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Comparative Evaluation of Minimally Invasive and Conventional Access on The Biofilm Elimination of Enterococcus Faecalis From the Root Canal System

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Abstract

Background: The access cavity is a fundamental step in endodontic treatment. Traditionally, it is performed by completely removing the pulp chamber and all its contents. Recently, the introduction of technologies such as the operating microscope, ultrasound, and Ni-Ti instrumentation systems has allowed this phase to be performed conservatively through minimal invasive access. However, further evidence is needed to



determine whether these techniques allow complete elimination of microorganisms within the root canal system.

Objectives: The aim of this study was to evaluate the effects of minimally invasive access (MIA) compared with conventional access (CA) on the disinfection and elimination of biofilm *Enterococcus faecalis* in root canal therapy.

Methods: Forty-four first molars were selected and randomly divided in groups: MIA and CA (n= 15 each), positive control (n= 8), negative control (n= 6). After access cavity, the root canals were contaminated with *E. faecalis* for 30 days. Bacterial sampling was performed with sterile paper points before (S1) and after (S2) instrumentation with the TruNatomy system to quantify the bacterial load. Colony-forming units per milliliter (CFU/mL) and scanning electron microscopy (SEM) were used for bacterial reduction assessment and elimination of biofilm. Statistical comparisons were conducted using Student's t-test or Mann-Whitney U test, with significance set at 5%.

Results: Significant reductions in *E. faecalis* were observed in all groups, regardless of cavity design. However, the lowest bacterial reduction was found in the MIA group, while the CA group achieved the highest reduction.

Conclusions: Conventional access resulted in significantly better disinfection compared with minimally invasive access. These findings suggest that the design of the access cavity influences bacterial load reduction and biofilm elimination within root canals.

Keywords: Bacterial reduction, biofilm elimination, minimally invasive access, conventional access, TruNatomy

Introduction

The access cavity (AC) is the initial phase of root canal treatment. Traditionally, its objectives include the removal of caries from the pulp chamber, localization of root canals, and establishment of a straight-line pathway for cleaning, shaping, and sealing (1,2).

In traditional or conventional access, it is necessary to remove the entire roof of the pulp chamber, which often results in substantial loss of pericervical dentin (3). Its advantages include that by offering a straight path to the canals, transoperative accidents such as instrument fracture, perforations, canal omission, and step formation are significantly reduced (4).

However, minimally invasive dentistry is currently a trend among oral health professionals and the endodontics is no exception; in

2010 Clark and Khademi proposed the execution of minimally invasive accesses as an alternative to conventional accesses, which are characterized by the partial removal of the chamber roof, which allows not removing the pericervical dentin (5). The term 'pericervical' refers to an area roughly 4 mm coronal to the crestal bone and 6 mm apical to the crestal bone. It is critical dentine for tooth strength and should be conserved as much as possible to ensure long-term retention of the tooth (6,7). Preservation of pericervical dentin is one of the most important aspects when considering maintaining the normal function, aesthetics, and durability of an endodontically treated tooth (8).

There is currently a vast variety of minimally invasive access designs. Shabbir et al. in 2021 proposed a broader classification including anterior and posterior teeth, in addition to addressing types of access designs not previously mentioned. The nomenclature considered 4 main geometric types of AC designs: traditional access cavity (TradAC), conservative access cavity (ConsAC), ultraconservative access cavity (UltraAC) and truss access cavity (TrussAC) (9). ConsAC in posterior teeth: preparation usually starts at the central fossa of the occlusal surface and extends with smoothly convergent axial walls to the occlusal surface, only as far as necessary to detect the canal orifices, preserving part of the pulp chamber roof (10,11). Within the UltraAC are the 'ninja' and 'truss' (NAC and TrussAC, respectively). The NAC is a small cavity on the occlusal surface that should allow the clinician to find and access all the orifices of the root canal system (12,13). While TrussAC aims to preserve the dentinal bridge between two or more small cavities prepared to access the canal orifice(s) in each root of multi-rooted teeth. In mandibular molars, for example, two or three individual cavities can be created to access the mesial and distal canals (14).

Technological advances have made more conservative access cavity preparations a viable option, including the use of magnification, cone beam computed tomography (CBCT), irrigation activation devices and solutions, as well as improved metallurgy for the fabrication of endodontic shaping instruments, resulting in increased flexibility and fracture resistance (15).

Recently, rotary instrumentation systems have been introduced for conservative shaping of the root canal system. TruNatomy (Dentsply Sirona, Charlotte, United States) instruments are given in three sizes: small (20/0.04), prime (26/0.04), and medium (36/0.03) (16). These files have a NiTi wire of 0.8 mm diameter and an off-center parallelogram square cross-section design and a variable regressive taper (17).

Despite the advances and studies carried out, exhaustive research that compares the effects of access cavity preparation is still



essential. Therefore, the present study aims to evaluate the effects of minimally invasive access versus conventional access on the degree of disinfection and elimination of *Enterococcus faecalis* in root canal therapy implemented with conservative instrumentation with the TruNatomy system. The proposed null hypothesis was that there would be no difference between minimally invasive access and conventional access in reducing the microbial load and elimination biofilm of *E. faecalis*.

Materials and Methods

Sample Size Calculation

Approval for this study was obtained from the Ethical Committee of the Faculty of Stomatology of the Autonomous University of San Luis Potosi (CEI-FE-046-021). The sample size was calculated; the probability level of $\alpha = 0.05$ and a statistical power of 0.9 were considered.

Sample Selection

Forty-four human permanent first molar teeth were selected. Were included teeth without caries or defects on the occlusal surface and curvatures less than 20 degrees of mesial canals; the root canal curvature was determined by Schneider's method (18). Teeth with extensive restorations or incomplete roots were excluded. The selected samples were disinfected with 2.5% NaClO and maintained in sterile saline solution. Then were randomly divided into two groups (n=15): Group 1: Molars with minimally invasive access (MIA); Group 2: Molars with conventional access (CA). Further, 8 molars were included as a positive control group, 4 for the MIA group and 4 for the CA group. For the negative control group, 6 molars were included, 3 for the MIA group and 3 for the CA group.

The access cavity for the MIA group was opened using an Endoguide EG1A bur (SS White Dental, Lakewood, New Jersey, United States) with water-cooling at high speed. The central fossa of the molars was taken as a reference point to prepare the access cavity, eliminating only the necessary dental tissue to allow the identification of the canals, and only partially removing the roof of the pulp chamber through a dental microscope was used throughout the procedure (19).

The access cavities of the teeth in the CA group were opened with carbide ball burs (Kavo Kerr, Brea, California, United States), as well as the endo Z burr (J Jota International, Rüthi, Swiss). The central fossa of the occlusal surface of the molars is used as a reference point to begin the perforation of the endodontic access. With a number 2 or 4 bur, the surface of the molar began to be

worn down until the sensation of emptiness was felt. Subsequently, with the endo Z drill, the roof of the pulp chamber was completely removed.

After opening the access cavity in teeth for all groups, the apical patency of mesial root canals was checked using a #10 K-file (Dentsply Sirona, Charlotte, United States); the teeth were subjected to a treatment with an ultrasonic bath (Biosonic UC-50, Coltene/Whaledent, Inc., Altstätten, Switzerland) with 17% EDTA for 4 min and 5.25% NaClO for 4 min and, subsequently, sterilized at 121°C and 15 psi for 20 min (20).

Biofilm formation of *E. faecalis*

To infect the root canals, a pure culture suspension of *E. faecalis* (ATCC 29212) was used. McFarland suspension was prepared by adding 1.5 mL pure *E. faecalis* into brain-heart infusion broth (BHI) (BD BBL; Cuautitlán Izcalli, Estado de México, México) and then obtaining a bacterial suspension of 1.5×10^6 colony-forming units (CFU). This process was carried out in a laminar flow chamber (Labconco, Kansas City, Missouri, United States). After initial inoculation, the samples were incubated at 37°C for 48 h. After this period, the culture medium was changed by transferring the molars to flasks with fresh BHI culture medium. The replacement process was carried out in the laminar flow hood, and the procedure was repeated every 48 h for 30 days. The pureness of the cultures was checked by Gram staining and colony morphology.

After the inoculation of teeth, for both groups the working length was established with an #8 K-file (Dentsply Sirona, Charlotte, United States), one millimeter before the apex. Subsequently, the samples were mounted on a metal base, fixed with the help of a silicone Z plus (Zhermack, GmbH, Deutschland), and the teeth were isolated with an absolute isolation technique. Afterwards, a sterile cotton swab was placed inside the access cavities; subsequently, a disinfection protocol of the operating field was carried out with 30% hydrogen peroxide (H₂O₂), 5.25% NaClO, and 10% sodium thiosulfate (Na₂S₂O₃) for one minute for each solution. The procedure used for taking the bacterial sample from the canals was performed before (S1) and after (S2) instrumentation. Before instrumentation with a sterile Pasteur micropipette, 0.05 mL of BHI culture broth was placed in the access cavity. Subsequently, two sterile #15 paper points were introduced, one point for each mesial root canal, and left there for one minute; then they were removed and introduced into a tube with 10 mL of BHI culture medium.

Instrumentation of the canals was carried out using TruNatomy files (Dentsply Sirona, Charlotte, United States), following the



manufacturer's guidelines. The master apical file size used was 26/04. During the instrumentation, the canals were irrigated with 2.5% NaClO after each file. Subsequently, a final irrigation protocol was performed with 5 mL of 2.5% NaClO, 5 mL of saline solution, and 5 mL of 17% EDTA. To take second samples (S2) from the root canals in all groups, the field disinfection protocol and sampling of the mesial canals were carried out again in the same way mentioned above.

Quantification of the Bacterial Load

The paper points, from before S1 and S2, were incubated at 37°C for 24 h under anaerobic conditions. Serial dilutions were then performed under sterile conditions in a laminar flow hood; 1 mL of the culture was taken from each sample and poured into a tube with 9 mL of sterile saline solution, and it was vortexed for 30 s. After $\times 10^{-7}$ serial dilutions within the sterile saline solution, a 10 μ L sample was cultivated on Agar-Sheep Blood. The cultivated samples were incubated at 37°C for 24 h, and the number of bacterial colonies was counted as CFU/mL.

Scanning Electron Microscopy Analysis

The elimination and disinfection of *E. faecalis* between the MIA and CA groups were evaluated by scanning electron microscopy (SEM) (Jeol JSM-6610LV; Jeol Ltd., Tokyo, Japan). The mesial root was separated and longitudinally sectioned, then placed in a 2% glutaraldehyde and 1% alcian blue stain for fixation over 24 h; after, a gradual dehydration protocol was made with ethyl alcohol at 20, 40, 60, 80, 90%, and absolute for 5 min in each solution. For critical point drying (Leica EM CPD030; Leica Microsystems, Wetzlar, Germany), the samples were submerged in acetone and placed inside the critical point drying chamber for seven cycles of washing with liquid carbon dioxide.

Finally, the pieces were covered with gold in an automatic fine coater (JEOL JFC-1100; Jeol Ltd., Tokyo, Japan). The samples were then observed by SEM at different magnifications. Data on the cleanliness of the apical zone was reported as the permeability of dentinal tubules using the modified Rome scale at 3 mm in the apical zone.

Statistical Analysis

The statistical analyses were performed using SPSS software (Version 18.0; IBM Corp, Armonk, NY), and $p < 0.05$ was considered significant. The normality of the variables' distribution was analyzed using the Shapiro-Wilk test. The data regarding the decrease of microbial load and cleanliness are expressed as the mean \pm standard deviation or as the median and interquartile range, according to their normal or non-normal distribution, respectively. Comparisons among the groups were performed by using Student's t-test or the U Mann-Whitney test as required.

Results

After instrumentation with the TruNatomy system was performed in all the samples with different cavity designs (MIA, CA), significant decreases in the microbial load of *E. faecalis* expressed in CFUs/mL were observed. In Table 1, the mean and standard deviation values are expressed for the MIA group before (S1) and after (S2) the instrumentation 10.31 ± 0.44 and 7.07 ± 4.44 , as well as for the CA group 10.83 ± 0.48 and 0.00 ± 0.00 , respectively. When comparing the elimination percent of the microbial load in the group MIA, there was a statistically significant difference $p < 0.05$ ($p=0.001$) (Figure 1); for the group CA, a significant difference in the percent elimination of microbial load was also observed $p < 0.05$ ($p=0.000$) (Figure 2).

Table 1 Microbiological analysis on intracanal bacterial counts before (S1) and after (S2) preparation with TruNatomy in Teeth with minimally invasive access (MIA) and conventional access (CA).

Groups	n	Mean \pm SD	Min	Max	p- value	
MIA	S1	15	10.31 ± 0.44	9.28	10.77	
	S2	15	7.07 ± 4.44	0.00	10.48	0.001*
CA	S1	15	10.83 ± 0.48	10.05	11.44	
	S2	15	0.00 ± 0.00	0.00	0.00	0.000*



MIA, minimally invasive access; conventional access (CA).

*Represent statistically significant differences between counting bacterial load in CFU'S ($p < 0.05$).

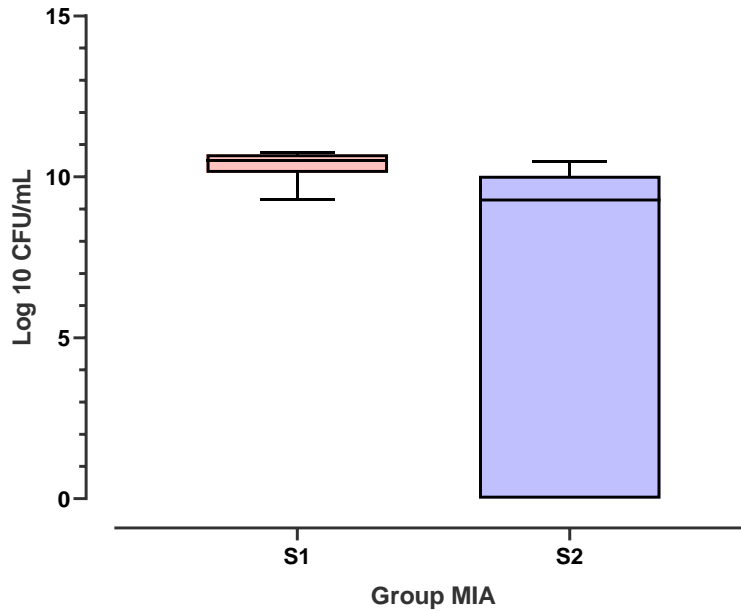


Figure 1 Diagram of the logarithmic reduction of the bacterial load in minimally invasive access group.

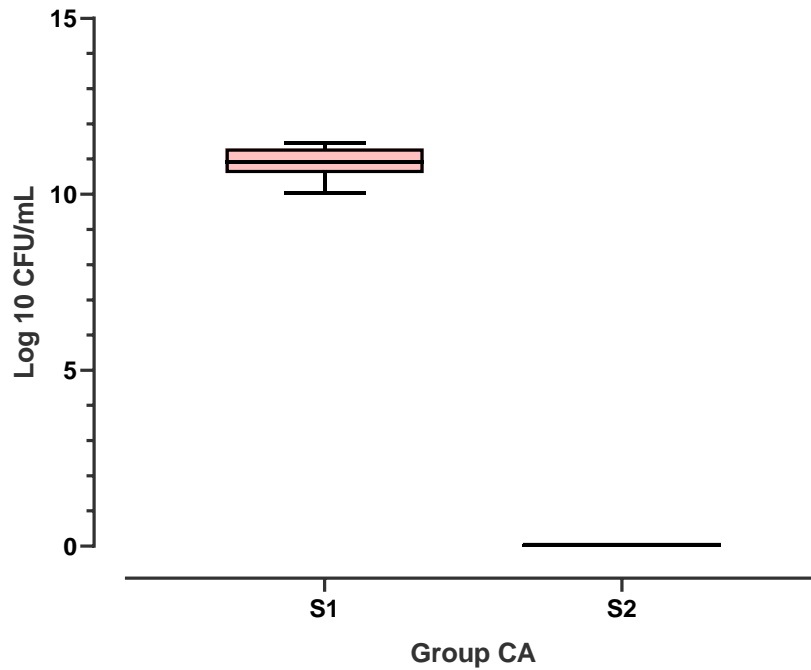


Figure 2 Diagram of the logarithmic reduction of the bacterial load in conventional access group.



In addition, when comparing the effectiveness of minimally invasive access versus conventional access on the biofilm elimination of *E. faecalis* in the root canal system (Table 2) (Figure 3), the group of conventional access presented a greater diminution (CFU/mL) of *E. faecalis* with a statistically significant difference of $p < 0.05$ ($p = 0.0001$).

Table 2 Mean, standard deviation and median (IQR 25%-75%) values minimally invasive access (MIA) and conventional access (CA) for biofilm elimination of *E. faecalis* in the root canal system (CFU/mL).

Groups	n	Mean ± SD	Min	Max	Percentiles			p - value
					25	50 (Median)	75	
MIA	15	3.24 ± 4.35	0.03	10.70	0.40	0.67	9.28	0.0001*
CA	15	10.83 ± 0.48	10.05	11.44	10.60	10.91	11.30	

* Intergroup comparisons analysis (U Mann-Whitney test) showed statistically significant difference ($p < 0.05$).

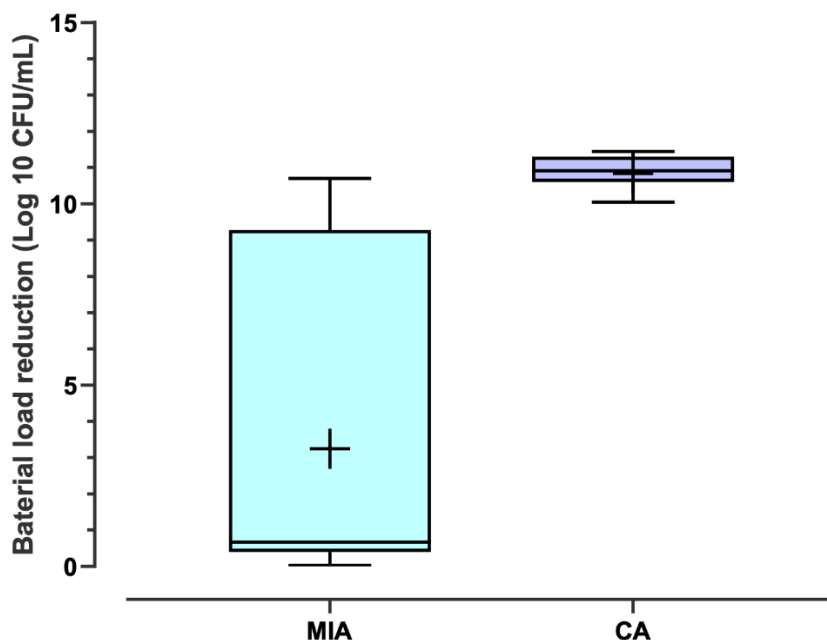


Figure 3 Diagram of the logarithmic reduction of the bacterial load after instrumentation with TruNatomy between minimally invasive access versus conventional access group.

Regarding the evaluation of dentin permeability. Dentin permeability of the apical third was evaluated by SEM as indicated in the methods. There were no significant differences in the percentage of permeability of the dentinal tubules evaluated at 3 mm in the different cavity design access (MIA, CA) $p > 0.05$ ($p = 0.061$) (Table 3). Nevertheless, in the CA group, a higher percentage of patent dentinal tubules was observed in the SEM images obtained (Figure 4).



Table 3 Analysis of permeability of the dentinal tubules evaluated at 3 millimeters of apical zone between cavity design access MIA and CA.

Groups	Mean ± SD	<i>p</i> - value
MIA	3.5 ± 0.57	0.061
CA	2.5 ± 0.57	

The permeability of dentinal tubules was evaluated using the modified scale Rome (Score 0: No smear layer and no erosion of tubules, 1: No smear layer and erosion of tubules, 2: Minimum smear layer, 3: Moderate smear layer, 4: Heavy smear layer).

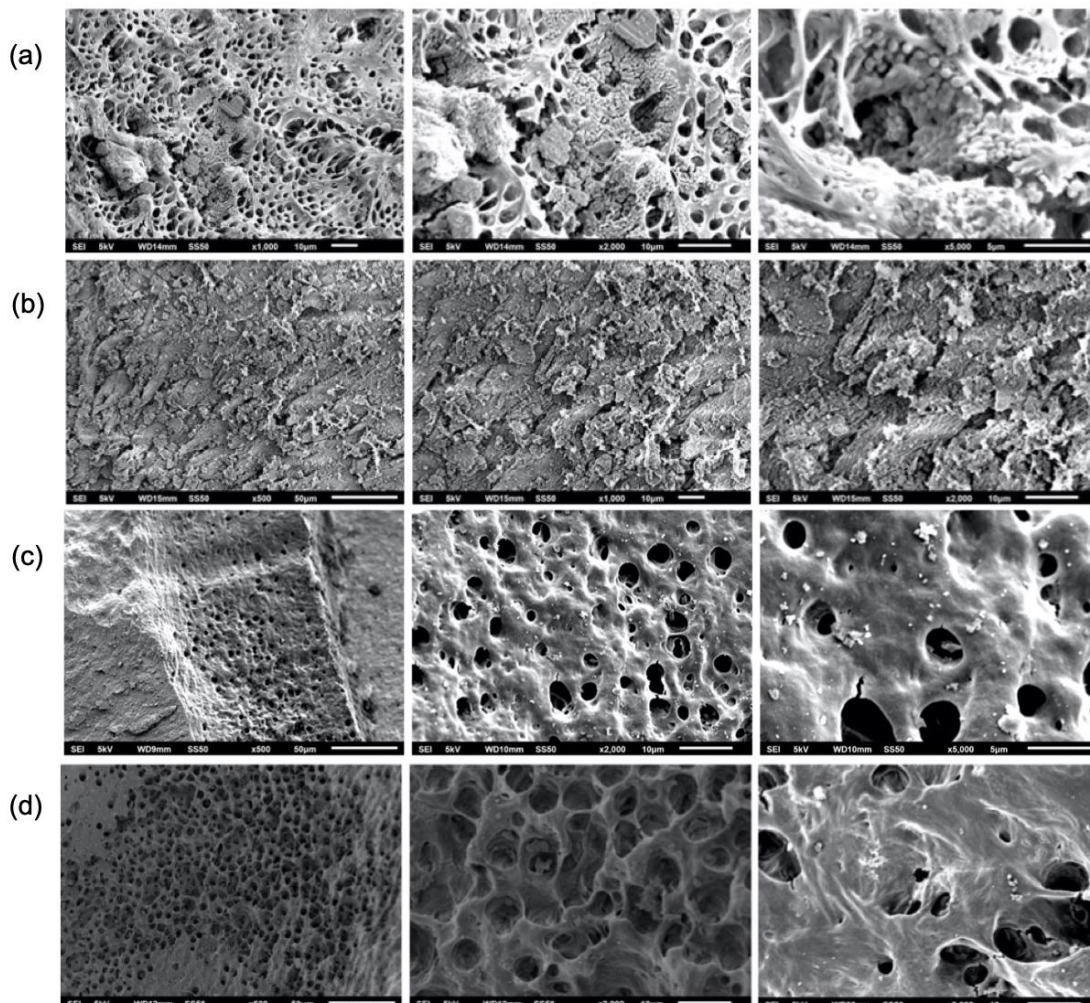


Figure 4 Scanning Electron Microscopy (SEM) images. (a) In vitro formation of the biofilm of *E. faecalis*, controls without instrumentation were employed to evaluate the in vitro formation of the biofilm on the root canals as positive control. (b) Root canals without biofilm formation and without instrumentation was employed as negative control. (c) Permeability of the dentinal tubules at 3 millimeters for the group MIA at 500x, 2000x and 5000x of magnification. (d) Permeability of the dentinal tubules at 3 millimeters for the group CA at 500x, 2000x and 2000x of magnification.



Discussion

This study was developed with the main purpose of comparing the microbial load of biofilm of *E. faecalis* associated with two different types of endodontic access cavities: minimally invasive access (MIA) and conventional access (CA). All groups were associated with a significant percentage of microbial reduction; however, there was a significant difference only between the MIA and CA groups when compared, with a larger percentage reduction in the CA group.

In the study of Barbosa et al., the traditional endodontic access (TEC) presented a smaller percentage of unprepared surface area in comparison with the conservative endodontic access (CEC), they suggest that smaller access cavities may have a negative influence on the preparation of root canals and potentially on the success of root canal treatment (21).

One of the most important stages of endodontic treatment is the instrumentation of root canals; proper cavity access should enable the removal of all tissue remnants and serve as a gateway for disinfecting irrigants and allow adequate access for the introduction of filling materials (22). Regarding root canal filling, Rover et al., in their results, reported that a minimally invasive endodontic access was associated with significantly more voids in root canal fillings (23).

Tüfenkçi et al. examined the effects of instrumentation using different file systems in teeth with a traditional access cavity (TEC) and a contracted access cavity (CEC) on the decrease of *E. faecalis* within the root canal system. Contrary to our study, they mentioned that the bacterial reduction counts of *E. faecalis* were similar in TEC and CEC. We can attribute this to the fact that, unlike the present investigation, the instrumentation systems presented conicities of 0.06 and 0.08, in addition to final activation of the irrigant with the file XP-Endo Finisher (24).

Similarly, in our study, Viera et al. demonstrated that treatment with conventional access cavities promoted significantly better disinfection than minimally invasive access cavities. These findings indicate that the size and design of the access cavity can influence root canal disinfection (25).

Minimally invasive endodontics (MIE) is an endodontic technique that aims to maintain as a tooth structure as possible including the coronal, cervical, and radicular thirds. Access opening, root canal cleaning and shaping, and surgical endodontics are all possible applications for MIE in endodontic treatment across minimally invasive access and conservative techniques of instrumentation (26).

Many studies have shown that residual bacteria after cleaning and shaping of the root canal system can spread from the main canal to other areas, including apical ramifications, lateral canals, isthmuses, and dentinal tubules, which represents a challenge for the clinician, who should be able to implement strategies to improve infection control in the canal system and increase the possibility of better treatment results (27, 28). Like other studies, bacterial sampling was performed using paper points, a technique well known to overestimate the disinfection ability of chemo-mechanical procedures, as it prevents the detection of bacterial biofilms on canal walls (29). Because of this limitation, the analysis of biofilm removal in this study was performed using scanning electron microscopy (SEM), with advantages like image quality, magnification and resolution, minimal sample loss, and actual sample structure preservation. The first step to make a morphological assessment of the effect of the various treatments on clinical biofilms is the production of images that faithfully reflect the structure of the sample (30).

In the present study minimally invasive access did not offer an advantage in comparison with conventional access in biofilm elimination of *Enterococcus faecalis* in the root canal system; however, current disinfection protocols in endodontics, in addition to chemo-mechanical instrumentation, allow improving the effectiveness of the treatment through the application of devices that activate irrigants such as passive ultrasonic irrigation (PUI), sonic activation, negative apical pressure, or antimicrobial photodynamic therapy (aPDT) (31,32), which represent an option to improve the effectiveness of treatments carried out under the concept of minimal invasibility.

An inconvenience of minimally invasive access compared to conventional access is limited vision and difficulty in entering instruments in a straight line, which increases the probability of accidents occurring during root canal treatment. However, the use of the operating microscope and coaxial lighting facilitates distinguishing visually and tactilely between a file penetrating in a root canal, resulting in significant advancements in the effectiveness of root canal therapy (33, 34).

Conclusions

The design of the access cavity significantly compromises the decrease in bacterial load and the elimination of biofilm within the root canal system. Regarding the TruNatomy instrumentation system, it presented good performance in the conventional access group, and we can infer that the type of instrument used does not affect disinfection, contrary to the design in the access cavity. Future studies are required that



evaluate the decrease in bacterial load through different access cavities, using irrigant activation systems, such as passive ultrasonic activation, in addition to the evaluation of the elimination of bacteria within the dentinal tubules through minimally invasive versus conventional access using confocal laser microscopy.

Ethical Considerations

Ethical Approval: Approval for this study was obtained from the Ethical Committee of the Faculty of Stomatology of the Autonomous University of San Luis Potosi (CEI-FE-046-021).

Conflict of Interest: The authors declare that they have no conflict of interest.

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