International Journal of Dental Sciences
www.dentaljournal.in
ISSN: 2663-4708
Received: 12-09-2026, Accepted: 12-10-2026, Published: 27-10-2026
Volume 8, Issue 4, 2026, Page No. 22-25

Antimicrobial effectiveness of passive and continuous ultrasonic irrigation against a multispecies biofilm: An *in vitro* study

Jessica Fernanda Ibarra De La Vega, Alex Vinicio Sánchez Ramirez*
Faculty of Health Sciences, Universidad Hemisferios Quito, Ecuador
Corresponding Author: Alex Vinicio Sánchez Ramirez
DOI: https://doi.org/10.66856/ijds.2026.8.4.8147

Abstract
Objective: To compare the antimicrobial effectiveness of passive ultrasonic irrigation (PUI) and continuous ultrasonic irrigation (CUI) against a multispecies biofilm by counting colony-forming units per milliliter (CFU/mL).
Methods: An *in vitro* experimental study was conducted using 60 human single-rooted premolars. Two experimental groups were established: PUI (n = 20) and CUI (n = 20), along with 10 positive controls and 10 negative controls. The multispecies biofilm consisted of Enterococcus faecalis, Candida albicans, and Staphylococcus aureus. Following chemomechanical preparation with reciprocating instrumentation and 5.25% sodium hypochlorite, the corresponding ultrasonic activation protocols were applied. Microbial load was assessed at three time points: baseline, after chemomechanical preparation, and after ultrasonic activation. Nonparametric statistical tests were used for within-group and between-group comparisons.
Results: Both groups showed a statistically significant reduction in microbial load across the assessed stages ($p < 0.001$). In the PUI group, the median decreased from 1.24×10^7 CFU/mL at baseline to 4.90×10^2 CFU/mL after activation. In the CUI group, it decreased from 7.20×10^6 CFU/mL to 2.55×10^1 CFU/mL. The between-group comparison after activation showed a statistically significant difference ($p < 0.001$; $r = 0.61$), with a lower residual microbial load in the CUI group. Counts below 100 CFU/mL were found in 85% of CUI specimens, compared with 25% of PUI specimens.
Conclusions: Both techniques significantly reduced the microbial load of the multispecies biofilm. Under the experimental conditions of this study, CUI resulted in a lower residual microbial load than PUI; however, these findings do not directly establish clinical superiority.

Keywords: Passive ultrasonic irrigation, continuous ultrasonic irrigation, multispecies biofilm, endodontic irrigation, microbial load colony-forming units

Introduction

The anatomy of the root canal system includes irregularities that make complete cleaning through mechanical instrumentation difficult. Isthmuses, accessory canals, apical ramifications, and dentinal tubules may limit instrument contact with canal walls and allow microorganisms to persist (Versiani *et al*., 2023) [14].

Endodontic infections may involve multispecies biofilms, whose microbial structures promote persistence in the presence of antimicrobial agents. The microorganisms considered in this context include Enterococcus faecalis and Candida albicans (Siqueira & Rôças, 2008; Ricucci & Siqueira, 2010) [9, 11].

Sodium hypochlorite is used as an endodontic irrigant because of its ability to dissolve organic tissue and eliminate microorganisms. However, its effectiveness depends on contact with the surfaces of the root canal system and its ability to reach areas that cannot be accessed directly by instruments.

Passive ultrasonic irrigation (PUI) activates the irrigant previously introduced into the canal by means of an ultrasonic tip. Continuous ultrasonic irrigation (CUI), in turn, combines ultrasonic activation with a continuous supply of the solution. Both approaches aim to improve irrigant distribution and facilitate cleaning in areas that are difficult to access (van der Sluis *et al*., 2007; Castelo-Baz *et al*., 2016) [5, 13].

Although both techniques have been studied as methods to improve endodontic irrigation, comparing their antimicrobial effectiveness against multispecies biofilms remains relevant to experimental research.

Therefore, the aim of this study was to compare the reduction in microbial load achieved with PUI and CUI in an *in vitro* multispecies biofilm model, quantified by CFU/mL counts and expressed on a logarithmic scale.

Materials and Methods

Study Design and Sample Selection

An *in vitro* experimental study was conducted. The sample was selected using non-probability convenience sampling.

Initially, 100 donated human single-rooted premolars were collected. The teeth were assessed using periapical radiographs in an orthoradial projection, and 40 that did not meet the established criteria were excluded. The final sample consisted of 60 teeth, randomly allocated to four groups: PUI (n = 20), CUI (n = 20), positive control (n = 10), and negative control (n = 10).

Single-rooted premolars with one canal and no calcifications, fractures, or cracks were included. Teeth with

previous endodontic treatment, root microdontia, apical curvatures greater than 15°, or internal or external root resorption were excluded.

Specimen Preparation

The teeth were standardized to a length of 16 mm by horizontal sectioning below the cementoenamel junction. The apices were sealed with epoxy resin, and the root surfaces were coated with two layers of clear varnish.

Initial manual instrumentation was then performed to 1 mm short of the apical foramen using sizes 10, 15, and 20 K-files. The teeth and instruments were sterilized in an autoclave at 121 °C for 20 minutes.

Preparation and Inoculation of the Multispecies Biofilm

The strains used were Enterococcus faecalis ATCC 29212, Candida albicans ATCC 10231, and Staphylococcus aureus ATCC 25923. Each microorganism was activated in its corresponding culture medium, and the suspensions were standardized to 0.5 on the McFarland scale.

The multispecies inoculum was prepared using 2,000 µL of Brain Heart Infusion (BHI) broth and 1,000 µL of each microbial suspension, yielding a final volume of 5,000 µL.

Each canal received 0.01 mL of human saliva previously sterilized by filtration through a 0.22-µm filter and approximately 0.01 mL of the multispecies inoculum, which was distributed using a sterile size 15 K-file.

The teeth were sealed and incubated at 37.5 °C for 21 days. Every 48 hours, 0.01 mL of sterile BHI broth was added to maintain biofilm viability. Microbial growth was subsequently verified by culturing on specific media and by microscopic examination following Gram staining.

Experimental Procedure

The 40 experimental specimens were instrumented using the WaveOne Gold reciprocating system up to a size 35/06 file. During preparation, 5.25% sodium hypochlorite was used, with a total volume of 2 mL per tooth, delivered through a 30-gauge NaviTip side-vented needle inserted passively to 1 mm short of the working length.

At the end of instrumentation, 17% EDTA was applied for one minute. Subsequently, 2 mL of 10% sodium thiosulfate was used for one minute to neutralize residual hypochlorite, followed by rinsing with 1 mL of sterile saline.

In the PUI group, the 2 mL of 5.25% sodium hypochlorite was activated using an E99 tip and a Woodpecker U6 ultrasonic unit for three 20-second cycles, with intermediate irrigation. The tip was kept passively at 1 mm short of the working length, without direct contact with the canal walls.

In the CUI group, continuous irrigation with simultaneous ultrasonic activation was performed using the Woodpecker U6 unit and an E99 tip for a total of one minute. Each tooth received 2 mL of 5.25% sodium hypochlorite, while the tip was kept out of direct contact with the canal walls and 1 mm short of the working length.

After each procedure, residual hypochlorite was neutralized with 10% sodium thiosulfate for one minute, followed by rinsing with 1 mL of sterile saline.

Microbiological Assessment

Microbial load was assessed at three stages: baseline, after chemomechanical preparation, and after ultrasonic activation.

For sampling, sterile paper points were placed in microtubes containing 1,000 µL of phosphate-buffered saline (PBS). The samples were homogenized by vortexing for one minute.

Depending on the expected microbial load, direct plating or serial decimal dilutions were performed. A volume of 100 µL was plated on blood-supplemented Mueller–Hinton agar, and the plates were incubated at 37.5 °C for 24 hours for colony-forming unit counts.

Positive controls were used to confirm the presence and viability of the biofilm, whereas negative controls were used to verify the absence of contamination during the procedure.

Statistical Analysis

Results were expressed as CFU/mL and on a logarithmic scale. Descriptive statistics, the Friedman test to compare the three experimental stages, and the Wilcoxon test for within-group comparisons with Bonferroni correction were used.

Between-group comparisons of PUI and CUI were performed using the Mann–Whitney U test. Fisher's exact test was also used to compare the proportions of specimens below the detection limit and below 100 CFU/mL. Sensitivity analyses were conducted to assess the robustness of the results. The two-sided significance level was set at 0.05.

Results

Experimental Controls

No microbial growth was observed in any of the 10 negative-control teeth, with counts of 0 CFU/mL. Growth was observed in all 10 positive-control specimens, with a median of 9.65×10^6 CFU/mL, a minimum of 1.07×10^6 CFU/mL, and a maximum of 1.35×10^9 CFU/mL.

Microbial Load by Experimental Group

In the PUI group, the baseline median was 1.24×10^7 CFU/mL. This decreased to 4.10×10^4 CFU/mL after chemomechanical preparation and reached 4.90×10^2 CFU/mL after ultrasonic activation.

In the CUI group, the baseline median was 7.20×10^6 CFU/mL. This decreased to 5.55×10^3 CFU/mL after chemomechanical preparation and reached 2.55×10^1 CFU/mL after continuous ultrasonic activation.

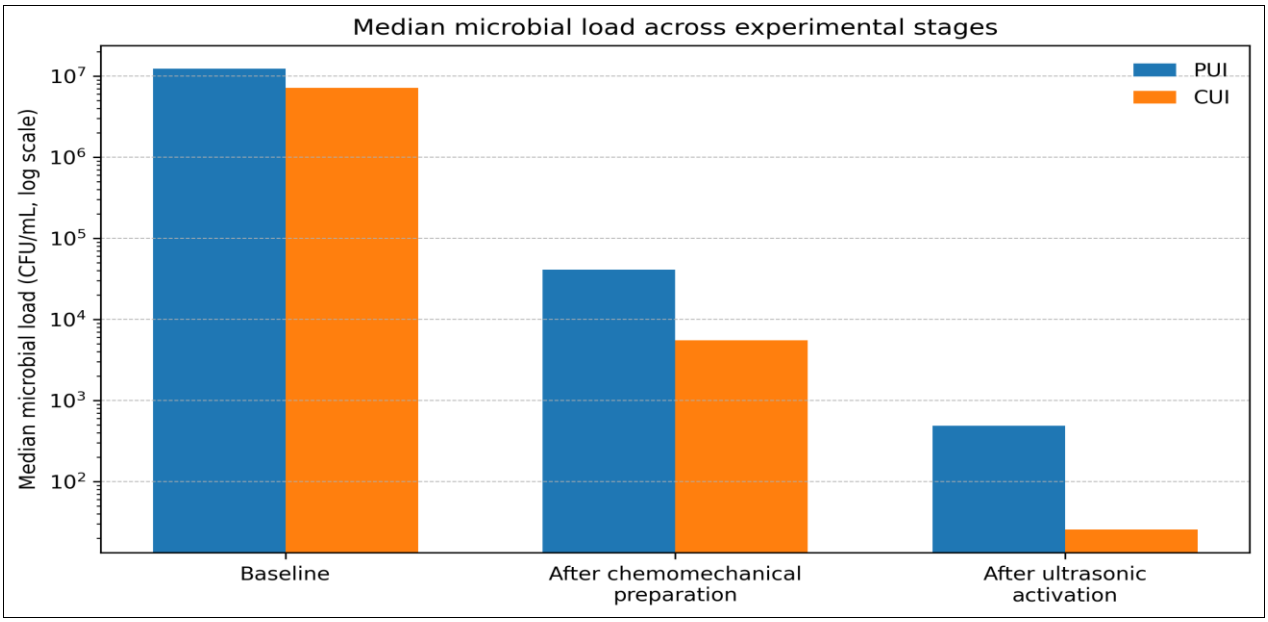


Fig 1: Median microbial load across experimental stages in the PUI and CUI groups. The vertical axis is logarithmic.

Table 1: Median microbial load and cumulative reduction by group.

Experimental stage / outcome	PUI	CUI
Baseline (CFU/mL)	1.24×10^7	7.20×10^6
After chemomechanical preparation (CFU/mL)	4.10×10^4	5.55×10^3
After ultrasonic activation (CFU/mL)	4.90×10^2	2.55×10^1
Total median reduction ($\log_{10}$)	4.50	6.36

Note: Values are medians. PUI = passive ultrasonic irrigation; CUI = continuous ultrasonic irrigation; CFU/mL = colony-forming units per milliliter.

Reduction in Microbial Load

The median cumulative reduction was 4.50 $\log_{10}$ CFU/mL for PUI and 6.36 $\log_{10}$ CFU/mL for CUI.

In the PUI group, chemomechanical preparation produced a median reduction of 2.65 $\log_{10}$, and ultrasonic activation provided an additional reduction of 2.06 $\log_{10}$.

In the CUI group, chemomechanical preparation produced a median reduction of 3.45 $\log_{10}$, and continuous activation provided an additional reduction of 2.79 $\log_{10}$.

Differences across the three stages were statistically significant in both groups (Friedman test: $\chi^2(2) = 40.00$; $p < 0.001$). Post hoc Wilcoxon comparisons with Bonferroni correction also showed significant differences between all stages (adjusted $p < 0.001$).

Comparison Between PUI and CUI

No statistically significant differences between groups were observed in baseline microbial load ($p = 0.602$) or after chemomechanical preparation ($p = 0.211$).

After ultrasonic activation, the between-group difference was statistically significant ($U = 336.5$; $p < 0.001$; $r = 0.61$). The Hodges–Lehmann estimated difference was 1.35 $\log_{10}$ CFU/mL in favor of CUI (95% CI: 0.97–2.06), corresponding to an approximately 22-fold lower residual microbial load.

The cumulative microbial reduction also differed significantly between groups ($p < 0.001$), with an estimated difference of 1.78 $\log_{10}$ CFU/mL (95% CI: 0.76–2.64).

Distribution of Residual Microbial Load

After activation, none of the PUI specimens had counts below the detection limit of 10 CFU/mL. In the CUI group, eight specimens (40%) had counts below this limit. This difference was statistically significant ($p = 0.003$).

Similarly, five PUI specimens (25%) and 17 CUI specimens (85%) had counts below 100 CFU/mL. The difference was statistically significant ($p < 0.001$).

Discussion

This study compared the antimicrobial effectiveness of PUI and CUI against a multispecies biofilm consisting of Enterococcus faecalis, Candida albicans, and Staphylococcus aureus. The results showed a significant reduction in microbial load with both protocols after chemomechanical preparation and ultrasonic activation. However, after activation, the CUI group had a lower residual microbial load.

The reduction observed after chemomechanical preparation demonstrates the effect of instrumentation combined with 5.25% sodium hypochlorite. Nevertheless, the recovery of microorganisms after this stage shows that chemomechanical preparation did not completely eliminate the microbial load. This finding is related to the limitations of instrumentation in reaching all surfaces of the root canal system, as described by van der Sluis *et al*. (2007) [13].

Ultrasonic activation produced an additional reduction in both groups. The estimated reduction from the post-instrumentation stage to the activation stage was 1.87 $\log_{10}$ CFU/mL for PUI and 2.79 $\log_{10}$ CFU/mL for CUI. These results indicate an additional effect of activation beyond chemomechanical preparation.

The PUI findings are consistent with those reported by Bhuva *et al*. (2010) [1], who evaluated the reduction of Enterococcus faecalis biofilm using passive ultrasonic irrigation. However, the studies differ methodologically: the

present study used a multispecies biofilm and quantified microbial load using CFU/mL.

The main difference between groups was observed after ultrasonic activation. CUI had a residual median of 2.55×10^1 CFU/mL, compared with 4.90×10^2 CFU/mL for PUI. In addition, 85% of CUI specimens had fewer than 100 CFU/mL, compared with 25% of PUI specimens.

One possible explanation for this behavior is the continuous replenishment of irrigant during CUI. However, this explanation should be interpreted as a possibility rather than direct evidence of the mechanism responsible for the observed difference.

The results should also be interpreted in the context of the variability across studies investigating ultrasonic activation. Căpută *et al* . (2019) [3] reported that microbiological findings from *in vitro* studies were variable and that the available evidence was insufficient to establish strong clinical recommendations.

One limitation of this study is its *in vitro* design. In addition, CFU/mL counts represent the microorganisms recoverable using the sampling procedure employed and do not necessarily reflect all microorganisms present in the root canal system. Values below the detection limit of 10 CFU/mL indicate no growth detectable using the method employed, but they do not establish the complete elimination of microorganisms.

Overall, both protocols significantly reduced microbial load. Under the experimental conditions of this study, CUI resulted in a lower residual microbial load and a higher proportion of specimens with low counts. However, these results do not directly establish the clinical superiority of CUI over PUI.

Conclusions

Passive ultrasonic irrigation and continuous ultrasonic irrigation significantly reduced the microbial load of the multispecies biofilm across the experimental stages assessed.

PUI reduced the median microbial load from 1.24×10^7 CFU/mL at baseline to 4.90×10^2 CFU/mL after activation.

CUI reduced the median microbial load from 7.20×10^6 CFU/mL at baseline to 2.55×10^1 CFU/mL after activation.

In addition, 40% of specimens had counts below the detection limit of 10 CFU/mL, and 85% had counts below 100 CFU/mL.

After ultrasonic activation, a statistically significant difference was observed between groups, with a lower residual microbial load in CUI ($p < 0.001$).

These results should be interpreted within the specific conditions of the *in vitro* experimental model and cannot be directly extrapolated to establish the clinical superiority of continuous ultrasonic irrigation.

References

1. Bhuva B, Patel S, Wilson R, Niazi S, Beighton D, Mannocci F. The effectiveness of passive ultrasonic irrigation on intraradicular *Enterococcus faecalis* biofilms in extracted single-rooted human teeth. International Endodontic Journal,2010:43(3):241–250. https://doi.org/10.1111/j.1365-2591.2009.01672.x
2. Cachovan G, Schiffner U, Altenhof S, Guentsch A, Pfister W, Eick S. Comparative antibacterial efficacies of hydrodynamic and ultrasonic irrigation systems *in vitro*. Journal of Endodontics,2013:39(9):1171–1175. https://doi.org/10.1016/j.joen.2013.06.008
3. Căpută PE, Retsas A, Kuijk L, Chávez de Paz LE, Boutsioukis C. Ultrasonic irrigant activation during root canal treatment: A systematic review. Journal of Endodontics,2019:45(1):31–44. e13. https://doi.org/10.1016/j.joen.2018.09.010
4. Castelo-Baz P, Martín-Biedma B, Cantatore G, Ruíz-Piñón M, Bahillo J, Rivas-Mundiña B, *et al. In vitro* comparison of passive and continuous ultrasonic irrigation in simulated lateral canals of extracted teeth. Journal of Endodontics,2012:38(5):688–691. https://doi.org/10.1016/j.joen.2011.12.032
5. Castelo-Baz P, Varela-Patiño P, Cantatore G, Domínguez-Perez A, Ruíz-Piñón M, Miguéns-Vila R, *et al* . *In vitro* comparison of passive and continuous ultrasonic irrigation in curved root canals. Journal of Clinical and Experimental Dentistry,2016:8(4):e437–e441. https://doi.org/10.4317/jced.53023
6. Galván-Pacheco J, Vitales-Noyola M, González-Amaro AM, Bujanda-Wong H, Aragón-Piña A, Méndez-González V, *et al* . Evaluation of *in vitro* biofilm elimination of *Enterococcus faecalis* using a continuous ultrasonic irrigation device. Journal of Oral Science,2020:62(4):415–419. https://doi.org/10.2334/josnusd.19-0399
7. García Delgado A, Martín-González J, Castellanos-Cosano L, Martín Jiménez M, Segura-Egea JJ. Sistemas ultrasónicos para la irrigación del sistema de conductos radiculares. Avances en Odontoestomatología,2014:30(2):79–94.
8. Kluge M, Trüschler J, Elamin F, Anderson A, Hellwig E, Altenburger M, *et al.* Improving the microbial sampling and analysis of secondary infected root canals by passive ultrasonic irrigation. Clinical Oral Investigations,2022:26(6):4575–4586. https://doi.org/10.1007/s00784-022-04424-x
9. Ricucci D, Siqueira JF Jr. Biofilms and apical periodontitis: Study of prevalence and association with clinical and histopathologic findings. Journal of Endodontics,2010:36(8):1277–1288. https://doi.org/10.1016/j.joen.2010.04.007
10. Silva EJNL, Rover G, Belladonna FG, Herrera DR, De-Deus G, da Silva Fidalgo TK. Effectiveness of passive ultrasonic irrigation on periapical healing and root canal disinfection: A systematic review. British Dental Journal,2019:227(3):228–234. https://doi.org/10.1038/s41415-019-0532-z
11. Siqueira JF Jr, Rôças IN. Clinical implications and microbiology of bacterial persistence after treatment procedures. Journal of Endodontics,2008:34(11):1291–1301.e3. https://doi.org/10.1016/j.joen.2008.07.028
12. Tonini R, Salvadori M, Audino E, Sauro S, Garo ML, Salgarello S. Irrigating solutions and activation methods used in clinical endodontics: A systematic review. Frontiers in Oral Health,2022:3:838043. https://doi.org/10.3389/froh.2022.838043
13. van der Sluis LWM, Versluis M, Wu MK, Wesselink PR. Passive ultrasonic irrigation of the root canal: A review of the literature. International Endodontic Journal,2007:40(6):415–426. https://doi.org/10.1111/j.1365-2591.2007.01243.x
14. Versiani MA, Martins J, Ordinola-Zapata R. Anatomical complexities affecting root canal preparation: A narrative review. Australian Dental Journal,2023:68(Suppl. 1):S5–S23. https://doi.org/10.1111/adj.12992