical and in vitro studies have demonstrated that root canal irrigation is variably effective in removing debris and microorganisms from the root canal system (3–14). Specifically, irrigation has been shown to be more efficacious with increased canal preparation size and taper (7, 14–16), closer proximity of the irrigant to the apex (10–13), and increased volume of irrigant (13). However, these studies are limited to those using straight root canals.

Histological studies have demonstrated that complete cleaning of curved root canal systems is difficult, if not impossible, to accomplish regardless of instrumentation techniques (20–22). In an effort to address this problem, innovative instruments and instrumentation techniques have been developed and evaluated specifically for curved root canal systems (17–24). In contrast, universal irrigation protocols are adopted for both straight and curved root canals. A more comprehensive understanding of the mechanical effectiveness of irrigation on bacterial debridement could provide new information to facilitate irrigation regimens for curved root canals.

There are no quantitative data on the mechanical efficacy of irrigation in the removal of bacteria from curved canals. The aim of this study was to quantitatively analyze and compare the mechanical efficacy of irrigation on bacterial debridement in curved and straight root canals over sequential treatment procedures using real-time imaging of bioluminescent bacteria. The hypotheses tested were that the mechanical efficacy of irrigation is dependent on: (a) root canal curvature, and (b) canal preparation size.

Materials and Methods

Teeth

Thirty-three single-rooted mandibular bicuspids with clinically intact crowns, no restorations, no cracks, and no caries were used. During the tooth selection process, each tooth was radiographed from both mesial and buccal views to assess canal morphology. Radiographs were digitized and stored electronically. Root canal curvature was determined based on angle of curvature initiated at the coronal aspect of the apical third of the root using established criteria (25). Angles of curvature were measured using an image analysis program (ImageJ 1.33u, NIH, Bethesda, MD) and images were stored electronically. Only those teeth identified as having normal anatomy and narrow single canals (estimated under ×10 magnification from both buccal and mesial views to be less than #27/.04 taper, corresponding to the smallest instrumentation size to be used) were used in the investigations. Tooth length was standardized to 22 mm to prevent the introduction of confounders that might contribute to variations in the efficacy of irrigation. At the conclusion of the selection process, three groups of 11 teeth were identified with distinct ranges of angles of root canal curvature: Group 1 (straight) 4 to 8 degrees, group 2 (intermediate curvature) 15 to 19 degrees, and group 3 (greatest curvature) 24 to 28 degrees.

Coronal access openings were prepared using tungsten carbide burs in a high-speed handpiece with constant water spray. Root canal working length (WL) was set at 21 mm which corresponded to 1 mm short of the apical foramen. Canals were instru-

mented using Orifice openers and Profile .04 Series 29 Rotary Ni-Ti files (Dentsply Tulsa Dental Products, Tulsa, OK) as previously described (14).

Preparation of Teeth for Sequence 1 (size 27/.04), 2 (size 36/.04), and 3 (size 46/.04)

All teeth were instrumented to a final apical size 27/.04 for the first experiment (sequence 1). Copious irrigation with 5.25% sodium hypochlorite was used during instrumentation. The smear layer was removed from canal walls by ultrasonic treatment for 4 minutes each in 17% EDTA and 5.25% NaOCl (26), after which canals were rinsed copiously with sterile distilled water and ultrasonically treated for 4 minutes with distilled water. The apices of teeth were coated with clear nail varnish to seal the apical foramen. Teeth were autoclaved at 121°C for 15 minutes at 26 psi and stored aseptically in 100% humidity at 25°C until use. Canals were dried with sterile paper points (Kerr USA, Romulus, MI) immediately before each experiment.

The above procedures were repeated on the same 33 teeth for the experiments using final preparation sizes 36/.04 (sequence 2) and 46/.04 (sequence 3).

Bacteria

All investigations used *Pseudomonas fluorescens* 5RL, a salicylate inducible bioluminescent reporter strain harboring the plasmid pUTK21, a *nah sal-lux* reporter plasmid (approximate size 120 kb) that contains a salicylate-inducible *luxCDABE* gene cassette from *Vibrio fischeri* (27, 28). Bioluminescent bacteria cultures were prepared as previously described (14). Subcultures were grown to OD_{600} 0.190 measured using a spectrophotometer (Spectronic 20D+, Thermo Electron Corporation, Pittsburgh, PA) with corresponding bioluminescence of 7.5×10^5 relative light units (RLU) measured using a single tube luminometer (Sirius-0, Zylux Corp., Oak Ridge, TN). Viable cell counts were determined for each sequence as previously described (14).

Standard microbiological negative controls were employed for each experiment to confirm noncontamination of culture media and suspension dilutants. *P. fluorescens* 5RL growth was confirmed on selective media [LB agar supplemented with salicylate (50 μg/ml) and tetracycline (16 μg/ml)], and Gram stained preparations were made from broth cultures to confirm expected cell morphology and Gram reaction. As a positive control to confirm the maintenance of bioluminescent activity of *P. fluorescens* 5RL over the duration of each experiment, bioluminescence was measured in additional aliquots of inoculum bacterial suspension in test tubes at the start and conclusion of each experiment using a single tube luminometer.

Undiluted bacterial suspension in LBS (15 μl) was pipetted into each root canal using sterile plastic pipets with an external diameter of 0.25 mm (Fisherbrand, Fisher Scientific, Pittsburgh, PA). The pipet tips were confirmed to fit loosely at the WL in root canals prepared to size 27/.04. Pipet tip placement for inoculation of bacteria was at WL for each experimental sequence.

Irrigation

A standardized irrigation protocol was followed. A new sterile needle and syringe combination was used for each procedure for each tooth. Irrigation (6 ml) with sterile H_2O/Sal was delivered at a rate of 1 ml/20 second via a sterile 30 gauge safety-ended endodontic irrigating needle (Max-I Probe, Dentsply Limited, Waybridge, Surrey, England) attached to a 10 ml sterile plastic syringe (BD Syringe, Becton Dickinson and Company, Franklin Lakes, NJ). The needle tip was placed 1 mm short of the WL. After irrigation, contents were immediately aspirated. An aseptic technique was strictly adhered to at all times as previously described (12, 13).

Measurement of Bioluminescence in Teeth

Bioluminescent imaging (BLI) was performed at the Michigan Small Animal Imaging resource (Department of Radiology, University of Michigan, Ann Arbor, MI) as previously described (12). Images and photon counts were obtained before inoculation of bacteria (background and negative control), after inoculation of bacteria, and after irrigation. Photons from bioluminescent bacteria in each tooth after inoculation and irrigation were obtained by subtracting background photon counts. A $\log_{10}$ transformation of bacterial photons was performed as previously described (12).

One tooth in each group served as a positive control to confirm the continuation of bioluminescent activity of *P. fluorescens* 5RL over the duration of each experiment. Positive control teeth were inoculated and imaged concurrently with experimental teeth.

Statistical Analysis

A repeated measures ANOVA was performed, which included the main effects of root canal curvature (a between-tooth factor) and canal preparation size (a within-tooth factor), and the interactions between root canal curvature and canal preparation size. The linear mixed-effects model (MIXED) procedure in SPSS 13.1 (SPSS, Inc., Chicago, IL) was used for the analysis. The Bonferroni adjustment method was used for post hoc comparisons of the estimated marginal means for the effect of canal preparation size within each root canal curvature group, and the effect of root canal curvature within each canal preparation size. Significance was set at $p < 0.05$.

Results

Viable counts confirmed that approximately 1.5×10^6 bacterial cells were inoculated in each canal for each experimental sequence. Results from all controls were as expected. Data were lost for five samples in group 2 and one sample in group 3 during sequence 1 and were therefore unavailable for analysis.

The percentage, estimated numbers, and $\log_{10}$ reduction of bacteria remaining in the root canals after irrigation are shown in Table 1. Statistical analyses were carried out on the log-log scores, which were calculated by taking the $\log_{10}$ of the original log-change score, to improve the normality of the residuals. The interactions between the main effects of preparation size and root canal curvature indicated that there was a tendency for the effects of preparation size on irrigation efficacy to differ for the various root canal curvature groups ($p = 0.099$) (Table 2). Bonferroni post hoc tests showed that the efficacy of irrigation in teeth that exhibited the greatest root canal curvature (24-28 degrees) was significantly less for apical preparation sizes of 27/.04 compared to 46/.04 ($p = 0.007$) (Table 3); Fig. 1 shows images of a representative tooth in this group. The mechanical efficacy of irrigation of canals in teeth with the least (group 1) or intermediate (group 2) root canal curvatures showed no significant difference with increasing apical preparation sizes ($p > 0.05$) (Table 2).

Discussion

This study quantitatively evaluated the effect of root canal curvature on the mechanical efficacy of irrigation to reduce bacteria in curved canals in real-time. Only in canals with the greatest root curvatures of 24 to 28 degrees (group 3) was apical preparation size a contributing factor to irrigation efficacy. Approximately 50% of bacteria remained in canals with curvatures of 24 to 28 degrees instrumented to size 27/.04 after 6 ml of irrigation. This finding suggests that increased root canal

TABLE 1. Bacteria remaining in root canals following irrigation of sequentially enlarged canals of different curvature

Root canal curvature	Preparation size		
	27/.04	36/.04	46/.04
A. Mean $\log_{10}$ reduction of bacteria in root canal~			
4°–8°			
Mean	0.61	0.78	0.61
SD	0.37	0.77	0.51
15°–19°			
Mean	0.57	0.79	0.52
SD	0.49	0.59	0.42
24°–28°			
Mean	0.39	0.51	0.70
SD	0.35	0.21	0.25
B. Percentage of bacteria remaining in root canal (%)			
4°–8°			
Mean	32.42	33.10	35.37
SD	22.93	25.17	20.42
15°–19°			
Mean	38.80	26.80	40.80
SD	30.60	21.92	29.51
24°–28°			
Mean	50.86	34.30	22.08
SD	37.69	16.83	12.44
C. Estimated numbers of bacteria remaining in root canal*			
4°–8°			
Mean	4.9.E + 05	5.0.E + 05	5.3.E + 05
SD	3.4.E + 05	3.8.E + 05	3.1.E + 05
15°–19°			
Mean	5.8.E + 05	4.0.E + 05	6.1.E + 05
SD	4.6.E + 05	3.3.E + 05	4.4.E + 05
24°–28°			
Mean	7.6.E + 05	5.1.E + 05	3.3.E + 05
SD	5.7.E + 05	2.5.E + 05	1.9.E + 05

~Statistical analyses were conducted using "Mean $\log_{10}$ reduction" data. The percentage and estimated numbers of remaining bacteria are included for clarification.

*Based on viable counts, approximately 1.5×10^6 bacteria were inoculated into each root canal.

curvature impedes the flow of irrigant, thereby reducing its flushing ability and decreasing its mechanical efficacy. This is supported by the finding that increasing the apical preparation size to 46 with .04-tapered files significantly improved irrigation efficacy (Table 2, Fig. 1). The latter could be attributable to a combination of both the larger apical preparation size and straightening of the root canal consequent to enlargement with a continuous .04-tapered file. A straighter line access for the irrigation needle might allow more effective flow of the irrigant to mechanically flush bacteria from the root canal system (10).

Factors previously shown to play an important role in the efficacy of root canal irrigation include narrower gauge and depth of apical penetration of the irrigation needle (10, 11, 13), apical enlargement of root canals (14), and increasing irrigation volume (13). Chow (10) suggested the use of a 30-gauge needle for irrigating fine curved canals. The 30-gauge needle used in this study had an external diameter of 0.30 mm. Because all the teeth had a minimum canal preparation size of 27/.04, at 1 mm from WL, the internal canal size (because of a continuous .04-taper increase) corresponded to 0.31 mm, which facilitated placement of a 30 gauge irrigation needle tip to within 1 mm from WL. Narrower gauge (25 gauge) needles were more effective than larger gauge (23 gauge) needles in removing debris from simulated canals; this may have been due in part to a smaller needle able to penetrate further apically (10). However, a narrower gauge needle provides more resistance to flow, if the same pressure is applied, thereby increasing the time needed to deposit irrigant (10). In pilot studies, expression of 6 ml irrigant using a 30-gauge needle required approximately 2 minutes, almost four times longer compared to 30 second using a 28 gauge needle as previously reported (13, 14). Regardless, the efficacy of irrigation to reduce a comparable bacterial load in straight cuspids prepared to 36/.04 was similar using 28 gauge (14) and 30 gauge (this study) needles; after 6 ml irrigation the remaining bacterial load was 27% and 33% (Table 1), respectively. These observations suggest that factors other than needle gauge may be more important to the mechanical efficacy of irrigation.

In contrast to previous studies that evaluated curved canals using histological methods (20–22), the present study utilized a protocol allowing real-time imaging of bioluminescent bacteria (12). The nondestructive in vitro method uses real-time optical biophotonic imaging and luminometry to visualize and quantify photon emission from the bioluminescent reporter strain *P. fluorescens* 5RL after it has been inoculated into root canals (12). Thereafter a nonantimicrobial irrigant

TABLE 2. Statistical analyses of the combined effects of root canal curvature and preparation size on the efficacy of irrigation

Repeated analysis of variance (ANOVA)	Tests of fixed effects*			
Source	Numerator df	Denominator df	F	Sig†
Preparation size	2	43.386	2.123	0.132
Root canal curvature type	2	22.128	0.135	0.875
Preparation size * root canal curvature type	4	43.409	2.088	0.099

*Dependent variable: $\log\_\log_{10}$.

†The mean difference is significant at the 0.05 level.

TABLE 3. Bonferroni pairwise comparisons of the effects of root canal curvature and preparation size on the efficacy of irrigation

Bonferroni pairwise comparisons	Preparation size		
Root canal curvature	27 versus 36	27 versus 46	36 versus 46
4°–8°	NS	NS	NS
15°–19°	NS	NS	NS
24°–28°	NS	p = 0.007*	NS

*Significance was set at $p < 0.05$.

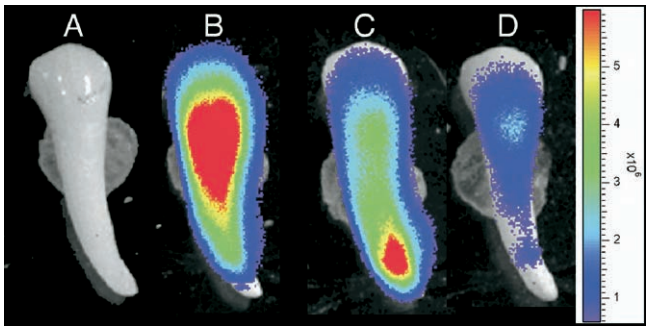

Figure 1. Images of a representative tooth from group 3 showing that irrigation was less effective in teeth with apical preparation sizes of 27/.04 compared to 46/.04 ($p = 0.007$). (*A*) Background, no bacteria, (*B*) 1.5×10^6 *P. fluorescens* 5RL in root canal; (*C*) after 6 ml irrigation using 30 gauge Max-I-Probe needle positioned 1 mm from WL at preparation size 27/.04; (*D*) after 6 ml irrigation in the same tooth prepared to 46/.04. Color bar on right gives luminescence image units (photons/sec/cm^2/sr).

is used to flush out the inoculated bacteria. Notably, each tooth sample serves as its own control throughout sequential instrumentation procedures, thus eliminating the potential impact of variations between individual teeth.

Data analysis was performed using a repeated measures ANOVA on the log-log scale using the linear mixed-effects model (MIXED). This procedure is able to take into account groups with unequal sample sizes and therefore allowed the analysis of incomplete data sets for those teeth for which data were lost. The standard deviations reported in this study were higher than in previous studies using the same method (Table 1; 13, 14), despite strict adherence to a standardized protocol. While log-log transformation of the raw data successfully normalized the residuals for the ANOVA, the reasons for the higher than expected standard deviations are not clear. One possible explanation may lie in the complexity of mandibular premolar anatomy itself. Although the uniformity of canal morphology and preparation shapes were confirmed radiographically, mandibular premolars may present with diverse and intricate ramifications of the main root canal (29), potentially influencing the ability to effectively irrigate these teeth.

Previously it was shown that 6 ml of irrigation delivered 1 mm from the WL using a 28 gauge needle was significantly less effective in teeth with straight canals prepared to size 36/.04 when compared to sizes 60/.04 and 77/.04 (14). In that study, preparation sizes 27/.04 and 46/.04 were not assessed. The present study is the first to evaluate the influence of preparation sizes 27/.04 and 46/.04 on the efficacy of root canal irrigation in variably curved root canals in real-time. Attempts were made to also study preparation size 60/.04 to allow direct comparisons with previous data using the same method (12–14) but instrumentation to size 60/.04 invariably resulted in apical perforation in curved root samples. From a clinical perspective, although increasing apical preparation sizes in curved root canal systems may allow for more effective irrigation, this is not always practical or desirable because of the potential for root perforation, root fracture, or weakening (7, 15, 30). In cases where an enlarged apical preparation is desired in curved root canals, the Lightspeed file system may offer an advantage over a continuous tapered file system because the cutting edge does not remove cervical root structure during the apical preparation, thus minimizing the excess removal of tooth structure (7).

In conclusion, this study demonstrates that instrumentation and apical enlargement of root canal systems with increasing root canal curvatures may facilitate the efficacy of endodontic irrigation. While specific instrumentation techniques have been developed to enhance curved root canal preparation (17–24), consideration could be given to the development of irrigation protocols for curved root canals.

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