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Biofilm removal from oval root canals using three irrigation activation protocols

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Abstract:

Persistent biofilm in oval root canal recesses remains a major cause of incomplete endodontic disinfection because conventional syringe irrigation cannot reliably reach fins, isthmuses and flattened buccolingual extensions. Therefore, it is of interest to evaluate the XP-Endo Finisher, a Gentle Wave-inspired negative-pressure multisonic irrigation protocol and passive ultrasonic irrigation for biofilm removal from 90 standardized oval mandibular premolar canals using paired micro-computed tomography and confocal laser scanning microscopy. After four-week multispecies biofilm maturation, canals were instrumented to a common apical size and randomly allocated to three irrigation activation groups; residual hard-tissue debris volume, biofilm-covered canal wall area, viable bacterial percentage and dentinal tubule penetration of live cells were recorded. The Gentle Wave-inspired protocol showed the highest debris reduction ($91.8 \pm 4.9\%$) and lowest viable biofilm percentage ($9.7 \pm 4.3\%$), followed by XP-Endo Finisher ($84.6 \pm 6.3\%$; $15.8 \pm 6.1\%$) and passive ultrasonic irrigation ($78.2 \pm 7.5\%$; $23.5 \pm 8.6\%$), with significant intergroup differences ($p < 0.001$). Thus, data shows that fluid dynamics combining negative pressure and multisonic agitation may improve disinfection of oval canals, although XP-Endo finisher also achieved substantial cleaning beyond passive ultrasonic irrigation.

Keywords: XP-Endo Finisher, Gentle Wave, passive ultrasonic irrigation, oval canals, biofilm, micro-CT, confocal microscopy

Background:

Endodontic success depends on the combined control of microbial infection, tissue remnants and chemically altered debris within a complex three-dimensional canal system [1]. Persistent intraradicular biofilm may remain clinically silent during instrumentation but can later sustain apical inflammation when viable cells are protected within lateral extensions, recesses, dentinal tubules, or accumulated hard-tissue debris [2]. Oval canals are particularly challenging because the buccolingual dimension exceeds the mesiodistal dimension, leaving untouched walls even after preparation with modern nickel-titanium systems [3]. Therefore, irrigation activation is no longer considered an optional adjunct; it is an essential strategy for moving irrigants into spaces that instruments do not contact. The biological limitation of mechanical preparation is closely related to the spatial organization of endodontic biofilm. Microbial communities embedded in an extracellular matrix behave differently from planktonic cells, showing higher tolerance to irrigants and mechanical shear [4]. Conventional syringe irrigation improves chemical delivery but is limited by vapor lock, needle position, canal curvature and restricted irrigant exchange in the apical third [5]. These limitations have encouraged the use of acoustic, hydrodynamic and shape-adaptive activation systems that aim to increase irrigant replacement, streaming velocity and contact with complex canal walls [6]. Passive ultrasonic irrigation creates acoustic streaming and transient cavitation around an oscillating file or tip that remains free within the canal. It has been widely investigated and is considered more effective than syringe irrigation for smear layer, debris and intracanal medicament removal in many experimental models [7]. However, the effectiveness of passive ultrasonic irrigation depends on tip size, activation time, irrigant volume, free oscillation and the ability of the tip to remain centered without wall contact. In oval canals, the ultrasonic field may not uniformly shear biofilm from flattened extensions distant from the tip. XP-Endo Finisher was introduced as a shape-adaptive finishing instrument manufactured from a

proprietary nickel-titanium alloy that expands at body temperature. Its spoon-like motion and high flexibility allow it to contact areas that are not prepared by shaping instruments. Micro-computed tomography and *ex vivo* biofilm studies have shown that this system can reduce accumulated hard-tissue debris and improve cleaning of anatomical irregularities [8]. *In vitro* biofilm models have also reported enhanced removal of infected material from apical root canal complexities when XP-Endo Finisher is used as an agitation system [9]. Multisonic and negative-pressure irrigation systems were developed to improve irrigant exchange through broad-spectrum acoustic energy and apical fluid movement while reducing the risk of extrusion. The Gentle Wave platform is a clinically available example, but its device-specific configuration is difficult to reproduce in every laboratory. A Gentle Wave-inspired protocol using apical negative pressure, warmed irrigant exchange and multisonic-like fluid agitation may offer a useful experimental model for testing whether coordinated hydrodynamic movement provides additional cleaning in oval canals. Existing systematic evidence has compared XP-Endo Finisher and passive ultrasonic irrigation for intracanal medicament and debris removal, yet direct comparison against a negative-pressure multisonic-inspired strategy remains limited [10]. Micro-computed tomography provides non-destructive volumetric assessment of internal debris before and after irrigation, while confocal laser scanning microscopy allows viable and non-viable biofilm assessment in dentinal tubules and canal wall regions [11]. Therefore, it is of interest to compare XP-Endo Finisher, a Gentle Wave-inspired irrigation protocol and passive ultrasonic irrigation for biofilm and debris removal from oval root canals.

Materials and Methods:

This *in vitro* comparative study was conducted on 90 freshly extracted single-rooted mandibular premolars with radiographically confirmed oval canals. Teeth were collected after informed patient consent for research use, cleaned of adherent soft tissue and stored in 0.1% thymol for no longer than

four weeks. Teeth with immature apices, previous endodontic treatment, resorption, cracks, calcifications, or more than one canal were excluded. The buccolingual to mesiodistal canal ratio was measured on preliminary CBCT sections and only canals with a ratio greater than 2.0 at 5 mm from the apex were included. All teeth were decoronated to a standardized root length of 16 mm. Working lengths was established by inserting a size 10 K-file until visible at the apical foramen and subtracting 1 mm. Canals were prepared with a rotary nickel-titanium system up to size 35/04 using 2.5% sodium hypochlorite between instruments. The smear layer was removed using 17% EDTA for 1 minute, followed by sterile saline. The outer root surfaces were coated with two layers of nail varnish except at the access opening sterilized by ethylene oxide and incubated to confirm sterility. A multispecies biofilm suspension containing *Enterococcus faecalis*, *Streptococcus gordonii*, *Fusobacterium nucleatum* and *Candida albicans* was adjusted to 0.5 McFarland standards, introduced into each canal and incubated anaerobically at 37°C for 28 days. Fresh brain-heart infusion broth was replenished every 48 hours. After biofilm maturation, specimens were scanned with micro-computed tomography at an isotropic voxel size of 10 micro meters to quantify baseline internal hard-tissue debris and canal volume. The specimens were then randomly allocated into three groups of 30 each using computer-generated random numbers. Group 1 received XP-Endo Finisher activation. The instrument was operated at 800 rpm and 1 Ncm torque for three cycles of 30 seconds with continuous replenishment of 2.5% sodium hypochlorite. Group 2 received a Gentle Wave-inspired irrigation protocol consisting of warmed sodium hypochlorite delivered through negative-pressure exchange with a multisonic agitation handpiece positioned at the canal orifice for three cycles of 45 seconds, followed by EDTA and saline. Group 3 received passive ultrasonic irrigation with a non-cutting ultrasonic file placed 1 mm short of working length and activated at medium power for three 30-second cycles. Irrigant volume was standardized to 12 mL sodium hypochlorite, 3 mL EDTA and 5 mL saline in all groups. After final irrigation, specimens were rescanned with the same micro-CT parameters. Residual debris volume and percentage reduction were calculated using registered pre - and post-irrigation datasets. Roots were then split longitudinally, stained with LIVE/DEAD BacLight and examined using confocal laser scanning microscopy at coronal, middle and apical thirds. The biofilm-covered canal wall area, viable bacterial percentage and maximum depth of viable biofilm penetration

into dentinal tubules were recorded by two blinded examiners. Intra-examiner and inter-examiner reliability were evaluated with intraclass correlation coefficients. Data were analyzed using SPSS version 26. Normality was assessed using the Shapiro-Wilk test. Continuous variables were expressed as mean $\pm$ standard deviation. Baseline canal characteristics were compared using one-way ANOVA. Intergroup differences in micro-CT and CLSM outcomes were analyzed using one-way ANOVA followed by Tukey post-hoc tests. Regional differences among canal thirds were analyzed using repeated-measures ANOVA. Categorical outcomes were compared using chi-square testing. A p value of less than 0.05 was considered statistically significant.

Results:

All 90 specimens completed biofilm maturation, irrigation activation, and micro-CT scanning and confocal microscopy. Baseline canal morphology was comparable among the three groups. Mean canal volume ranged from $7.84 \pm 0.76 \text{ mm}^3$ in the passive ultrasonic group to $7.92 \pm 0.81 \text{ mm}^3$ in the Gentle Wave-inspired group, with no statistically significant difference ($p = 0.888$). Baseline debris volume and buccolingual-to-mesiodistal canal ratios were also similar across groups, confirming adequate randomization. The Gentle Wave-inspired protocol produced the greatest mean reduction in hard-tissue debris volume ($91.8 \pm 4.9\%$), followed by XP-Endo Finisher ($84.6 \pm 6.3\%$) and passive ultrasonic irrigation ($78.2 \pm 7.5\%$). The intergroup difference was statistically significant ($p < 0.001$). Tukey analysis showed significant differences between Gentle Wave-inspired irrigation and passive ultrasonic irrigation ($p < 0.001$), Gentle Wave-inspired irrigation and XP-Endo Finisher ($p = 0.002$) and XP-Endo Finisher and passive ultrasonic irrigation ($p = 0.011$). Residual debris was highest in the apical third in all groups. Confocal microscopy showed that viable biofilm percentage differed significantly among groups ($p < 0.001$). The Gentle Wave-inspired group recorded the lowest viable biofilm percentage ($9.7 \pm 4.3\%$), while passive ultrasonic irrigation recorded the highest ($23.5 \pm 8.6\%$). Biofilm-covered wall area and depth of viable cell penetration followed the same pattern. Complete or near-complete apical third biofilm clearance was observed in 22 specimens (73.3%) in the Gentle Wave-inspired group, 16 specimens (53.3%) in the XP-Endo Finisher group and 10 specimens (33.3%) in the passive ultrasonic irrigation group ($p = 0.006$). Baseline canal characteristics, micro-CT debris reduction and confocal microscopy outcomes are summarized in **Tables 1, 2 and 3** respectively.

Table 1: Baseline characteristics of oval root canal specimens

Parameter	XP-Endo Finisher (n=30)	Gentle Wave-inspired (n=30)	Passive ultrasonic irrigation (n=30)	p value
Root length after decoronation (mm)	16.0 ± 0.0	16.0 ± 0.0	16.0 ± 0.0	1.000
Canal volume before irrigation (mm ³)	7.88 ± 0.79	7.92 ± 0.81	7.84 ± 0.76	0.888
Baseline debris volume (mm ³)	0.92 ± 0.18	0.94 ± 0.17	0.93 ± 0.19	0.912
Buccolingual/mesiodistal canal ratio	2.41 ± 0.28	2.45 ± 0.31	2.39 ± 0.27	0.721
Baseline viable biofilm (%)	87.9 ± 5.4	88.3 ± 5.1	87.1 ± 5.7	0.684

Table 2: Micro-CT comparison of debris reduction after irrigation activation

Outcome	XP-Endo Finisher	Gentle Wave-inspired	Passive ultrasonic irrigation	p value
Post-irrigation residual debris (mm ³)	0.14 ± 0.06	0.08 ± 0.04	0.20 ± 0.08	<0.001
Overall debris reduction (%)	84.6 ± 6.3	91.8 ± 4.9	78.2 ± 7.5	<0.001
Coronal third reduction (%)	89.7 ± 5.1	95.1 ± 3.6	84.8 ± 6.8	<0.001

Middle third reduction (%)	85.6 ± 6.7	92.6 ± 4.7	78.9 ± 7.2	<0.001
Apical third reduction (%)	78.4 ± 8.9	87.5 ± 6.4	70.9 ± 9.8	<0.001

Table 3: Confocal laser scanning microscopy outcomes

Outcome	XP-Endo Finisher	Gentle Wave-inspired	Passive ultrasonic irrigation	p value
Biofilm-covered canal wall area (%)	18.9 ± 7.4	11.6 ± 5.8	28.4 ± 9.2	<0.001
Viable biofilm cells (%)	15.8 ± 6.1	9.7 ± 4.3	23.5 ± 8.6	<0.001
Maximum viable penetration depth (micro meters)	132.5 ± 40.7	86.4 ± 31.2	184.6 ± 51.5	<0.001
Near-complete apical clearance, n (%)	16 (53.3%)	22 (73.3%)	10 (33.3%)	0.006
Examiner ICC for CLSM measurements	0.91	0.93	0.90	-

Discussion:

The present study showed that all three irrigation activation methods improved cleaning of oval canals, but the magnitude of biofilm and debris reduction differed significantly. The Gentle Wave-inspired protocol showed the greatest micro-CT debris reduction and the lowest residual viable biofilm on confocal microscopy. This outcome is biologically plausible because negative-pressure exchange, warmed irrigant and multisonic-like agitation can increase irrigant replacement and shear stress in flattened canal extensions without relying on direct wall contact [13]. Oval canals contain recesses that are frequently untouched by shaping files and improved hydrodynamic exchange is therefore critical for dislodging biofilm from protected spaces [14]. XP-Endo Finisher performed better than passive ultrasonic irrigation in most outcomes. The shape-adaptive file is designed to expand and sweep through irregular spaces during rotation, which may explain its ability to reduce biofilm-covered area and viable penetration depth. Earlier micro-CT investigations have shown similar benefits in reducing accumulated hard-tissue debris from oval canals, although complete cleanliness remains uncommon [15]. The present results support the same concept: the instrument improved the cleaning effect but did not eliminate all viable cells, particularly in the apical third. Passive ultrasonic irrigation also achieved substantial debris reduction, confirming the clinical value of acoustic streaming. Nevertheless, its comparatively lower performance may be explained by the geometry of the oval canals. Ultrasonic activation is strongest around the oscillating tip and may be reduced when the tip contacts a wall or when irrigant movement is restricted in extensions those are not aligned with the tip. Experimental reviews have emphasized that passive ultrasonic irrigation is technique sensitive and depends on tip size, power, and canal taper and free oscillation [16]. The difference between micro-CT and confocal outcomes is important. A canal with low residual debris volume can still harbor viable cells within dentinal tubules and a canal with reduced viable staining can still contain organic matrix attached to walls. Therefore, the combination of micro-CT and CLSM gives a more balanced view than either method alone. This approach is consistent with contemporary endodontic research, where structural and microbial outcomes are increasingly combined to evaluate disinfection [17]. The Gentle Wave-inspired protocol should not be interpreted as a direct substitute for the commercial Gentle Wave system. The study intentionally used an experimentally reproducible protocol incorporating the principles of negative-pressure fluid exchange and broad-spectrum agitation rather than a proprietary clinical device.

Even so, the results align with the broader observation that enhanced fluid dynamics can improve cleaning in complex canals [18]. Future work should directly compare manufacturer-specific systems with standardized laboratory analogs, include extrusion assessment and evaluate safety parameters. The apical third remained the most difficult region in all groups. This is consistent with the reduced diameter, limited taper, vapor lock and lower irrigant exchange commonly seen in apical anatomy. Persistent apical biofilm has direct clinical relevance because this region is closest to periapical tissues and may influence healing if microbial products remain active [19]. Increasing activation time may improve outcomes, but longer activation may also raise concerns about dentin erosion, irrigant extrusion and thermal effects. Thus, improved cleaning should always be balanced with biological safety. The study has limitations. The bio film model was standardized and does not fully reproduce the polymicrobial ecology, host tissue proteins and nutritional gradients of clinical infection. Extracted teeth also differ in dentin age, sclerosis and canal morphology. Although micro-CT and confocal microscopy provide precise laboratory data, they cannot predict clinical healing by themselves. The sample size was sufficient for primary outcomes, but larger studies using curved oval canals and simulated periodontal ligament models could improve external validity [20]. Within these limits, the findings suggest that hydrodynamic irrigation strategies may provide measurable advantages in oval canals. The data also indicate that XP-Endo Finisher is a clinically meaningful adjunct when advanced multisonic or negative-pressure systems are not available. Passive ultrasonic irrigation remains useful, inexpensive and accessible, but may require careful optimization to approach the cleaning levels achieved by more adaptive or fluid-exchange systems [21]. Micro-CT evidence has also showed accumulation of hard-tissue debris in untouched canal regions, supporting the relevance of volumetric debris assessment in the present model [12]. A 2025 lateral-canal study likewise reported method-dependent differences in biofilm removal across activation protocols, reinforcing the importance of irrigant activation in anatomically protected spaces [21]. A 2024 study of ultrasonic and Er, Cr: YSGG laser-activated irrigation also showed substantial biofilm reduction with activated protocols, supporting continued comparison of adjunctive activation strategies [22].

Conclusion:

The Gentle Wave-inspired protocol achieved high debris and biofilm reduction in oval root canals. XP-Endo Finisher outperformed passive ultrasonic irrigation, but no protocol

achieved complete disinfection. Advanced activation may improve cleaning of complex anatomy, with clinical safety and validation still required.

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