Research Article

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Comparative Evaluation of Conventional Syringe Irrigation, Manual Dynamic Agitation, Sonic Activation, Negative-pressure Irrigation and Ultrasonic Activation for the Elimination of Enterococcus Faecalis from Infected Root Canals: An in Vitro Study

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ABSTRACT

Background: Microorganisms persisting within anatomically inaccessible areas of the root canal system can compromise endodontic treatment. Enterococcus faecalis is frequently investigated because of its ability to penetrate dentinal tubules, survive adverse conditions and persist after chemomechanical preparation. Irrigant activation may improve antibacterial effectiveness by promoting irrigant penetration and fluid exchange. Aim: To compare the effectiveness of conventional syringe irrigation, manual dynamic agitation, sonic activation, negative-pressure irrigation and ultrasonic activation in reducing E. faecalis from experimentally infected root canals. Materials and Methods: Sixty-five extracted human mandibular first premolars with single canals and closed apices were prepared to ProTaper F2, sterilised and inoculated with E. faecalis. After seven days of incubation, the specimens were randomly assigned to five groups (n=13): conventional syringe irrigation, manual dynamic agitation, sonic activation, negative-pressure irrigation and ultrasonic activation. Following irrigation, microbiological samples were collected using sterile paper points, serially diluted and cultured on blood agar. Colony-forming units per millilitre (CFU/mL) were calculated. Data were analysed using one-way analysis of variance and post-hoc pairwise comparisons at a significance level of 5%. Results: The representative baseline bacterial count was 1559.50±56.77 CFU/mL. Mean residual counts were 761.31±580.28 CFU/mL with conventional irrigation, 532.15±302.62 CFU/mL with manual dynamic agitation, 368.77±246.30 CFU/mL with sonic activation, 90.92±72.01 CFU/mL with negative-pressure irrigation and 17.77±18.63 CFU/mL with ultrasonic activation. The overall difference was statistically significant ($F=12.49$; $p<0.001$). Ultrasonic activation produced the greatest reduction, followed by negative-pressure irrigation. Conclusion: Ultrasonic activation and negative-pressure irrigation achieved greater

bacterial reduction than the other tested methods. Nevertheless, no technique consistently rendered every root canal culture-negative.

INTRODUCTION

Microbial infection is the principal cause of pulpal and periapical disease. The classic experimental work of Kakehashi et al. demonstrated that pulpal exposure produced periapical disease in conventionally raised animals but not in germ-free animals, establishing the essential role of microorganisms in endodontic pathosis.[1] Consequently, successful root canal treatment depends on reducing the intracanal microbial burden, removing necrotic tissue and preventing subsequent reinfection.

Complete disinfection is difficult because the root canal system contains fins, isthmuses, ramifications, recesses and dentinal tubules that may remain untouched by instruments. Persistent microorganisms can therefore survive chemomechanical preparation. *Enterococcus faecalis* has frequently been isolated from previously treated teeth with persistent periapical disease.[2] Its ability to withstand nutrient deprivation, form biofilms, invade dentinal tubules and tolerate unfavourable environmental conditions makes it a useful organism for evaluating root canal disinfection.[3]

Mechanical instrumentation reduces microbial contamination but cannot predictably contact the entire canal surface. Irrigation is therefore required to carry antimicrobial agents into areas beyond the reach of instruments, flush out debris and facilitate dissolution of organic material. Sodium hypochlorite remains the principal endodontic irrigant because of its broad antimicrobial activity and tissue-dissolving properties.[4] However, its effectiveness depends on concentration, replenishment, contact time, temperature and penetration into the apical portion of the canal.[5]

Conventional syringe irrigation is widely used but may provide limited fluid replacement beyond the needle tip. Increasing irrigant movement through manual, sonic, ultrasonic or pressure-controlled methods could enhance contact between the solution and intracanal microorganisms.[6] Manual dynamic agitation uses repeated movement of a fitted gutta-percha cone to generate hydrodynamic displacement. Sonic systems operate at relatively low frequencies and produce broad tip movement. Passive ultrasonic irrigation creates high-frequency acoustic energy that can improve fluid movement, while negative-pressure systems deliver irrigant coronally and aspirate it apically, facilitating irrigant exchange with a reduced risk of apical extrusion.[7]

Although numerous activation systems are available, their relative antibacterial performance varies according to canal anatomy, experimental design and microbiological method.[8] The present study therefore compared five commonly used irrigation approaches under standardised conditions. The null hypothesis was that there would be no difference in residual *E. faecalis* counts among conventional syringe irrigation, manual dynamic agitation, sonic activation, negative-pressure irrigation and ultrasonic activation.

MATERIALS AND METHODS

Study design and selection of specimens

This laboratory investigation was designed as a comparative in vitro study. Sixty-five extracted human mandibular first premolars were included and distributed equally among five irrigation groups, with 13 specimens in each group. Freshly extracted, intact mandibular first premolars having a single root canal and closed apex were selected. Teeth with open apices, caries, visible craze or fracture lines, calcified canals, developmental anomalies or previous endodontic treatment were excluded. Adherent soft tissue and external deposits were removed before specimen preparation.

Access preparation and working-length determination

Standard endodontic access cavities were prepared. Apical patency was confirmed with a size 10 K-file. A size 15 K-file was advanced until its tip became visible at the apical foramen. The working length was established by subtracting 1 mm from this measured length.

Root canal preparation

All canals were mechanically prepared using the ProTaper rotary system up to the F2 instrument. During instrumentation, 1 mL of 5.25% sodium hypochlorite was used between successive instruments. Canal patency was maintained throughout preparation. The external apical foramen of each specimen was subsequently sealed with epoxy resin to prevent bacterial or irrigant leakage through the apex. Prepared specimens were autoclaved at 121°C for 20 minutes. They were handled under aseptic conditions after sterilisation to avoid environmental contamination.

Preparation of the bacterial inoculum

A pure strain of *E. faecalis* was cultured on blood agar at 35°C for 48 hours. Representative colonies were transferred into sterile brain–heart infusion broth and incubated at 37°C for 24 hours. Visible turbidity was used

to confirm bacterial growth. Colony characteristics, Gram-staining appearance and biochemical findings were used to verify the identity of the organism.

Root canal inoculation

Ten microlitres of the prepared *E. faecalis* suspension were introduced into each root canal. The inoculated specimens were covered with artificial saliva and incubated for seven days to permit bacterial colonisation. Representative samples were obtained to establish the baseline bacterial load before the irrigation procedures.

Group allocation and irrigation procedures

The 65 specimens were randomly allocated to the following five groups:

Group I—Conventional syringe irrigation: Irrigation was performed using a 27-gauge side-vented needle. The needle was positioned 1 mm short of the established working length without binding against the canal walls. The solution was delivered using gentle pressure while maintaining needle movement.

Group II—Manual dynamic agitation: The canal was filled with irrigant, and an F2 gutta-percha cone was inserted 2–3 mm short of the working length. Repetitive short push-and-pull strokes were used to agitate and displace the solution within the prepared canal.

Group III—Sonic activation: The irrigant was activated for one minute with a sonic device using a size 20/.02 polymer tip positioned 1 mm short of the working length. The tip was maintained without intentional binding against the canal wall.

Group IV—Negative-pressure irrigation: Irrigation was performed using a negative-pressure delivery system. The aspiration tip was positioned 1 mm short of the working length. Irrigant was delivered into the pulp chamber and drawn apically through the canal by negative pressure.

Group V—Ultrasonic activation: The irrigant was ultrasonically activated for one minute. The ultrasonic tip was positioned 2–3 mm short of the working length and operated freely within the irrigant-filled canal without purposeful contact with the canal walls.

Post-irrigation microbiological sampling

After completion of the assigned irrigation procedure, a sterile paper point was placed inside each canal for one minute. The paper point was transferred into a tube containing 500 μL of sterile brain–heart infusion broth and retained for 15 minutes. Each tube was vortexed to disperse the collected microorganisms.

Fifty microlitres of the resulting suspension were serially diluted in sterile brain–heart infusion broth. Appropriate dilutions were plated on blood agar and incubated aerobically at 37°C for 48 hours. Visible colonies were counted, and the bacterial concentration was expressed as CFU/mL. Colony morphology, Gram staining and biochemical characteristics were assessed to confirm that the recovered colonies represented *E. faecalis*.

The primary outcome was the residual viable *E. faecalis* count after irrigation, expressed as CFU/mL. The secondary outcome was the percentage reduction relative to the representative mean baseline bacterial count. Because a common representative baseline was used, these percentages did not represent paired pre-irrigation and post-irrigation measurements from every individual specimen.

STATISTICAL ANALYSIS

Data were entered into Microsoft Excel and analysed using IBM SPSS Statistics version 25. Quantitative variables were summarised using the mean, standard deviation, median, minimum and maximum. Distributional characteristics and homogeneity of variance were evaluated, including assessment with Levene's test. Mean post-irrigation CFU counts were compared using one-way analysis of variance. Post-hoc pairwise comparisons were subsequently performed. All tests were two-sided, and $p<0.05$ was considered statistically significant.

RESULTS

The representative baseline mean *E. faecalis* count was 1559.50±56.77 CFU/mL. The recovery of viable bacteria before irrigation confirmed successful contamination of the prepared specimens.

The post-irrigation bacterial counts differed significantly among the five methods ($F=12.49$; $p<0.001$). Ultrasonic activation produced the lowest mean residual count of 17.77±18.63 CFU/mL. Negative-pressure irrigation produced the second-lowest mean count of 90.92±72.01 CFU/mL. Conventional syringe irrigation had the highest residual count and the greatest variability.

Table 1: Residual *E. faecalis* counts and reductions according to irrigation technique

Irrigation technique	n	Mean±SD (CFU/mL)	Median (minimum–maximum)	Reduction from representative baseline	Efficacy rank

Conventional syringe irrigation	13	761.31±580.28	711 (1–1824)	51.18%	5
Manual dynamic agitation	13	532.15±302.62	455 (149–1141)	65.88%	4
Sonic activation	13	368.77±246.30	316 (22–792)	76.35%	3
Negative-pressure irrigation	13	90.92±72.01	58 (2–210)	94.17%	2
Ultrasonic activation	13	17.77±18.63	10 (0–48)	98.86%	1
F=12.49; p<0.001*					

Ultrasonic activation demonstrated the greatest numerical reduction in viable bacteria, followed by negative-pressure irrigation as seen in Table 1. Sonic activation and manual dynamic agitation produced intermediate reductions, whereas conventional syringe irrigation was the least effective method. The percentage reductions were calculated relative to the common representative baseline mean of 1559.50 CFU/mL.

The difference between conventional syringe irrigation and manual dynamic agitation was not significant (p=0.223) as shown in Table 2. Similarly, manual dynamic agitation and sonic activation did not differ significantly (p=0.145). All other reported pairwise comparisons were statistically significant.

Table 2: Pairwise comparisons of mean residual bacterial counts

Pairwise comparison	Mean difference (CFU/mL)*	P value
Conventional versus manual dynamic agitation	229.15	0.223 (NS)
Conventional versus negative pressure	670.38	0.001*
Conventional versus sonic activation	392.54	0.039*
Conventional versus ultrasonic activation	743.54	<0.001*
Manual dynamic agitation versus negative pressure	441.23	<0.001*
Manual dynamic agitation versus sonic activation	163.38	0.145 (NS)
Manual dynamic agitation versus ultrasonic activation	514.38	<0.001*
Negative pressure versus sonic activation	−277.85	0.002*
Negative pressure versus ultrasonic activation	73.15	0.033*
Sonic activation versus ultrasonic activation	351.00	<0.001*

Mean difference was calculated as the mean of the first technique minus that of the second technique.

The 73.15 CFU/mL difference between negative-pressure irrigation and ultrasonic activation was statistically significant, favouring ultrasonic activation. Culture plates from the ultrasonic group showed the greatest degree of bacterial clearance. Nevertheless, residual growth occurred in at least some specimens from every group; therefore, none of the tested methods consistently achieved complete sterility. Recovered colonies were small and non-haemolytic. Gram staining revealed Gram-positive cocci arranged predominantly in pairs and short chains, and biochemical findings confirmed *E. faecalis*.

DISCUSSION

The present study rejected the null hypothesis because residual *E. faecalis* counts differed significantly among the five irrigation techniques. Ultrasonic activation produced the lowest residual bacterial count and the greatest reduction relative to the representative baseline (98.86%). Negative-pressure irrigation ranked second (94.17%), followed by sonic activation (76.35%), manual dynamic agitation (65.88%) and conventional syringe irrigation (51.18%). The superior performance of ultrasonic activation was consistent with Ada et al., who reported greater bacterial elimination with passive ultrasonic irrigation than with sonic activation, manual dynamic agitation and conventional syringe irrigation.[9] Ultrasonic oscillation generates vigorous irrigant movement and improves solution circulation and renewal within canal irregularities. This hydrodynamic activity may detach bacterial aggregates and increase irrigant contact with anatomical areas beyond the direct reach of instruments.[9,10]

Guerreiro-Tanomaru et al. similarly found that passive ultrasonic irrigation significantly reduced *E. faecalis* in an

ex vivo root canal model.[10] Nevertheless, residual CFUs were detected in some specimens in the present ultrasonic group. Ultrasonic activation should therefore be regarded as a method of improving bacterial reduction rather than achieving predictable root canal sterilisation. Microorganisms may persist within deep dentinal tubules, isthmuses, fins and protected biofilm structures where irrigant penetration and mechanical shear remain inadequate.[10,16] Although ultrasonic activation performed significantly better than negative-pressure irrigation, both produced markedly lower residual counts than the remaining techniques. Ultrasonic irrigation primarily increases fluid movement and intracanal shear stress, whereas negative-pressure irrigation improves apical irrigant delivery and evacuation.[10,11] Their different mechanisms may explain why both approaches demonstrated strong antibacterial performance.

Negative-pressure irrigation performed significantly better than conventional syringe irrigation, manual dynamic agitation and sonic activation. Nielsen and Baumgartner demonstrated that the EndoVac negative-pressure system provided improved debridement at apical canal levels compared with needle irrigation.[11] The system draws irrigant towards the apical region instead of delivering it under positive pressure, thereby improving apical fluid exchange and reducing irrigant stagnation.[11] In the present investigation, the negative-pressure tip was positioned 1 mm short of the working length. This placement may have facilitated irrigant exchange in an area where conventional positive-pressure delivery can be restricted by the apical vapour lock. Alkahtani et al. reported favourable canal debridement and reduced apical extrusion with negative-pressure irrigation compared with conventional delivery systems.[12] However, the current study did not measure irrigant extrusion. Therefore, its findings demonstrate antibacterial effectiveness but cannot establish the clinical safety of negative-pressure irrigation.

Sonic activation achieved a significantly greater bacterial reduction than conventional syringe irrigation. Sonic devices produce lower-frequency oscillation and broad movement of a flexible polymer tip, which promotes irrigant displacement within the prepared canal. Ghoddusi et al. demonstrated that sonic activation reduced intratubular *E. faecalis*, although complete bacterial elimination was not achieved.[13] This finding was consistent with the residual bacterial growth detected in the present sonic group. Sonic activation was less effective than ultrasonic and negative-pressure irrigation. Its lower operating frequency, variable energy transmission and damping of tip movement following contact with canal walls may reduce its hydrodynamic effectiveness.[9,13] The single one-minute activation cycle used in this study could also have limited irrigant contact. These results should consequently be interpreted according to the tested tip size, position and activation duration.

Manual dynamic agitation produced an intermediate bacterial reduction but did not differ significantly from either conventional syringe irrigation or sonic activation. Repetitive movement of a fitted gutta-percha cone generates intracanal pressure changes, promotes irrigant movement and may disrupt the apical vapour lock.[9] Although the method is inexpensive and technically simple, its effectiveness depends on cone adaptation, stroke amplitude, agitation frequency and operator consistency.[9] Variability in these factors may have contributed to its intermediate performance. Conventional syringe irrigation produced the highest mean residual count and the widest range, extending from 1 to 1824 CFU/mL. Although the side-vented needle was positioned 1 mm short of the working length, syringe irrigation generates limited hydrodynamic activity compared with active agitation systems.[9,11] Irrigant exchange may remain concentrated around the needle outlet, leaving fins, recesses and dentinal tubules less effectively exposed.[11] The wide dispersion of values may also have resulted from natural variations in root canal anatomy and bacterial colonisation.

The ranking observed in this study has not been demonstrated universally. Flores Orozco et al. reported that passive ultrasonic activation did not produce significantly greater microbial reduction than conventional irrigation in teeth with primary root canal infections.[14] The difference from the present findings may be explained by the complex polymicrobial composition of naturally infected canals, anatomical variability, host-related factors and differences in microbiological sampling. In contrast, the present experiment used a standardised single-species *E. faecalis* model. Nogueira et al. demonstrated that the apparent effectiveness of irrigant agitation techniques may vary according to the microbiological assessment method used.[15] Culture identifies viable organisms capable of growth under the selected incubation conditions but may not detect viable non-culturable cells. Molecular methods provide greater detection sensitivity but may identify DNA from both viable and non-viable microorganisms.[15] The CFU method used in the present study quantified cultivable bacteria but did not determine their anatomical location or characterise the remaining biofilm. The seven-day incubation period permitted *E. faecalis* colonisation; however, clinical biofilms may be older, multispecies and more resistant. Seghayer et al. demonstrated that irrigation activation technologies differed in their effectiveness against *E. faecalis* biofilms in the apical third of root canals.[16] Their findings highlight the importance of

evaluating irrigation systems against mature biofilms situated within anatomically protected regions.

The strengths of this study included standardised tooth selection, instrumentation endpoint, irrigant concentration, microbial species and equal allocation of 13 specimens per group. Nevertheless, the percentage reductions were calculated using a common representative baseline rather than paired baseline counts for every specimen. Additional limitations included anatomical variation, paper-point sampling and culture-based detection. Bacterial penetration depth, biofilm biomass, smear-layer removal, irrigant extrusion and dentinal changes were not assessed. Furthermore, an in vitro single-species infection cannot reproduce the biological complexity of clinical endodontic disease.

Within these limitations, ultrasonic activation provided the greatest bacterial reduction, followed by negative-pressure irrigation. Neither technique achieved complete sterility in every specimen. Future investigations should employ paired microbiological sampling, mature multispecies biofilms, standardised canal anatomy and complementary culture, molecular and imaging techniques.

CONCLUSION

Within the limitations of this in vitro study, the method used to deliver or activate the irrigant significantly influenced the reduction of *Enterococcus faecalis* in infected root canals. Ultrasonic activation produced the lowest residual bacterial count and the greatest reduction relative to the representative baseline, followed by negative-pressure irrigation. Sonic activation demonstrated intermediate effectiveness, while manual dynamic agitation provided a modest improvement over conventional syringe irrigation; however, the difference between these two methods was not statistically significant. Conventional syringe irrigation produced the highest and most variable residual bacterial counts. Despite the marked effectiveness of ultrasonic and negative-pressure methods, viable organisms were recovered from some specimens in every group. Thus, none of the evaluated techniques consistently sterilised the root canal system. Ultrasonic activation may be preferred when maximising intracanal bacterial reduction is the principal objective, whereas negative-pressure irrigation represents an effective alternative for enhanced apical irrigant exchange.

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