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ORIGINAL ARTICLE

Evaluation of two endodontic irrigation needles on curved root canal disinfection: an ex vivo study

ABSTRACT

Aim: *The study aimed to compare the effectiveness of two different irrigation needles in eliminating* E. faecalis *from contaminated curved root canals and to test their difference in flexibility.*

Materials and methods: *Thirty-five extracted multirooted teeth with visible curved canals were instrumented, autoclaved at 121 °C for 25 minutes, and contaminated with 10 µL of the bacterial suspension. The teeth were randomly divided into three groups: A (IrriFlex), B (Navitip), and C (Control). After an incubation period of 21 days at 37 °C, group C was irrigated with 20 µL of sterile saline solution. Groups A and B were irrigated with 5 ml of 5% sodium hypochlorite (NaOCl) solution by inserting the respective needle tip until the apical third, neutralized with 5 mL of 5% sodium thiosulfate and rinsed with 5 mL of sterile saline. Three sterile paper points for each sample were taken, transferred into a brain heart infusion (BHI) medium under aseptic conditions, and frosted immediately at -20 °C. Colony-forming unit (CFU) counting was assessed after microbiological culture on selective media for* E. faecalis. *A customized device was used to test the bending behavior of the tips at 3 mm, 6 mm, and 9 mm. Kruskal-Wallis and Dunn tests were used for statistical analysis.*

Results: *IrriFlex needle demonstrated higher flexibility with no statistical difference at 3 and 6 mm compared to NaviTip. NaOCl irrigation with NaviTip Tip and IrriFlex effectively reduced* E. faecalis *CFU count.*

Conclusions: *NaOCl irrigation with NaviTip Tip and IrriFlex demonstrated high and comparable efficacy in removing* E faecalis *from curved canals of multirooted teeth.*

Giulia Malvicini[1]

Crystal Maruganti[1]

Riccardo Tonini[2]

Francesco Santoro[3]

Simone Grandini[1*]

Carlo Gaeta[1]

[1]*Department of Medical Biotechnologies, Unit of Endodontics and Restorative Dentistry, University of Siena, Siena, Italy*

[2]*Private practice in Brescia, Italy*

[3]*Department of Medical Biotechnologies, Laboratory of Molecular Microbiology and Biotechnology, University of Siena, Siena, Italy.*

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Corresponding Author
Simone Grandini | University of Siena, Viale Bracci, Siena, 53100 | Italy
Phone: +39 3357045335 E-mail address: grandini@unisi.it

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Introduction

Enterococcus faecalis is a Gram-positive coccus frequently isolated from secondary endodontic infections (1). *E. faecalis* can survive despite prolonged periods of nutrient deficiency (2), and it was demonstrated to be resistant to endodontic antimicrobials during endodontic treatment (3). This feature is related to *E. faecalis* proficiency to form dense biofilms and to invade dentinal tubules and root canal complexities (3), thus surviving the antibacterial action of sodium hypochlorite (NaOCl) (4). However, even though NaOCl can quickly oxide the membranes of those bacteria located more superficially within the biofilm, it is less effective on deeply embedded bacteria that remain protected from the direct action of chlorine (4). Microorganisms should be eliminated from the root canal system or reduced as much as possible to be compatible with healing to obtain a successful treatment outcome (5).

Although mechanical instrumentation may not completely eliminate microorganisms, particularly in cases featuring isthmuses, deltas, lateral canals, ramifications, and complex anatomies (4,6,7). Notably, complete microbial eradication from the apical third of the canal remains a considerable challenge, as 10-50% of the canal walls remain untouched by root canal instruments (8), regardless of the chosen instrumentation technique (9).

Microorganisms that persist despite chemo-mechanical preparation are widely recognized as the leading cause of failure after primary treatment and non-surgical retreatment (10). Therefore, effective endodontic irrigation is essential for root canal debridement, serving as the primary means to clean areas inaccessible to mechanical instrumentation (11). Achieving complete eradicate *E. faecalis* from the endodontic system requires the penetration of irrigating solutions deep into dentinal tubules (12).

In clinical practice, irrigation with a syringe and a needle remains the most used procedure to inject the solution deep into the root canal (13), although its efficacy in the apical region is uncertain (14). Available evidence suggests that the size, design, and insertion depth of the irrigation needle tip are essential in eliminating bacterial biofilms (15). Irrigation needles can be classified into two primary categories: *open-ended* needles that eject the irrigant directly through their tip and *closed-vented* needles with a closed tip expelling the irrigant through one or more side vents (10). Closed-vented needles must be positioned within 1 mm of working length (WL), while open-ended needles should be inserted 2-3 mm or less from WL (14). In recent years, alongside conventional stainless steel irrigation needles, other irrigation tips have emerged, aiming to enhance irrigation at the apical level, particularly in severely curved canals (16). Prominent examples include NaviTip (Ultradent, South Jordan, UT, USA) and IrriFlex (Produits Dentaires SA, Vevey, Switzerland). NaviTip is available in three different designs: NaviTip Tips, NaviTip Sideport Tips, and NaviTip FX Tips. NaviTip Tip is a stainless-steel open-ended cannula, slightly stiff at the base and centre but flexible at the end to facilitate penetration into curved canals. The tip is designed to reach deep into the canal to deliver the irrigant effectively. Tipically, irrigants advance about one mm past the delivery tip within the canal. Each NaviTip Tip has a smoothed-out, rounded end to navigate through curvatures without scratching or potentially causing ledges as it moves through the canal.

On the contrary, IrriFlex (27mm, 30G, 0.04 taper) is a unique irrigation needle composed of a double-side-vented soft polypropylene. It adapts smoothly to the anatomy of root canals, and has been proposed as an alternative to traditional metal needles. Furthermore, the flexibility of the polypropylene cannula allows for apical access without resistance or damage to the dentinal walls. It ensure reaching the predetermined WL, wihile avoiding the risk of extrusion in the periapical area. This feature enable balanced irrigant expulsion through two precise jets oriented directly against the dentinal walls (4,15).

The impact of irrigants on biofilm has received far less attention than other topics, such as debris and smear layer removal, which have been extensively investigated

(12). Moreover, the capability of IrriFlex and NaviTip Tip to reduce the mean *E. faecalis* colony-forming units (CFU) count in curved canals of multirooted teeth has not been quantified and compared yet in recent literature. Although IrriFlex is a widely used endodontic irrigation needle and is well known for its higher flexibility compared to conventional needles, no studies have quantified its actual bending ability. Furthermore, its flexibility has never been compared with that of NaviTip, which, as claimed by the manufacturer, is designed to ensure that the cannula's tip easily reaches the apical portion of any canal. Notably, adequate syringe irrigation depends on the proximity of the needles to the apical terminus of the root canal, especially in cases of severe curvature (12,16). The present study aimed to compare two irrigation needles' effectiveness in reducing E. faecalis CFU counts in in vitro contaminated root canals and assess their flexibility. Two null hypotheses were established: there was no statistically significant difference between the irrigation systems tested in eradicating *E. faecalis* from the root canal, and there was no statistically significant difference in flexibility between the two cannulas.

Methodology

Sample Collection and Preparation

This *ex vivo* study was approved by the ethical committee of Azienda-Ospedaliero Universitaria Senese number 7/2021. Thirty-five multirooted teeth extracted for either orthodontic or periodontal reasons were collected and stored in phosphate buffered saline (PBS) until use. Each tooth was subjected to a radiological examination to assess the presence of walkable curved canals. Furthermore, a preliminary bidimensional radiographic examination was performed in bucco-lingual and mesio-distal directions to ensure comparative anatomy among teeth. Only those with an angle of curvature ranging from 25° to 40° degrees, according to Pruett et al. evaluation method, were selected (17). Teeth with decay or fractures below the cemento-enamel junction, internal or external resorption, open apices, or previous root canal therapy were excluded. The teeth surface was scraped to clean the soft tissue residues and disinfected with 5% NaOCl solution for 20 min. The cuspids of all teeth were removed until a uniform plain was established to promote a repeatable WL. A diamond bur mounted on a high-speed handpiece equipped with a water-cooling system was used for access cavity preparation. Access was obtained following the design of the traditional access cavities to locate all the canal orifices. Then, each canal's WL was established with a size 10 K-File (Dentsply Maillefer, Ballaigues, Switzerland). The canals were then prepared with Reciproc R25 files (VDW, Dentsply Maillefer, Ballaigues, Switzerland) to the full WL using a 6:1 reduction handpiece (Sirona Dental Systems GmbH, Bensheim, Germany) connected to an electric motor (X-Smart Plus, Dentsply Maillefer) using the Reciproc ALL program (300 rpm, 150° REV–30° FWD) with an up-and-down pecking motion as suggested by the manufacturer. Each instrument was used to prepare only one canal and then discarded.

To ensure a perfect apical seal, the apices of the prepared roots of the teeth were sealed using flowable composite resin (Filtek Flow, 3 M-ESPE, St-Paul, MN, USA). At the same time, the orifices of the untreated canals were sealed with flowable resin composite. Subsequently, each tooth was placed in a stub created with putty-consistency silicone impression material (Zhermack, Badia Polesine, Veneto, Italy) to maintain it vertically in a glass jar. Samples were submerged in 10 mL of PBS and autoclaved at 121 °C for 25 min. Teeth were randomly divided into four groups: A (IrriFlex group), B (Navitip group), and C (Control group).

Laboratory Assessment

An *E. faecalis* strain (BE34) isolated from a chemo-mechanically treated root canal was grown at 37 °C in microaerophilic conditions (5% CO_2) on brain heart infusion (BHI, Oxoid-Thermo Fisher, Italy) broth supplemented with 1.5 % Agar (BD, Italy) and 5% defibrinated horse blood (Liofilchem, Italy). Starter cultures were grown in BHI broth at 37 °C until an OD_{600} of 0.4 and subsequently frozen at -70 °C in BHI with 10% glycerol.

Root Canal Inoculum

Each root canal was inoculated with $5x10^5$ bacterial CFUs of *E. faecalis* BE34 in 10 µl. The bacterial suspension was introduced into the whole length of the canal using an IrriFlex needle mounted on a P20 micropipette, the tip was inserted as deep as possible in the canal, and the suspension was released with a gentle pumping motion. The inoculated teeth were incubated at 37 °C in a 5% CO_2 atmosphere for 21 days [18]. The teeth were randomly allocated into three groups, with 10 specimens in both groups A and B, and 5 specimens in the control group. Initial microbial assessment (S1) was carried out after the incubation period. Each root canal was filled with 20 µL sterile saline solution, and a #20 K-file (Dentsply Maillefer, Ballaigues, Svizzera) was inserted into the root canals to reach the WL with a gentle filling motion. To detach the microorganisms from the inner root surfaces, an ultrasonic tip was placed in contact with the file shank and activated for 1 min; then, the root canals were sampled. Eventually, bacteria were recovered from the root canal using a series of three sterile paper points (size ISO 25, Dentsply), which were, in turn, rubbed against the walls of the root canals (Figure 1). The paper points were allowed to draw up their full capacity of liquid before being transferred into a tube containing PBS with 10% glycerol broth under aseptic conditions and frozen at -70 °C. After this stage, the contaminated vials were exchanged with empty sterile vials.

Figure 1
The image shows the sampling procedure. Sterile paper points were used to collect viable bacteria from the root canals of multirooted extracted teeth.

Irrigation Procedure

Irrigation with 5 ml of 5.25% NaOCl solution at room temperature was performed in groups A and B, respectively, inserting IrriFlex (30 G, 22mm, 0.04 taper) and NaviTip Tip (30 G, 21 mm) (Figure 2). NaviTip Tip was introduced into the canals up to 2 mm from the WL; IrriFlex, after reaching the WL, was retracted by 1 mm before irrigant ejection, according to manufacturer instructions. The irrigant exposure time for groups A and B was 30 seconds. The procedure was performed using digital pressure with the forefinger only, and the needle was gently moved back and forth in the canal, ensuring that the needle did not bind in the canal itself. At the end of irrigation, the canals were rinsed copiously with a sterile saline solution to flush away residual irrigants.

After chemical preparation, samples in groups A and B were irrigated with 5 mL of 5% sodium thiosulfate as a neutralizer and then rinsed with 5 mL sterile saline. Final sampling (S2) was performed using a similar method to initial sampling. *E. faecalis* CFUs were enumerated by plating serial dilutions with a multilayer plating method on BHI agar. The same cohort of teeth was used throughout the experiments, and all irrigation experiments were conducted in triplicate.

Flexibility Test

The equipment used for the test was a customized device (Figure 3) validated in a recently published study (19) with a stainless steel (SS) alloy platform, initially built for Ni-Ti instruments and then adapted for testing irrigation needle tips. In the current study, to eliminate experience variations, the same skilled operator conducted each test. All the needle tips were examined at a 45-degree angle and in three distinct positions: 3, 6, and 9 mm from the tip.

Statistical analysis

The sample size was determined based on the data of a previous study regarding the NaviTip ability to eradicate *E. faecalis* from the root canal system (20). Therefore, a total of 24 samples were indicated as the

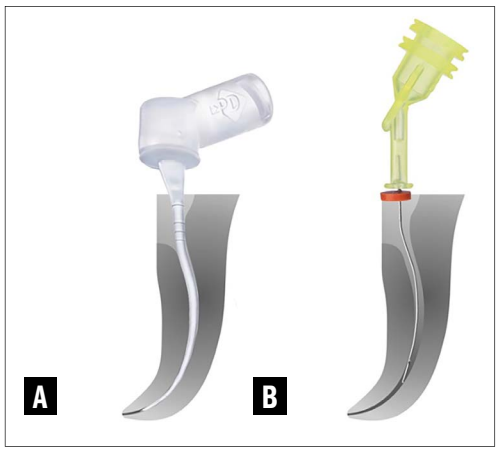


Figure 2
The two tips (IrriFlex and NaviTip Tip) containing NaOCl are visible in this image. IrriFlex is shown on the right (A). NaviTip Tip is shown on the left (B).

ideal size required for noting significant differences using G-Power v3.1 (Heinrich Heine, University of Düsseldorf, Düsseldorf, Germany) by selecting the analysis of variance (ANOVA) test and setting an alpha-type error of 0.05, a beta power of 0.90, and an effect size of 0.80. However, an additional 11 samples (5 for groups A, B, and 1 for the control group) were added to compensate for unexpected values of IrriFlex because there were no data in the literature regarding its ability to eradicate *E. faecalis* strain (BE34). According to this, a total of 35 samples were selected. Data were analyzed using an ad hoc statistical software (STATA BE, version 17.1, StataCorp LP, TX, USA), setting the level of significance at α=0.05. After verification of data distribution using Shapiro-Wilk test, Kruskal-Wallis test was conducted to assess the overall differences in CFU and flexibility among the three groups. Dunn test was used for pairwise comparison.

Results

Irrigation procedure
A mean of $7.8x10^4$ CFUs of *E. faecalis* BE34 was recovered from the control samples, while the use of NaviTip and IrriFlex produced a reduction in bacterial viability below the limit of detection of 2.5 CFUs (Figure 4) without a statistical difference between the two irrigation needles ($p > 0.05$) (Table 1). The bacterial load recovered from control samples was slightly lower than the actual inoculum of $5x10^5$ CFUs. Bacterial viability was significantly reduced after NaOCl irrigation using NaviTip and IrriFlex compared to controls. The mean log CFUs of 5.89 reported for untreated teeth turned out to be much higher compared to the test groups, as shown in Table 1.

Flexibility test
The results of the flexibility test are shown in table 2. Statistical analysis showed:
No significant difference between NaviTip and IrriFlex at 3mm and 6mm.
A significant difference between NaviTip and IrriFlex at 9 mm.

Discussion

The aim of the current study was to assess how two different endodontic irrigation

Table 1

***E. faecalis* count after root canal irrigation expressed in mean log CFU**

Needles	Bacteria Recovered (mean log CFU)	N	Standard Deviation	Variance
Control group	5.89	5	34.94	1220.87
IrriFlex	1.25	15	-	-
NaviTip	1.25	15	-	-

Abbreviations: CFU, colony-forming units; N, number of samples per group

Table 2

Results of the flexibility test conducted at three distinct positions from the instrument tips: 3, 6, and 9 mm

Needles	Flexibility [grams] (mean ± standard deviation)		
	3mm	6mm	9mm
NaviTip Tip	13.6 ± 1.14*	24.2 ± 2.59*	35.8 ± 3.11†
IrriFlex	9.4 ± 1.81†	26.8 ± 2.77†	55.2 ± 4.66†

**,† In each column, means sharing the same symbol are related by a statistically significant difference (P<0.05).*

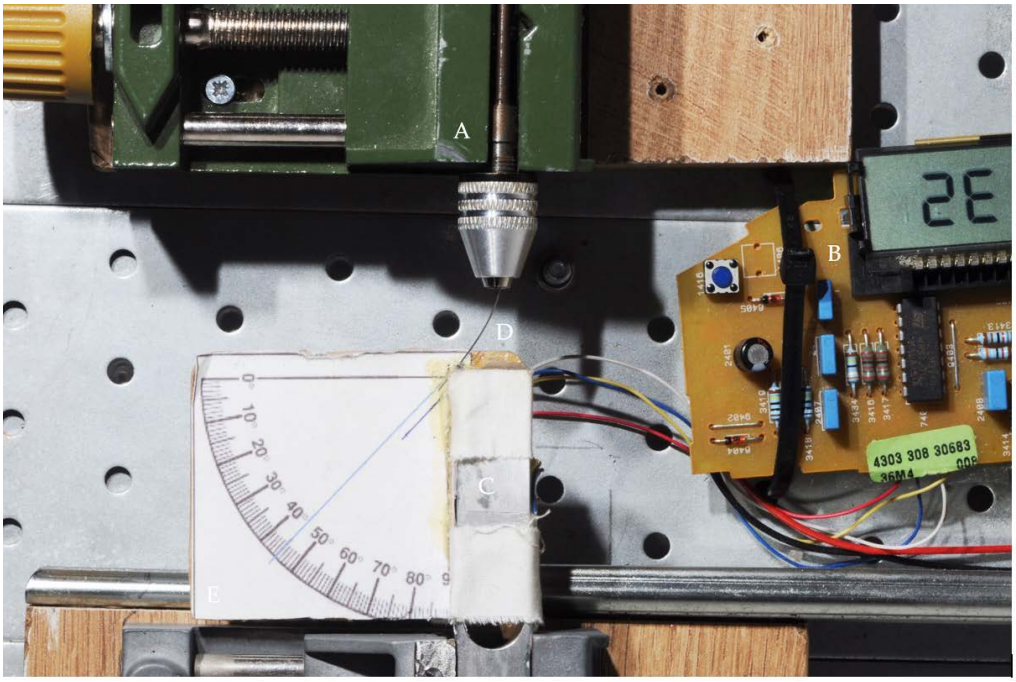
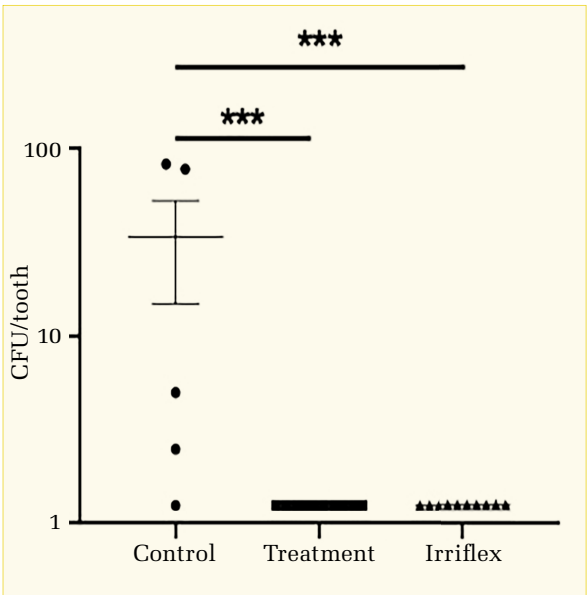


Figure 3
Bending device. A load cell (C) connected to a digital display (B) was put on a stainles-steel platform, together with an analog protractor (E) and a mobile device that enabled reproducible positioning of the needle tip (A, D) on the load cell. The analog protractor measured various bending angles, while the mobile device measured the bending resistance at multiple points on the tip (3 mm, 6 mm, 9 mm).

needles affected the elimination of *E. faecalis* from curved canals of multirooted teeth contaminated *in vitro* with *E. faecalis*. The two tested needles were IrriFlex (flexible double-sided polypropylene needles) and NaviTip Tips (open-ended steel needles). Additionally, the study tested and compared the flexibility of these needle tips at three different positions from their extremities: 3 mm, 6mm, and 9 mm. The first null hypothesis was accepted as no statistically significant difference emerged between NaviTip and IrriFlex (Table 1). However, the second null hypothesis was only partially rejected because irriFlex showed a higher but not significant banding ability at 3 and 6 mm (Table 2). On the contrary, at 9 mm, a statistically significant difference was highlighted with NaviTip demonstrating greater flexibility compared to IrriFlex. This difference was attributed to their varying taper, which impacts the bending ability of the needle tip in the coronal third.

Figure 4
Mean count in CFUs. Bacterial viability after passive irrigation using NaviTip and IrriFlex compared to controls.



Effective infection control during endodontic treatment requires direct contact between the irrigant and the entire canal wall surface, particularly in the apical region (21). To fulfil this requirement, an effective delivery system is necessary (10). In the current study, both NaviTip and IrriFlex systems demonstrated a comparable capacity to significantly reduce bacterial counts below the positivity threshold.

These results align with a recent study conducted on single-rooted teeth, which demonstrated that both NaviTip and IrriFlex are more efficient at removing mature bacterial biofilms compared to another needle, with a slight but not significant advantage for IrriFlex (4). Methodological differences, such as the choice of different E. faecalis strains with a two-week-old biofilm and viability assessment, may have contributed to this slight variation in results. IrriFlex and NaviTip bending abilities allow the needle tip to easily reach the apex in case of important canal curvatures

(10). Current literature suggests that the extrusion of the irrigant close to the WL results in a more efficient irrigation (6). The current study demonstrated that IrriFlex is slightly more flexible than NaviTip without showing a statistically significant difference, this result confirms their comparable efficacy in CFUs reduction.

E. faecalis mature biofilms are frequently used as a model of endodontic infection in *ex vivo* studies (4). They have been employed in several previous studies to evaluate the effectiveness of endodontic irrigants (22). This Gram-positive bacterium can proliferate without synergistic support from other bacteria and survive under extended periods of nutrient deficiency, even after mechanical and chemical root canal preparation, due to its ability to form biofilm and penetrate dentinal tubules (2). However, recent studies have raised questions about its actual role in post-treatment apical periodontitis (23,24), demonstrating that it is often absent and, when detected, it is not among the most prevalent species (1). Nevertheless, *E. faecalis ability* to thrive under various growth conditions makes it a convenient model for laboratory research (22). In the present study, the clinical strain BE18 was employed because resistant to chemo-mechanical preparation (27).

The exposure time of the irrigant in this study appears to be shorter than what is typically used in clinical practice. Although, Dunavant et al. showed that the amount of *E. faecalis* elimination is not significantly affected by an exposure time ranging from 1 to 5 minutes (27).

Regarding the concentrations of NaOCl, there is no consensus, and values ranging from 0.5 to 8.25% are commonly used for root canal irrigation (28). Laboratory studies indicated that the effectiveness of NaOCl is related to its concentration (22). A recent systematic review demonstrated, with weak evidence, that higher concentrations may ensure an advantage (29). Nevertheless, recent clinical studies have not found a significant difference in the antimicrobial effect among different NaOCl concentrations(30,31). Verma et al. found no difference in healing after endodontic treatment when using 1% or 5.25% NaOCl for root canal irrigation (30). In the current study, a 5.25% NaOCl solution was used because it was demonstrated that higher concentrations of NaOCl reduce the time required to lower viable counts below the limit of detection (32). Moreover, *E. faecalis* was significantly more resistant to NaOCl (0.5, 1.0, 2.5, and 5.25%) when compared with the other species tested (*Actinomyces naeslundii*, *Candida albicans*) (32) . Additionally, in the present study, no irrigant activation was performed despite being the gold standard in order to effectively validate the disinfection ability of the two needles, excluding any other factors able to influence it.

The primary outcome in endodontics is the prevention or healing of apical periodontitis (14); However, the lack of evidence on root canal irrigation is probably due to the complexity of measuring this primary outcome (22). Authors often prefer to use surrogate end-points, which are easier to measure. The reduction of the intracanal microbial load is undoubtedly the most relevant surrogate end-point to study irrigants and irrigation systems (22) due to the critical role of bacteria in the development of pulpal and periapical diseases. In single-rooted teeth, this end-point is linked to the healing of apical periodontitis, and there was a need to prove these findings also in posterior teeth (22).

The use of extracted teeth in this study, previously employed in other research comparing various irrigation systems and solutions, provided better control over endodontic system contamination (12). Clinical studies, while representing a higher level of evidence, are subject to variations between teeth and uncontrollable parameters, acting as potential confounders (12). The efficiency of new irrigation systems or irrigants should not be immediately tested by *in vivo* studies. Instead, *in vitro* and *ex vivo* studies with rigorous control of confounders should be performed in order to select the appropriate candidates for *in vivo* studies (22).

Standardizing the insertion depth of the needle tip is essential (22), as it has been demonstrated in some previous studies on

the binding point of these components inside the root canal (22). However, the variability of this point may differ even in root canals with the same apical size and taper (33). Therefore, it is advisable to use the apical end of instrumentation as a reference to define the insertion depth (22). The constant in-and-out movement of these components within the root canal, as applied by some clinicians, is difficult to standardize in laboratory studies without the use of robotic arms (33).

While paper points can be employed for sampling when the biofilm is grown inside a root canal, they have limitations (22). Paper points can only detach planktonic bacteria from the root canal lumen and those bacteria that are only loosely adherent to the wall (22). Therefore, this sampling procedure excludes all the bacteria remaining in isthmuses, lateral canals, and other anatomic irregularities that are difficult to reach with instruments and irrigants (4). Furthermore, the vortexing movement used to recover the sampled bacteria with the paper point causes a loss of information on the exact localization of the bacteria in the root canal (2).

An additional limitation of our study was the use of a mono-species biofilm model. Although E. faecalis is often found in natural biofilm communities with multiple microorganisms, we opted for a mono-species model to ensure protocol simplicity and standardization. Multi-species biofilm models, despite their closer resemblance to real-life conditions, present challenges in managing competitive interactions among bacterial species. Indeed, to date no valid methods exist to manage each bacterial species within a multispecies biofilm (4).

Another weakness of the present study is the assessment of bacterial viability in our study relied on culture methods, which quantify only viable, cultivable bacteria by plating on agar plates (22) and quantifying CFUs. However, only viable bacteria, able to divide and form colonies, were quantified through this approach (22). In root canal infections, many bacteria are viable but non-culturable (VBNC), meaning that despite their inability to grow in culture media, they are virulent, metabolically active, and able to form a biofilm, although to a lesser degree than viable bacteria (22). To address these limitations, further experimental and clinical studies are necessary to better assess the efficacy of new devices in eradicating *E. faecalis* from canals with complex anatomy and establish a more direct link with clinical practice.

Conclusions

The results demonstrated that both irrigation systems effectively reduced the bacterial load, with no statistically significant differences between them. Although IrriFlex exhibited greater flexibility, there was no significant difference between the two tips at 3 and 6 mm.

Clinical Relevance

This study compared IrriFlex and NaviTip irrigation needles in eliminating E. faecalis from curved root canals. . Both IrriFlex and NaviTip Tip demonstrated comparable efficacy, significantly reducing E. faecalis colony-forming units. Additionally, IrriFlex exhibited higher flexibility at 3 and 6 mm compared to NaviTip. The findings suggest both needles are effective in bacterial removal, with IrriFlex offering enhanced flexibility, crucial for navigating complex canal systems.

Conflict of Interest

The authors declare no conflict of interest

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Azienda Ospedaliero-Universitaria Senese N 7/2021.
Data Availability Statement: the data used in this research has been properly cited and reported 321 in the main text.

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