

Efficacy of high-frequency sonic irrigation on removing debris from root canal isthmus: an in vitro study based on simulated root canals (#108216)

1

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Efficacy of high-frequency sonic irrigation on removing debris from root canal isthmus: an in vitro study based on simulated root canals

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Background. Infection control is important in root canal treatment. Effective cleaning and shaping are challenging due to complex anatomy, particularly in the isthmus4narrow connections between canals that can harbor bacteria. Conventional needle irrigation (CNI) is inadequate in this region, prompting the use of passive ultrasonic irrigation (PUI) and high-frequency acoustic instruments like EDDY. This study evaluates the cleaning effects of four irrigation protocols using 3D-printed isthmus models. **Methods.** Sixty digital root canal models with isthmuses in the crown, middle, and apical thirds were designed using Ansys 19.0 and 3D printer (20 specimens per isthmus location). Specimens were prepared to 30#, 0.04 without irrigation. Debris accumulation in the isthmus was photographed and analyzed using Image J to calculate the initial debris area (S1). Specimens were then irrigated using CNI, low-frequency sonic irrigation (EndoActivator, EA,Dentsply, Charlotte, NC, USA), PUI, or high-frequency sonic irrigation (EDDY), followed by re-imaging to calculate remaining debris area (S2). Debris reduction percentage was determined using the formula: $(S1-S2)/S1 \times 100\%$. **Results.** Debris reduction varied with isthmus position. In the crown third, EDDY achieved the highest debris reduction ($86.18 \pm 2.25\%$), followed by PUI, EA, and CNI, with significant differences among groups ($P < 0.05$). The same trend was observed in the middle third, with EDDY

showing the highest efficacy (73.96±6.75%). In the apical third, debris reduction was lower overall, with no significant difference between EDDY and PUI, but both outperformed EA and CNI. **Discussion.** Our results showed that EDDY demonstrated superior debris removal in the crown and middle thirds, but all irrigation protocols showed limited efficacy in the apical third.

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4

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38 **Abstract**

39 **Background.** Infection control is important in root canal treatment. Effective cleaning and
40 shaping are challenging due to complex anatomy, particularly in the isthmus narrow
41 connections between canals that can harbor bacteria. Conventional needle irrigation (CNI) is
42 inadequate in this region, prompting the use of passive ultrasonic irrigation (PUI) and high
43 frequency acoustic instruments like EDDY. This study evaluates the cleaning effects of four
44 irrigation protocols using 3D-printed isthmus models.
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47 were prepared to 30#, 0.04 without irrigation. Debris accumulation in the isthmus was
48 photographed and analyzed using Image J to calculate the initial debris area (S1). Specimens
49 were then irrigated using CNI, low-frequency sonic irrigation (EndoActivator, EA, Dentsply,
50 Charlotte, NC, USA), PUI, or high-frequency sonic irrigation (EDDY), followed by re-imaging
51 to calculate remaining debris area (S2). Debris reduction percentage was determined using the
52 formula: (S1-S2)/S1 100%.
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54 highest debris reduction (86.18–2.25%), followed by PUI, EA, and CNI, with significant
55 differences among groups (P<0.05). The same trend was observed in the middle third, with
56 EDDY showing the highest efficacy (73.96–6.75%). In the apical third, debris reduction was
57 lower overall, with no significant difference between EDDY and PUI, but both outperformed
58 EA
59 and CNI.
60 **Discussion.** Our results showed that EDDY demonstrated superior debris removal in the crown
61 and middle thirds, but all irrigation protocols showed limited efficacy in the apical third.

62 **Introduction**

63 Infection control is an essential goal of root canal treatment in order to prevent or cure apical
64 periodontitis caused by a polymicrobial infection of the root canal (Ricucci et al., 2018). During
65 the infection control procedure, chemo-mechanical cleaning and shaping of the root canal system
66 plays an important role in thoroughly eliminating the infection. Due to the complex anatomy of
67 the root canal system and diversity of root canal infection, the current method of debris removal
68 in complex anatomical structures is inadequate. The isthmus is a common complex anatomical
69 structure in the root canal system of maxillary premolars and first mandibular molars, a narrow
70 banded communication between two root canals (Vertucci, 2005), and usually contains dental
71 pulp tissue (Weller et al., 1995), which can be found in molars using Micro-CT with a
72 percentage up to 75.4% (Yin et al., 2021). Bacteria can infiltrate the isthmus and form a biofilm

Z komentarzem [us1]: The introduction does not state the null hypothesis.

(Villalta-Briones et al., 2021). However, the narrow width and irregular shape of the isthmus prevent direct contact with files (Kim et al., 2016). Moreover, the substantial debris caused by mechanical preparation may accumulate in the isthmus, shielding the underlying biofilm from exposure to irrigants (Paque et al., 2011). Furthermore, root canal filling is unable to fully obturate the isthmus space (Yu et al., 2024). All these factors allow bacteria to survive and pose a hidden risk that can potentially lead to the failure of root canal treatment in infected root canal systems (Kim et al., 2016).

Many root canal irrigation methods were applied in order to remove smear layer and dentine debris that occur following instrumentation of the root canal (Baugh & Wallace, 2005). Conventional syringe irrigation was one of the most widely-used irrigation methods. Because of the vapor lock phenomenon in the apical third of a root canal (Blanken et al., 2009), it is difficult to achieve ideal infection debridement in the apical region by conventional syringe irrigation alone. The unsatisfactory irrigation efficacy of conventional syringe irrigation in root canal has been reported by many studies (Chen et al., 2016, Rajamanickam et al., 2022, Vatanpour et al., 2022). Johnson et al. (2012) pointed out that the debridement efficacy of isthmus by conventional syringe irrigation in the apical one third should be further strengthened.

Passive ultrasonic irrigation (PUI) has been recommended for its better irrigation efficacy because of the ultrasonically activated files with high frequency between 25,000 Hz and 40,000 Hz (Plotino et al., 2007). PUI transfers energy through the vibration of the ultrasonic file in liquid and utilizes significant acoustic streaming and cavitation effect to achieve debridement (Gu et al., 2009). Malentacca et al. (2018) reported that PUI may help to remove the artificial pulp tissue from the isthmus of a transparent tooth model. However, none of the protocols of different irrigation methods including PUI were able to completely remove all the debris in the isthmus (de Mattos de Araujo et al., 2022).

The sonically driven EndoActivator (EA) canal irrigation system (Dentsply, York, PA, USA) uses disposable flexible polymer tips of different sizes. The activator tips can be operated at 2,000-10,000 cycles/min without damaging the root dentin. Compared with the uncurved file of PUI, EA can enter the middle and lower segments of the root canal, breaking the apical vapor lock. In shaped canals, some related results showed no statistically significant difference in canal cleanliness between EA and PUI (Klyn et al., 2010).

The high frequency acoustic irrigation instrument EDDY tip was flexible, made of a smooth polymer, and oscillates at 6,000Hz by means of a sonic scaler. It was found that EDDY tips had the ability to enter the middle and lower segments of the root canal, break the apical block, effectively remove the dentin debris from the apical third of root canals, and adapt well to the initial shape of root canals (Zeng et al., 2018). After mechanical preparation, the high-frequency acoustic irrigation instrument EDDY was used with a combination of irrigants to achieve effective contact with the root canal wall, with the help of a large amplitude generated by acoustic vibration that can promote the irrigation of the isthmus and other irregular areas of the root canal system. Urban et al. (2017) demonstrated that high-frequency acoustic irrigation can achieve root canal cleaning effects comparable to ultrasonic irrigation in single-rooted mandibular premolars. Using scanning electron microscopy, it was found that the abilities of

acoustic irrigation and ultrasonic irrigation of debris and smear layer removal in root canals were similar, and were both superior to low frequency acoustic irrigation (EndoActivator) and conventional needle irrigation. Linden et al. (2020) compared the volume changes of debris in the isthmus space in the mesial root canal system of extracted mandibular molars before and after root canal irrigation using Micro-CT scanning. The results showed that the ability of EDDY was not superior to that of conventional needle irrigation, but was weaker than that of ultrasonic irrigation on debris removal in the isthmus space. This study appears to indicate that EDDY has limitations in cleaning the isthmus. EDDY tips, with their greater taper, increase contact with root canal walls and may affect its free movement. However, EDDY may have greater efficiency than EndoActivator in removing dentin debris from the simulated isthmus (Plotino et al., 2023).

As a special and representative complex anatomical structure, the isthmus represents a considerable challenge for root canal disinfection. The thorough cleanliness of the isthmus could significantly enhance the effectiveness of root canal disinfection. Ultrasonic irrigation and acoustic irrigation may have relevant potential abilities in isthmus cleaning. However, an assessment of the different ultrasonically activated devices and acoustic irrigation and their cleaning abilities in the isthmus is still lacking. Therefore, the objective of this study was to evaluate the cleaning effects of four different irrigation protocols (CNI, PUI, EA, and EDDY) on the isthmus. A new realistic 3D-printed isthmus model based on the Micro-CT parameters of the maxillary first premolars was applied for irrigation and images under microscope were taken to compare the debris removal abilities.

134

Materials & Methods

Construction of isthmus models

A digital model was constructed using ANSYS 19.0 finite element analysis software (ANSYS, Pittsburgh, PA, USA). The geometric shape of the main root canal model was a truncated cone with a height of 15 mm, and the apical foramen diameter was set at 0.15 mm (size 15#). The apical foramen was closed (Johnson et al., 2012) and the root canal taper was 0.02. The isthmus model parameters utilized in this study, specifically a height of 3 mm (Swimberghe et al., 2019;

Park et al., 2023) and a width of 0.15 mm (Swimberghe et al., 2019), were derived from isthmus models previously constructed in earlier studies. Isthmus models were designed at the coronal 1/3, middle 1/3, and apical 1/3 of the root, with their upper surfaces positioned at distances of 2 mm, 5 mm, and 8 mm from the root canal orifice plane, respectively.

Preparation of the root canal isthmus model

The 3D-printed model's main root canal was prepared to working length using the size 15# file. Subsequently, mechanical preparation was carried out using M3-2017 rotary nickel-titanium

instruments (YiRui, Changzhou, China) until it reached size 30#, taper 0.04. During the preparation process, no root canal irrigation was performed to ensure the entry of as much debris as possible into the isthmus area. Following mechanical preparation, a stereomicroscope

Z komentarzem [us2]: Trade name of the file and country?

(Olympus, Tokyo, Japan) was used to photograph the distribution of debris in the isthmus space. The microscope brightness was adjusted to the maximum value during photography, and the objective distance from the surface of the root canal model was kept constantly at 4 cm. Using Image J software, the isthmus area was measured based on the isthmus parameters set in the digital model. For example, in the model with the isthmus located at the coronal 1/3, horizontal lines were drawn at 2 mm from the upper end of the model (model total length of 20 mm, isthmus upper surface 2 mm from the upper end of the model) as the upper boundary of the isthmus. Similarly, horizontal lines were drawn at 15 mm from the lower end of the model as the lower boundary of the isthmus. Vertical lines were drawn at the middle two quartile points of the model's transverse axis, defining the left and right boundaries of the isthmus. The closed rectangle formed by the intersection of these four lines represented the isthmus (Figure 1).

Due to the potential impact of brightness settings on defining the debris area during measurements, the study found that adjusting the color brightness threshold to 200 provided a clear and accurate display of debris areas within the isthmus under the stereomicroscope. Therefore, to ensure consistency in measurement conditions across all groups, the color brightness threshold of all images was uniformly set to 200. The software's automatic selection function was used to extract the debris containing parts within the isthmus and measure their area, which was denoted as S1.

Grouping and irrigation

Sample size estimation was performed using PASS for Windows software (version 15.0; NCSS, Kaysville, UT, USA) with a significance level set at 0.05 ($\alpha=0.05$), power at 0.90, and the number of irrigation groups set to 4, specifying equal sample sizes for each group. The minimum sample size for each group was calculated to be five. Therefore, the sample size for experiments at the same isthmus location was determined to be 20 and the total sample size of the three groups with different isthmus locations was 60. The 20 isthmus-models, labeled 1-20, were randomly assigned to four irrigation groups ($n=5$). The root canals were filled with sterile water and the following irrigation protocols were conducted:

d The conventional needle irrigation group (CNI, $n=5$): Using a 30-gauge side-vented irrigation needle (Kontour, Switzerland), the needle tip was placed 2 mm short of the working length of the root canal and irrigated with 1 mL of sterile water at a rate of 0.033 mL/s for 30 seconds, followed by a 30-second static period. This irrigation-static process was repeated twice, accumulating a total of 3 mL of sterile water irrigation for each root canal, and a total of 6 mL for each model with two root canals.

e Low-frequency sonic irrigation (Endo Activator, EA, $n=5$): The root canals and isthmus were filled with sterile water, and a #25/.04 Endo Activator sonic working tip (Dentsply, Charlotte, NC, USA) was used. The working tip was placed 2 mm short of the working length of the root canal, set at 10,000 cycles/min, and sonically irrigated for 30 seconds, followed by a 30-second static period. This sonic irrigation-static process was repeated twice for each root canal.

f The passive ultrasonic irrigation group (PUI, $n=5$): The root canals and isthmus were filled

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191 with sterile water, and a P5 ultrasonic dynamic system (Satelec, Viry-Ch tillon, France) with a
192 #25/.02 ultrasonic working tip (Satelec, Viry-Ch tillon, France) was used. The ultrasonic tip was
193 placed 2 mm short of the working length of the root canal, at the power setting of 7 out
194 of 20, and ultrasonically irrigated for 30 seconds, followed by a 30-second static period.
This ultrasonic 195 irrigation-static process was repeated twice for each root canal.

196 g High-frequency sonic irrigation (EDDY, n=5): The root canals and isthmus were filled with
197 sterile water, and a #20/.04 EDDY sonic working tip (VDW, Munich, Germany) was used. The
198 working tip was placed 2 mm short of the working length of the root canal and sonically
199 irrigated for 30 seconds, followed by a 30-second static period. This sonic irrigation-static
process was 200 repeated twice for each root canal.

201 During the 30-second activation process in e-g, the syringe irrigation needle tip was placed
202 horizontally at the root canal orifice, providing continuous irrigation of 1 mL of sterile water per
203 canal (0.033 mL/s). Each root canal received a cumulative irrigation of 3 mL of sterile water, and
204 each model's two root canals received a total irrigation of 6 mL of sterile water. The
same 205 protocol was followed for different isthmus locations.

206 Evaluation of isthmus cleaning and assessment criteria

207 After irrigation, each root canal was dried by three paper points, and the surface of the model
208 was allowed to air dry for a minimum of two hours. The dried root canal models were
209 photographed under a stereomicroscope. Image J software was then used to measure the area of
210 debris within the isthmus after irrigation, using the same method as described in section (2). The
211 area occupied by debris within the isthmus after irrigation was recorded as S2. The debris 212
removal rate within the isthmus was calculated as follows: Debris Removal Rate = (Initial 213
isthmus debris area S1 - Debris area after irrigation S2) / Initial isthmus debris area S1.

214 Statistical analysis

215 Statistical analysis of experimental data was performed using SPSS 20.0 software (SPSS,
216 Chicago, IL, USA). The normality of the initial isthmus debris area and debris removal rate 217
results for each group was first tested. If the data followed a normal distribution, a one-way
218 analysis of variance (ANOVA) was conducted, followed by Games-Howell post hoc tests for
219 pairwise comparisons. If the data did not follow a normal distribution, the Kruskal-Wallis non-
220 parametric test was used for overall and pairwise comparisons. A significance level of $P < 0.05$
221 was considered statistically significant.

222

223 Results

224 1. When the isthmus was located in the coronal 1/3, the EDDY system exhibited the most 225
optimal performance, followed by ultrasonic irrigation, EndoActivator, and irrigation needle in 226
descending order.

227 Representative images before and after irrigation for each group at the coronal 1/3 are shown in
228 Figure 2. The initial isthmus debris areas were relatively consistent among the groups, with the
229 pre-irrigation debris area for the CNI group at 4.05–0.27 mm², EA group at 3.82–0.36 mm², PUI
230 group at 3.73–0.32 mm², and EDDY group at 3.89–0.21 mm². The pre-irrigation debris areas
231 showed no statistically significant differences among the groups ($F=1.022$,

Z komentarzem [us4]: why the power setting 7 was
chosen

$P=0.409>0.05$), suggesting that the initial isthmus debris areas were at a similar baseline level for all groups.

The isthmus debris removal rates varied among the groups, with the CNI group exhibiting a removal rate of only 8.36–2.04%, EA group at 17.62–3.05%, PUI reaching 54.07–3.33%, and EDDY group achieving the highest rate at 86.18–2.25%. The isthmus debris removal rates showed statistically significant differences among the groups ($F=862.106$, $P=0<0.05$).

Specifically, when the isthmus was located in the coronal 1/3, the isthmus debris cleaning efficiency was ranked as follows: EDDY > PUI > EndoActivator > CNI. The isthmus debris area before and after completion of root canal irrigation for each group is presented in Table 1.

2. When the isthmus was located in the middle 1/3, the EDDY system demonstrated the most optimal performance, followed by PUI, EndoActivator, and CNI, in descending order. Representative images before and after irrigation for each group in the middle 1/3 are shown in Figure 3. The pre-irrigation isthmus debris areas were relatively consistent among the groups, with the CNI group at 3.47–0.41 mm², EndoActivator group at 3.05–0.35 mm², PUI group at 3.27–0.59 mm², and EDDY group at 3.14–0.11 mm². There were no statistically significant differences among the groups ($F=1.030$, $P=0.406>0.05$), suggesting that the initial isthmus debris areas were at a similar baseline level for all groups.

After irrigation, there was a reduction in isthmus debris area for each group. The isthmus debris removal rate was lower in the CNI group at 6.74–0.13%, EndoActivator group at 17.40–5.52%, PUI group at 49.84–6.32%, and the EDDY group exhibited the highest removal rate at 73.96–6.75%. The isthmus debris removal rates showed statistically significant differences among the groups ($F=162.673$, $P=0<0.05$). Specifically, when the isthmus was located in the middle 1/3, the isthmus debris cleaning efficiency was ranked as follows: EDDY > PUI > EndoActivator > CNI. The isthmus debris area before and after completion of root canal irrigation for each group is presented in Table 2.

3. When the isthmus was located in the apical 1/3, the cleaning efficacy of EDDY was comparable to that of PUI, and both were superior to other irrigation methods. In the apical 1/3, all groups exhibited relatively low debris before irrigation. The pre-irrigation isthmus debris areas were similar among the groups, with the CNI group at 1.76–0.28 mm², EndoActivator group at 1.77–0.43 mm², PUI group at 1.83–0.41 mm², and EDDY group at 2.16–0.35 mm². The pre-irrigation debris areas showed no statistically significant differences among the groups ($F=1.282$, $P=0.314>0.05$), indicating that the initial isthmus debris areas were at a similar baseline level for all groups.

The debris removal rates for each group were as follows: CNI 3.72–0.76%, EndoActivator group at 12.89–4.43%, PUI at 30.45–6.60%, and EDDY group at 31.78–5.74%. The isthmus debris removal rates showed statistically significant differences among the groups ($F=38.854$, $P=0<0.05$). Further pairwise comparisons revealed no statistically significant difference in cleaning efficiency between EDDY and PUI ($P=0.744>0.05$), while significant differences were

271 observed in pairwise comparisons between the other groups. Therefore, when the isthmus was
 272 located in the apical 1/3, the isthmus debris cleaning efficiency was equally EDDY = PUI > 273
 EndoActivator >CNI. The isthmus debris area before and after completion of root canal 274
 irrigation for each group is presented in Table 3.

275 The overall comparison of the cleaning effects of different irrigation methods on isthmus debris
 276 is illustrated in Figure 5. It is evident that regardless of the isthmus location in the coronal 1/3,
 277 middle 1/3, or apical 1/3, the irrigation needle demonstrated poor cleaning efficiency for isthmus
 278 debris. While the EndoActivator showed some improvement in isthmus cleaning efficacy
 279 compared to the irrigation needle, its performance remained at a relatively low level. When the
 280 isthmus was in the coronal 1/3 or middle 1/3, the ultrasonic and EDDY groups exhibited better
 281 cleaning efficiency. However, when the isthmus was located in the apical 1/3, both the PUI and
 282 EDDY groups showed a noticeable reduction in cleaning effectiveness.

283

284 Discussion

285 This study investigated standardized, transparent tooth root models with isthmuses to compare
 286 the cleaning efficiency of four irrigation methods and explore how isthmus position affects
 287 cleaning efficiency. Our findings indicate that high-frequency sonic waves (EDDY) were most
 288 effective on removing debris within isthmus models, followed by PUI, whereas low-frequency
 289 sonic waves (EA) and CNI showed less favorable outcomes, with all methods exhibiting
 290 limited efficacy in the apical region.

291 Currently, there has been a substantial amount of research on cleaning dentin debris within
 292 isthmuses; however, many studies have focused on ex vivo teeth (Johnson et al., 2012, Villalta-
 293 Briones et al., 2021). Due to the lack of uniformity in isthmus morphology, position, and other
 294 parameters in ex vivo teeth (Estrela et al., 2015, Xu et al., 2020), it is challenging to evaluate the
 295 cleaning efficacy of different techniques under the same conditions. There is limited research on
 296 isthmuses with identical parameters, with only a few studies utilizing 3D printing to construct
 297 root canal models with standardized isthmuses as research subjects (van der Sluis et al.,
 2007,
 298 Malentacca et al., 2018, Swimberghe et al., 2019). In this study, we employed digital modeling
 299 and 3D printing technology to design tooth root models with standardized isthmuses as research
 300 subjects and evaluate the cleaning efficacy of various irrigation techniques. Previous studies
 301 evaluating the cleaning efficacy of simulated isthmus models used indicators such as injecting
 302 hydrogel into the isthmus and assessing the efficiency of different irrigation methods in
 303 removing the hydrogel (Swimberghe et al., 2019, Robberecht et al., 2023); Malentacca et
 al.
 304 (2018) injected bovine pulp tissue into prepared isthmus models and used a stereomicroscope to
 305 observe the effectiveness of syringe irrigation, endovac, PUI, and ultrasonic negative pressure
 306 devices in clearing bovine pulp tissue from the isthmus, but the injected tissues may have had
 307 different distribution patterns compared with the debris accumulation procedure of the
 isthmus.

308 In order to closely simulate clinical conditions, we mechanically prepared the root canal models

Z komentarzem [us5]: Consider discussing the null hypotheses of the study to remind the reader of the study's purpose.

309 based on clinical operational steps. The removal rate of debris entering the isthmus during root
310 canal preparation was used as the evaluation indicator.

311 In this study, the high-frequency sound wave system EDDY demonstrated the most effective
312 cleaning of isthmuses. Except for the lack of statistically significant differences between EDDY
313 and PUI in cleaning efficacy in the apical 1/3, EDDY outperformed other irrigation methods in
314 all other conditions. This is consistent with the findings of Swimberghe et al. (2019). In that
315 study, the hydrogel removal from an artificial isthmus model with EDDY was significantly 316
greater than that with CNI and EA, but not significantly different from PUI. Robberecht et al.
317 (2023) also showed that EDDY achieved significantly better results than syringe irrigation in
318 isthmus cleaning. In their study, high-speed cameras recorded significant acoustic streaming 319
effects in the isthmus during EDDY irrigation, explaining its superior cleaning efficacy.

320 Additionally, based on the principles of vibration, the intensity of acoustic streaming is directly
321 proportional to the frequency and amplitude of the working tip's vibration. Given that high322
frequency sound waves vibrate at a higher frequency than low-frequency ones, the resulting
323 acoustic streaming effect was stronger. Compared to ultrasonics, high-frequency sound waves
324 also have a larger vibration amplitude, facilitating better irrigation fluid penetration into the
325 isthmus and achieving superior isthmus cleaning (van der Sluis et al., 2007). Otherwise, high-
326 speed imaging (100,000 fps) observations of activated EDDY tips make three dimensional
327 orbital movements, while ultrasonic files oscillate transversely in one plane (Swimberghe et al.,
328 2019). In the narrow space, the horizontal vibration of the working tip will be significantly
329 limited, so the amplitude of the ultrasonic working tip will be significantly reduced when it is
330 constrained by the root canal wall (Donnermeyer et al., 2024). However, the acoustic working tip
331 can still produce longitudinal vibration with larger amplitude when constrained, and its
332 weakening degree is smaller than that of ultrasonic (Chu et al., 2023).

333 There is limited research on the impact of isthmus parameters on cleaning efficacy. Robberecht
334 et al. (2023) explored the influence of isthmus anatomical parameters by designing models with
335 different width (0.4 mm, 0.15 mm) and length (2 mm, 4 mm) parameters, and demonstrated
336 increased difficulty in cleaning longer and narrower isthmuses. Alsubait et al. (2021) compared
337 different irrigation techniques in different positions within ex vivo teeth, and found no difference
338 in cleaning efficacy between positions 3 mm and 5 mm from the apex. There was also no
339 difference between EDDY and PUI at the same position, although both of which were superior to
340 manual irrigation. To the best of our knowledge, no study has compared the effect of isthmus
341 position on cleaning efficacy in simulated root canals. In this study, isthmuses with the same
342 parameters were designed in the coronal 1/3, middle 1/3, and apical 1/3 of the root. The results
343 showed that as the isthmus position approached the apex, the cleaning efficiency of all irrigation
344 methods tended to decrease. This trend aligns with the conclusions of Klyn et al. (2010) from
345 their ex vivo study. Ultrasonics and high-frequency sound waves (EDDY) exhibited significantly
346 lower cleaning efficacy in the apical 1/3 compared to the coronal 1/3 and middle 1/3. This might
347 be due to the narrower main canal in the apical segment, resulting in increased contact between
348 the irrigating instrument and the canal walls, reducing the EDDY current effect
generated by 349 mechanical vibration and subsequently diminishing cleaning efficacy
(Ahmad et al., 2009).

350 Additionally, when the lateral movement of the sonic working tip is restricted, its vibration mode
 351 may shift to a purely longitudinal vibration, leading to a reduction in the driving force of
 352 irrigation fluid into the isthmus and potentially weakening isthmus cleaning efficacy (Walmsley
 353 et al., 1989). In addition, there was not as much initial accumulation of debris in the apical 1/3,
 354 and since the preparation file had the ability of debris extrusion, it may have led to low
 removal
 355 rates.
 356 Given that this study employed a simulated root canal system model, the resin debris generated
 357 did not include microbial or dentin debris components. Therefore, the cleaning efficacy of resin
 358 debris may not entirely represent the cleaning efficacy of pulp tissues and bacteria in the
 isthmus
 359 and root canal system. Furthermore, as the resin model’s canal walls were relatively smooth, the
 360 attachment state of debris inside the canal may differ from the present state of infectious
 361 substances in a clinical scenario. Because of the limitation of water irrigation, the chemical
 362 action between irrigants and pulp tissues and bacteria were not involved in this study. An in vitro
 363 root canal model with isthmuses confirmed by Micro-CT and different commonly-used
 irrigants 364 may be needed for further investigation.

366
Conclusion

367 Under the experimental conditions employed in the current study, high-frequency sonic waves
 368 (EDDY) can be considered a suitable method for cleaning the isthmus within root canals.
 369 However, the results indicate that the efficacy of EDDY diminishes in the apical isthmus.
 Further 370 research is warranted to explore techniques that enhance the cleaning efficiency in the
 apical 371 third of the root canal system.

375
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Figure 1

Schematic illustration of isthmus debris area measurement

(a) The original image captured by the stereomicroscope. (b) The isthmus area selected based on the set parameters. (c) The image after adjusting the brightness parameters; the area of the black portion in (c) was measured as the debris area within the isthmus.

Figure 2

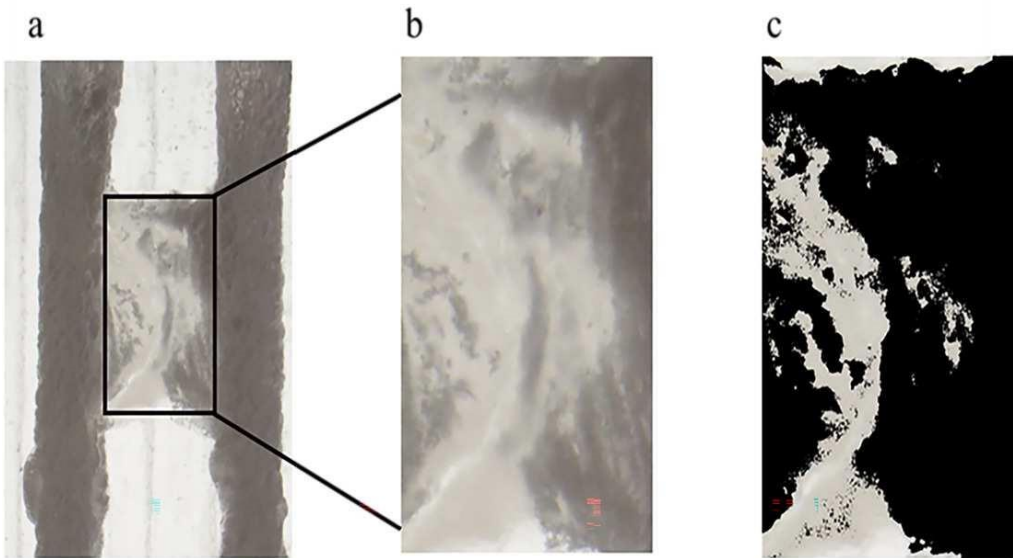


Figure 3

Representative diagrams of crown isthmus debris distribution before and after irrigation

(a) The vertical cross-sectional schematics and parameters of the digital model with coronal isthmus; (b)-(e) Illustration of the distribution of debris before irrigation for each group; (f)-(i) Depiction of the distribution of debris after irrigation. The black areas represent the proportion of debris.

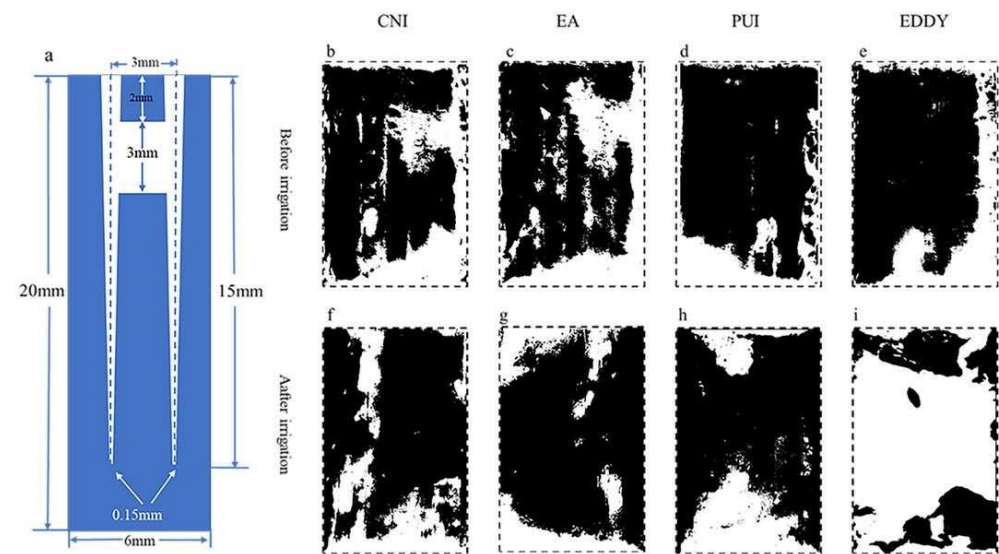


Figure 4

Representative diagrams of middle isthmus debris distribution before and after irrigation

(a) The vertical cross-sectional schematics and parameters of the digital model with middle isthmus; (b)-(e) Illustration of the distribution of debris before irrigation for each group; (f)-(i) Depiction of the distribution of debris after irrigation. The black areas represent the proportion of debris.

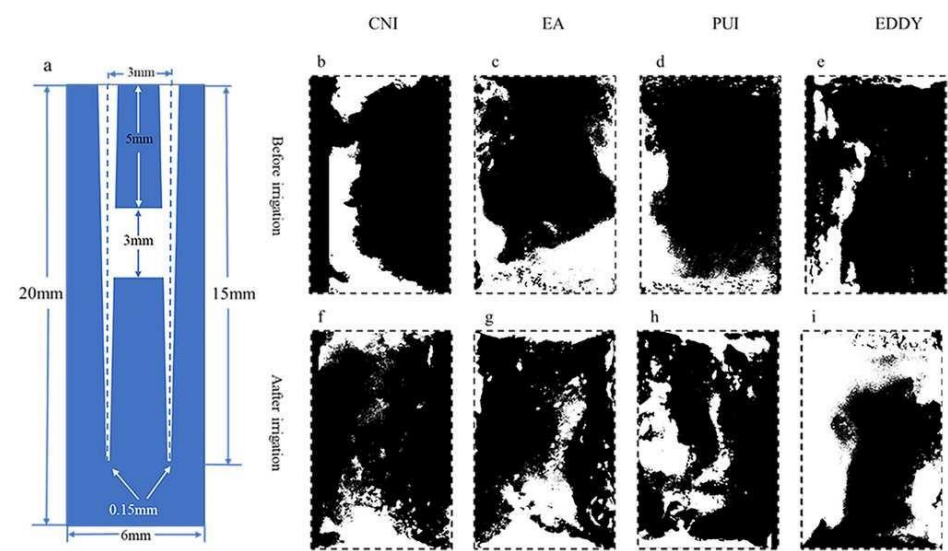


Figure 5

Representative diagrams of apical isthmus debris distribution before and after irrigation

(a) The vertical cross-sectional schematics and parameters of the digital model with apical isthmus; (b)-(e) Illustration of the distribution of debris before irrigation for each group; (f)-(i) Depiction of the distribution of debris after irrigation. The black areas represent the proportion of debris.

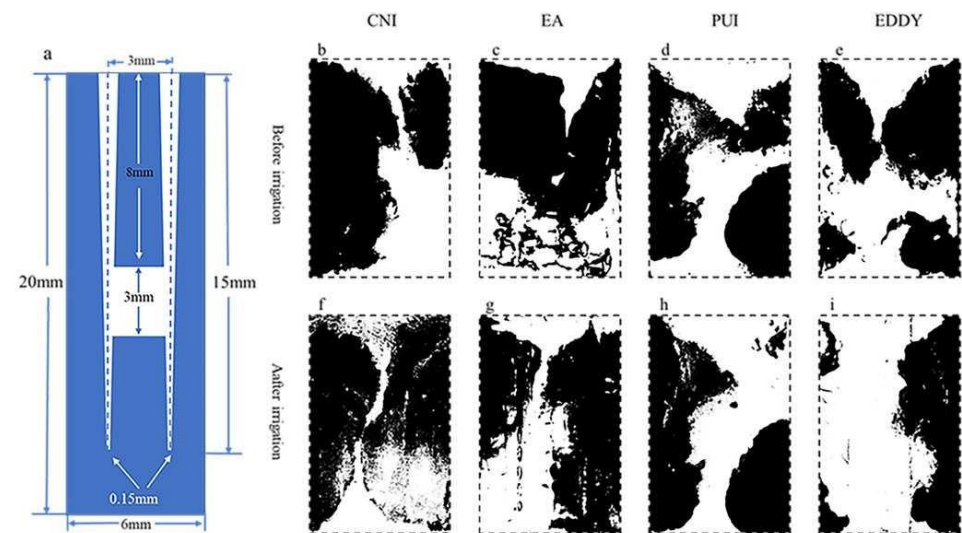
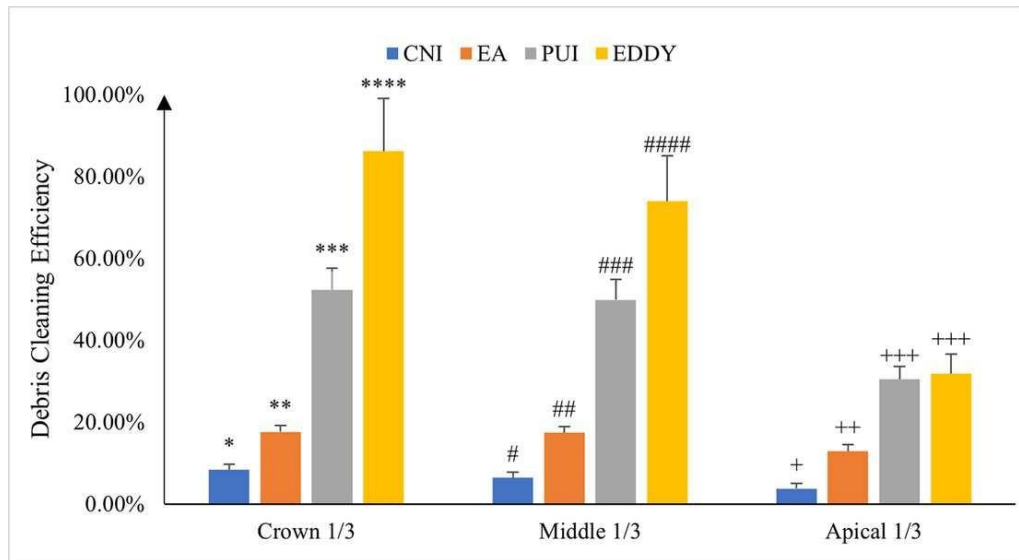


Figure 6

Debris cleaning efficiency of various irrigation methods at different isthmus positions



¹
Table 1

Crown isthmus debris area before and after root canal irrigation

(on next page)

Group	S1/mm2 (mean – SD)	S2/mm2 (mean – SD)	Debris removal rates % 0
CNI	4.05–0.27	3.71–0.30	8.36–2.04
EA	3.82–0.36	3.15–0.36	17.62–3.05
PUI	3.73–0.32	1.72–0.21	54.07–3.33
EDDY	3.89–0.21	0.53–0.06	86.18–2.25

¹
Table 2

Middle isthmus debris area before and after root canal irrigation

(on next page)

Group	S1/mm2 (mean – SD)	S2/mm2 (mean – SD)	Debris removal rates % 0
CNI	3.47–0.41	3.24–0.38	6.74–0.13
EA	3.05–0.35	2.52–0.34	17.40–5.52
PUI	3.27–0.59	1.64–0.34	49.84–6.32
EDDY	3.14–0.11	0.82–0.20	73.96–6.75

¹
Table 3

Apical isthmus debris area before and after root canal irrigation

(on next page)

Group	S1/mm2 (mean – SD)	S2/mm2 (mean – SD)	Debris removal rates % 0
CNI	1.76–0.28	1.70–0.26	3.72–0.76
EA	1.77–0.43	1.54–0.38	12.89–4.43
PUI	1.83–0.41	1.27–0.31	30.45–6.60
EDDY	2.16–0.35	1.47–0.24	31.78–5.74