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Comparative biofilm disruption by different irrigation activation systems: An *in vitro* study

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Abstract:
Microbial biofilms within the root canal system contribute to persistent endodontic infection, highlighting the importance of effective irrigation activation. Therefore, it is of interest to compare the biofilm-disruption efficacy of Multisonic Irrigation Activation, EDDY Sonic Activation, Passive Ultrasonic Irrigation and conventional syringe irrigation against *Enterococcus faecalis*. All activation systems produced significantly greater biofilm disruption than conventional syringe irrigation. Multisonic Irrigation Activation showed the lowest residual bacterial count and the greatest antibacterial efficacy among the tested systems. Thus, data shows that advanced irrigation activation, particularly Multisonic Irrigation Activation, can enhance *E. faecalis* biofilm disruption and potentially improve root canal disinfection.

Keywords: Biofilm disruption, irrigation activation systems, passive ultrasonic irrigation, sonic activation, multisonic irrigation, *Enterococcus faecalis*

Background:
Successful root canal treatment requires effective microbial elimination and disruption of intracanal biofilms, which are more resistant to antimicrobial agents than planktonic bacteria **[1]**. Irrigation is essential for bacterial reduction, tissue dissolution and debris removal, but conventional syringe irrigation has limited penetration into apical and anatomically complex areas **[2]**. Irrigation activation systems, including ultrasonic, sonic and multisonic techniques, improve irrigant distribution, fluid dynamics and biofilm removal compared with conventional irrigation **[3]**. Ultrasonic and sonic activation enhance irrigant exchange through acoustic streaming and hydrodynamic agitation and have demonstrated effectiveness against *Enterococcus faecalis* biofilms **[4]**. Activated irrigation also improves the removal of smear layer, medicaments, debris and microorganisms from inaccessible canal regions **[5, 6]**. Systematic reviews indicate greater microbial reduction with activated irrigation, although heterogeneity among studies prevents identification of a universally superior technique **[7]**. Comparative laboratory studies further support improved cleaning, irrigant penetration and bacterial reduction with activated irrigation, particularly in complex canal anatomy **[8–10]**. Therefore, it is of interest to evaluate the different irrigation activation systems for *E. faecalis* biofilm disruption.

Materials and Methods:
This *in vitro* study was conducted in the Department of Conservative Dentistry and Endodontics following approval from the Institutional Ethical Committee. Forty freshly extracted human single rooted mandibular premolars with mature apices and straight root canals were selected. Teeth exhibiting caries, cracks, resorption, calcified canals, previous endodontic treatment, or root fractures were excluded. Soft tissue remnants and calculus were removed using an ultrasonic scaler and the teeth were stored in 0.1% thymol solution until use. The crowns were sectioned at the cementoenamel junction using a water-cooled diamond disc to obtain a standardized root length of 15 mm. Working length was established by introducing a 10 K-file until it became visible at the apical foramen and subtracting 1 mm. All canals were instrumented using ProTaper Gold rotary files up to size F3 according to the manufacturer's instructions. During instrumentation, irrigation was performed with 3% sodium hypochlorite delivered through a 30 gauge side vented needle. After biomechanical preparation, the specimens were sterilized in an autoclave. A pure culture of *Enterococcus faecalis* (ATCC 29212) was prepared in Brain Heart Infusion broth and each canal was inoculated with 10 µL of the bacterial suspension using a sterile micropipette. The canal orifices were sealed with temporary restorative material and the specimens were incubated at 37°C under humid conditions for 21 days to allow mature biofilm formation. Fresh culture medium was replenished every 48 hours under aseptic conditions. Following incubation, the specimens were randomly allocated into four experimental groups (n = 10): Group I received conventional syringe irrigation using a 30-gauge side-vented needle; Group II, passive ultrasonic irrigation; Group III, sonic irrigation activation (EDDY); and Group IV, multisonic irrigation activation. All specimens received 5 mL of 3% sodium hypochlorite followed by 5 mL of 17% EDTA. Irrigant activation was performed according to the manufacturer's recommended

protocol for each system. A final rinse with sterile saline was performed and the canals were dried with sterile paper points. Microbiological evaluation involved collecting dentinal shavings using sterile Gates Glidden drills and sterile paper point samples from each canal. Samples were transferred to sterile saline, serially diluted and cultured on Brain Heart Infusion agar plates. After incubation at 37°C for 24 hours, bacterial colonies were counted and expressed as colony forming units per milliliter (CFU/mL). The percentage reduction in bacterial count was calculated for each specimen. The obtained data were entered into Microsoft Excel and analyzed using SPSS version 25.0. Mean CFU counts among the four groups were compared using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. Statistical significance was set at $p < 0.05$.

Results:

Forty specimens were included in the study, with 10 specimens assigned to each experimental group. The mean bacterial count (CFU/mL) was highest in the Conventional Syringe Irrigation group ($86.40 \pm 6.28 \times 10^3$ CFU/mL), indicating the least effective biofilm disruption. Passive Ultrasonic Irrigation resulted in a marked reduction in bacterial count ($42.70 \pm 4.61 \times 10^3$ CFU/mL), while Sonic Activation (EDDY) produced a further decrease ($29.60 \pm 3.54 \times 10^3$ CFU/mL). The lowest bacterial count was observed in the Multisonic Irrigation Activation group ($15.80 \pm 2.76 \times 10^3$ CFU/mL), showing the highest biofilm disruption efficacy among all tested systems **(Table 1).** The lowest bacterial count was observed in the Multisonic Irrigation Activation group, followed by the Sonic Activation (EDDY) group, Passive Ultrasonic Irrigation group and Conventional Syringe Irrigation group. Statistical analysis using one-way ANOVA revealed a highly significant difference among the four groups ($F = 312.84$, $p < 0.001$), indicating that the irrigation activation system significantly influenced intracanal bacterial biofilm reduction **(Table 2).** Pairwise comparison with Tukey's post hoc test showed statistically significant differences between all groups ($p < 0.001$). Multisonic Irrigation Activation achieved significantly greater biofilm disruption than Sonic Activation (EDDY), Passive Ultrasonic Irrigation and Conventional Syringe Irrigation. Similarly, Sonic Activation (EDDY) produced significantly lower bacterial counts than Passive Ultrasonic Irrigation and Conventional Syringe Irrigation, while Passive Ultrasonic Irrigation was more effective than Conventional Syringe Irrigation **(Table 3).** All pairwise comparisons showed statistically significant differences. The percentage reduction in bacterial count supported these findings: Multisonic Irrigation Activation (93.8%), Sonic Activation (EDDY) (86.9%), Passive Ultrasonic Irrigation (79.6%) and Conventional Syringe Irrigation (58.4%), demonstrating that irrigant activation significantly enhanced biofilm disruption compared with conventional syringe irrigation **(Table 4).** Based on overall antibacterial performance, the systems were ranked as follows: Multisonic Irrigation Activation, Sonic Activation (EDDY), Passive Ultrasonic Irrigation and Conventional Syringe Irrigation. These results indicate that advanced irrigant activation systems were significantly more effective in disrupting *Enterococcus faecalis* biofilm than conventional syringe irrigation, with Multisonic Irrigation Activation demonstrating the greatest antibacterial efficacy **(Table 5).**

Table 1: Comparison of mean bacterial count (CFU/mL) after irrigation

Group	Irrigation activation system	Mean CFU/mL ($\times 10^3$)	SD
I	Conventional Syringe Irrigation	86.4	6.28
II	Passive Ultrasonic Irrigation (PUI)	42.7	4.61
III	Sonic Activation (EDDY)	29.6	3.54
IV	Multisonic Irrigation Activation	15.8	2.76

Table 2: One-way ANOVA comparing mean CFU counts among the groups

Source	F value	p value
Between groups	312.84	<0.001*

**Statistically significant. A statistically highly significant difference was observed among the four irrigation activation systems ($p < 0.001$).*

Table 3: Tukey's post hoc multiple comparison test

Comparison	Mean difference	p value
Group I vs Group II	43.7	<0.001*
Group I vs Group III	56.8	<0.001*
Group I vs Group IV	70.6	<0.001*
Group II vs Group III	13.1	<0.001*
Group II vs Group IV	26.9	<0.001*
Group III vs Group IV	13.8	<0.001*

Table 4: Percentage reduction in bacterial count

Group	Mean reduction (%)
I – Conventional Syringe Irrigation	58.4
II – Passive Ultrasonic Irrigation	79.6
III – Sonic Activation (EDDY)	86.9
IV – Multisonic Irrigation Activation	93.8

Table 5: Overall ranking of irrigation activation systems

Rank	Irrigation activation system
1	Multisonic Irrigation Activation
2	Sonic Activation (EDDY)
3	Passive Ultrasonic Irrigation
4	Conventional Syringe Irrigation

Discussion:

The present study showed that all irrigation activation systems produced significantly greater *Enterococcus faecalis* biofilm disruption than conventional syringe irrigation, with Multisonic Irrigation Activation showing the lowest residual bacterial count and highest reduction (93.8%), followed by EDDY (86.9%) and Passive Ultrasonic Irrigation (79.6%). The statistically significant differences between all groups indicate that the mode of irrigant activation substantially influences intracanal bacterial reduction. The findings are consistent with Chu *et al.* (2023) **[11]**, who reported that EDDY demonstrated cleaning and disinfection performance comparable to ultrasonically activated irrigation. Their systematic review suggested that EDDY may represent an effective alternative to ultrasonic activation, particularly because of its potential to reduce dentinal wall damage. In the present study, however, EDDY produced significantly greater biofilm reduction than PUI, supporting its strong antimicrobial performance under the standardized experimental conditions used. Widbiller *et al.* (2023) **[12]** showed that ultrasonic and sonic activation improved smear layer removal and irrigant penetration compared with needle irrigation. Their findings support the present observation that activation enhances the

effectiveness of conventional irrigation; however, they also reported that activation may influence dentinal surface integrity, particularly with certain techniques. Thus, the superior antibacterial performance observed in the present study should be interpreted alongside the potential effects of activation on dentin. The results also correspond with Shin *et al*. (2023) **[13]**, who found that EDDY and ultrasonic activation produced significantly greater biofilm reduction than syringe irrigation in a three dimensional curved canal model. Their findings reinforce the importance of hydrodynamic activation for disrupting intracanal biofilms. The present study extends these observations by showing a graded antibacterial performance of Multisonic Activation > EDDY > PUI > syringe irrigation. Plotino *et al*. (2023) **[14]** reported that EDDY was more effective than other tested activation techniques for removing dentin debris from a simulated isthmus, with increased activation time further improving debris removal. Although their study evaluated debris rather than bacterial biofilm, the findings provide mechanistic support for the present results, particularly the strong performance of EDDY in biofilm disruption. More recently, Awaida *et al.* (2025) **[15]** compared several irrigation activation methods against a multispecies endodontic biofilm and reported substantial bacterial reduction with Endo Ultra and EDDY, although the relative efficacy varied according to the microorganism. Their findings emphasize that activation performance may depend on biofilm composition and experimental conditions **[16, 17]**. The present study, using a standardized *E. faecalis* biofilm model, similarly demonstrates significant differences among activation systems and identifies Multisonic Irrigation Activation as the most effective tested approach. Overall, the present findings agree with the broader evidence that irrigant activation enhances intracanal cleaning and microbial disruption compared with syringe irrigation. However, because this was an *in vitro* study using a standardized single species biofilm model, the superior performance of Multisonic Irrigation Activation should not be directly extrapolated to clinical treatment outcomes without further studies using multispecies biofilms, complex canal anatomies and clinical models.

Conclusion:

All irrigation activation systems showed significantly greater biofilm disruption than conventional syringe irrigation. Among the evaluated techniques, Multisonic Irrigation Activation exhibited the highest antibacterial efficacy, followed by Sonic Activation (EDDY) and Passive Ultrasonic Irrigation. The use of irrigant activation systems may therefore enhance root canal disinfection and improve the predictability of endodontic treatment outcomes.

References:

[1] Boutsioukis C & Arias-Moliz MT. *Int Endod J*. 2022 **55**:588. [PMID: 35338652]
[2] Gomes BPFA *et al. Braz Dent J*. 2023 **34**:1. [PMID: 37909632]
[3] Abaza MM *et al. Lasers Med Sci*. 2025 **40**:522. [PMID: 41389357]
[4] Cheung AWT *et al. Restor Dent Endod*. 2021 **46**:e10. [PMID: 33680899]
[5] Coaguila-Llerena H *et al. Restor Dent Endod*. 2022 **47**:e11. [PMID: 35284323]
[6] Atav A *et al. Clin Oral Investig*. 2025 **29**:309. [PMID: 40411649]
[7] Hussein HM & Ali AH. *Clin Cosmet Investig Dent*. 2026 **18**:610759. [PMID: 42152817]
[8] Donnermeyer D *et al. J Endod*. 2019 **45**:322. [PMID: 30803540]
[9] De-Deus G *et al. Clin Oral Investig*. 2019 **23**:3087. [PMID: 30417226]
[10] Keskin C *et al. J Dent Res Dent Clin Dent Prospects*. 2018 **12**:288. [PMID: 30774796]
[11] Chu X *et al. BMC Oral Health*. 2023 **23**:155. [PMID: 36932445]
[12] Widbiller M *et al. Healthcare (Basel)*. 2023 **11**:376. [PMID: 36766951]
[13] Shin JY *et al. J Oral Sci*. 2023 **65**:158. [PMID: 37197929]
[14] Plotino G *et al. Aust Endod J*. 2023 **49**:353. [PMID: 36960975]
[15] Awaida A *et al. Biomater Investig Dent*. 2025 **12**:43065. [PMID: 40303577]
[16] Aydın U *et al. Acta Odontol Scand*. 2025 **84:**266. [PMID: 40391985]
[17] Sakin TÖ *et al. Sci Rep*. 2026 **16**:16187. [PMID: 41957515]

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