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The Effect of Access Cavity Design and Three Single-File Rotary Systems on the Reduction of Enterococcus Faecalis Count in the Root Canal System

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ABSTRACT

Background and Objective: The main goal in root canal therapy is to minimize the microbial load as much as possible. Since Enterococcus faecalis multiplies and forms biofilms on the wall of root canal, the aim of this study was to investigate the effect of access cavity design and three single-file rotary systems on reduction of Enterococcus faecalis count in the root canal system.

Methods: This in vitro study evaluated 60 extracted sound single-rooted single-canal mandibular premolars with long oval root canal cross-section. The teeth were randomly assigned to two groups (n=30) for preparation of traditional endodontic cavity (TEC) and conservative endodontic cavity (CEC). The root canals were prepared, and inoculated with E. faecalis (ATCC29212) for 4 weeks. Each group was randomly divided into three subgroups (n=10) for instrumentation with Reciproc, OneShape, and Only One File rotary systems. Bacterial samples were taken before (S1) and after (S2) instrumentation, using sterile paper points. Reduction in microbial load was calculated and reported in colony forming units (CFUs/mL).

Findings: The reduction in microbial load was significantly greater in TEC design (99.85±0.04) compared to CEC design (99.76±0.06) ($p<0.05$). The reduction in microbial load was not significantly different between the three files in TEC or CEC design.

Conclusion: Based on the results of this study, TEC design resulted in significantly greater reduction in E. faecalis count in the root canal system, but the three single-file rotary systems had no significant difference in this respect in any cavity design.

Keywords: *Enterococcus Faecalis, Root Canal Therapy, Microbial Load*.

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Introduction

Apical periodontitis occurs as the result of bacterial infection of the root canal system. Thus, the main goal of endodontic treatment is to minimize the microbial load as much as possible (1, 2). Enterococcus faecalis (E. faecalis) is a Gram-positive coccus that well tolerates low-oxygen environments and complex ecologies of the root canal system (3). Since E. faecalis proliferates and forms a biofilm on root canal walls, it has high resistance to antimicrobial agents, and is considered as a refractory and resilient pathogenic microorganism in root canal infections (4, 5).

Preparation of a suitable coronal endodontic access cavity is the first step in root canal treatment (2, 6, 7). In the traditional endodontic cavity (TEC) design, the emphasis is placed on creation of a straight path to the root canal system to prevent procedural errors, and maximize the efficacy of root canal instrumentation and quality of obturation (6, 8). Removal of tooth structure following TEC preparation may compromise the fracture resistance of teeth against functional forces (9-11). Tooth fracture and subsequent extraction of endodontically treated teeth may compromise the patients' trust in their clinician and long-term benefits of endodontic treatment (11-13). As a result, the conservative endodontic cavity (CEC) design was introduced as an alternative to TEC, which emphasizes on the significance of preservation of tooth structure. CEC design is characterized by minimal removal of tooth structure, and preservation of part of the pulp chamber roof and peri-cervical dentin (14, 15).

Although irrigants play a pivotal role in preparation of the root canal system, they cannot solely remove the entire bacteria from the root canal system (16). Mechanical instrumentation of the root canal system has a fundamental role in elimination of microbial biofilm and reduction of microbial load in the root canal system (17, 18). Different nickel-titanium (NiTi) instruments with different characteristics in terms of cross-sectional design, variable taper, and kinematics are available for root canal preparation (16). Instrumentation of the root canal system with single-file rotary systems can save time and cost, and has gained increasing popularity (19). The Reciproc system (VDW GmbH, Munich, Germany) has a S-shaped cross-sectional design, two cutting blades, and a non-cutting tip, and is made of a specific NiTi alloy, known as the M-Wire, which has higher flexibility and fracture resistance than the traditional NiTi alloys (20, 21).

OneShape (Micro Mega, Besancon, France) single-file rotary system is made of austenite traditional NiTi alloy and has continuous rotational movement. It has a tip size of #25 and constant taper of 6%. Its design is the most important characteristic of this rotary system since it has an asymmetrical horizontal cross-section. It has three cutting blades, and a triangular cross-sectional design at the tip. Its cross-sectional design gradually changes from three cutting blades to two cutting blades from the apical towards the coronal region, and changes to a S-shaped cross-section with two cutting blades at the coronal region (22).

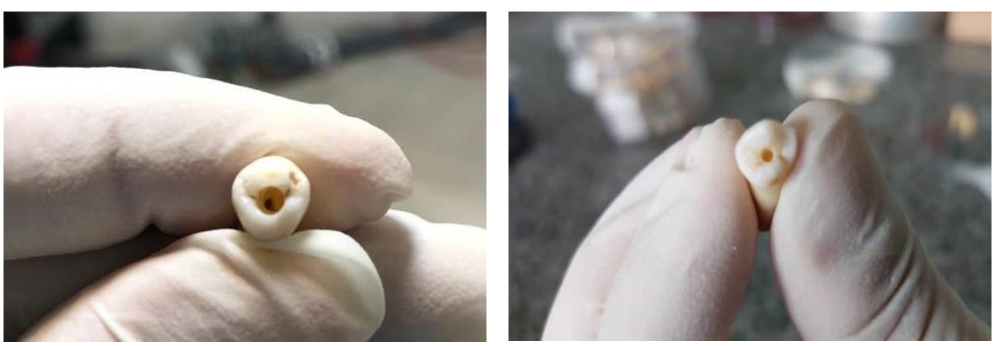
Only One File single-file rotary system (Shenzhen Denco Medical, Guangdong, China) was recently introduced, which has a reciprocal movement (30-degree clockwise and 150-degree counterclockwise). It is fabricated from heat-treated NiTi alloy; as a result, it has high fracture resistance. It is available in three sizes #25/0.08, #40/0.06, and #50/0.05.

Studies on the effects of CEC design on fracture resistance of endodontically treated teeth, and efficacy of different rotary instruments in root canal cleaning and shaping have reported controversial results (6, 11, 23-25). Furthermore, studies regarding the effect of CEC design on reduction of microbial load by endodontic treatment are highly limited, and the existing ones have reported contradictory results (21, 26, 27). Thus, further studies are warranted on this topic (28). The aim of this study was to assess the effect of root canal instrumentation with three single-file systems based on TEC and CEC designs on the reduction of E. faecalis load in the root canal system.

Methods

After approval by the Ethics Committee of Babol University of Medical Sciences with code IR.MUBABOL.HRI.REC.1400.186, this in vitro study was conducted on 60 extracted mandibular premolars extracted for orthodontic purposes. Inclusion criteria included: (I) sound single-rooted and single-canal mandibular premolars with long oval root canal cross-section at 5 mm from the apex (as confirmed on buccolingual and mesiodistal radiographs; accordingly, the buccolingual diameter had to be at least twice the mesiodistal diameter (29, 30), (II) sound crowns with no caries or restoration, (III) fully formed roots, (IV) similar root length, and (V) root canal curvature < 20 degrees as measured according to the Schneider's method (29). Exclusion criteria included teeth with internal or external root resorption or calcification. The teeth were randomly assigned to two groups of traditional endodontic cavity (TEC) and conservative endodontic cavity (CEC). The teeth in each of the CEC and TEC groups were then randomly divided into three subgroups (n=10) for root canal instrumentation with Reciproc, OneShape, and Only One File rotary systems. (The sample size was calculated to be 9 in each group according to a previous study (21), assuming $\alpha=0.05$, $\beta=0.20$ and study power of 80%; however, 10 samples were used in each group to increase the accuracy).

The collected eligible teeth were rinsed with saline, and all residual tissues were removed. The teeth were stored in distilled water until the experiment. The teeth were randomly assigned to two groups (n=30) for preparation of TEC and CEC. TEC was prepared by using a diamond fissure bur (Dia, Italy) and high-speed hand-piece under water spray according to the standard protocol (Figure 1A) (31, 32). The pulp chamber roof was completely removed, and a straight access to the coronal third of the root canal was created. CEC was prepared by using a diamond fissure bur (Dia, Italy) and high-speed hand-piece under water spray such that the bur was entered 1 mm buccal to the central fossa, and the cavity was prepared with minimal mesiodistal and buccolingual extension. Peri-cervical dentin and parts of the pulp chamber roof were preserved (Figure 1B) (11).



A B

Figure 1. Prepared access cavity. (A) TEC, (B) CEC

After access cavity preparation, a #10 K-file (Mani, Japan) was used to ensure patency. Also, a #10 K-file was introduced into the canal until its tip was visible at the apex; 1 mm was subtracted from this length to determine the working length. Next, each tooth was placed in an Eppendorf tube containing 1.5 mL of broth (Conda, Spain) and autoclave-sterilized (Euronda, Italy) at 121°C temperature and 15 Psi pressure for 15 minutes. The teeth were then incubated at 37°C for 48 hours to ensure no microbial contamination of the teeth.

Then, pure-culture E. faecalis (ATCC 29212) was obtained from the microbial bank to prepare a microbial suspension; 100 µL of E. faecalis suspension with 0.5 McFarland standard concentration containing 1.5×10^8 colony forming units (CFUs/mL) was delivered into the access cavity and reached to the working length by using a sterile #15 hand K-file. The teeth were then incubated at 37°C and 95% humidity for 4 weeks. Primary bacterial samples were then collected from the root canals (S1). To eliminate free bacteria, the root canals were rinsed with 1 mL of sterile saline, and two #15 paper points were consecutively introduced into the root canals to the working length and remained there for 1 minute to collect baseline (S1) samples. Complete contact of paper points with the root canal walls was ensured. Next, the paper points were placed in test tubes containing saline.

In the Reciproc subgroups, the root canals were prepared by using R25 (25/0.08) file of Reciproc single-file system (VDW GmbH, Munich, Germany) and an endo-motor in “Reciproc ALL” mode, as instructed by the manufacturer. The file was placed in the cervical third of the canal, and was used with three in-and-out pecking motions with 3-mm range, and also sweeping motion on root canal walls. After three movements, the file was removed from the canal, and debris was cleaned by a sterile gauze. This protocol was repeated until reaching the working length.

In the OneShape subgroups, OneShape rotary file (Micro Mega, Besancon, France) with #25 size and 0.06% taper was used with an endo-motor in continuous rotation movement at a speed of 350 rpm and 2.5 N/cm torque by the crow-down technique as instructed by the manufacturer.

In the Only One File subgroups, Only One File (Shenzhen Denco Medical, Guangdong, China) with #25 size and 0.08 taper was used with an endo-motor in “Reciproc ALL” mode as instructed by the manufacturer. It was used with three in-and-out pecking motions with 3-mm range. After three movements, the file was removed from the canal, and debris was cleaned by a sterile gauze. This protocol was repeated until reaching the working length. During instrumentation, the root canals were rinsed with 10 mL of sterile saline using a 30-gauge irrigation needle at 1 to 3 mm to the working length. Bacterial samples (S2) were then collected again from the prepared root canals using two #25 sterile paper points remained in the canal for 1 minute to the working length. Complete contact of paper points with the root canal walls was ensured. The paper points were then placed in test tubes containing saline.

The paper points (S1 and S2) were removed from saline and placed in test tubes containing 1 mL of 0.85% phosphate buffer, and were vortexed for 1 minute. They were then diluted with sterile saline, 0.1 mL of the contents of each tube was cultured on blood agar (Conda, Spain), and incubated at 37°C for 24 hours. The number of bacterial colonies was counted and reported as CFUs/mL. The difference between microbial load at S1 and S2 was calculated to assess the effect of interventions on microbial load.

Data were analyzed using SPSS version 26 (SPSS Inc., IL, USA). Normal distribution of data was ensured by Kolmogorov-Smirnov. Accordingly, independent samples t-test and one-way ANOVA were used to compare the effect of access cavity design and type of single-file rotary system on reduction of microbial load in the root canal system, and $p<0.05$ was considered significant.

Results

The average reduction in microbial load was significantly greater in TEC design compared to CEC when using Reciproc ($p<0.05$), OneShape ($p<0.05$) and Only One File ($p<0.05$). One-way ANOVA revealed no significant difference in microbial load reduction between the three rotary file subgroups, irrespective of access cavity design ($p<0.05$) (Table 1).

Table 1. Primary (S1) and secondary (S2) colony count (CFUs/mL) according to access cavity design and three rotary systems

File type	Access cavity design	S1 (CFUs/mL)	S2 (CFUs/mL)	Reduction (%)
Reciproc	**TEC**			
	Mean	172000000.000	237000.000	99.862
	Std. Deviation	10338708.280	71225.776	0.039
	CEC			
	Mean	174900000.000	397400.000	99.771
	Std. Deviation	13253511.065	108807.884	0.072
	Total			
	Mean	173450000.000	317200.000	99.816
	Std. Deviation	11664047.325	121579.777	0.073
OneShape	**TEC**			
	Mean	169200000.000	251800.000	99.851
	Std. Deviation	10747609.553	87678.453	0.053
	CEC			
	Mean	171200000.000	408600.000	99.762
	Std. Deviation	12647353.698	90848.103	0.052
	Total			
	Mean	170200000.000	330200.000	99.806
	Std. Deviation	11468951.221	118410.348	0.068
Only One file	**TEC**			
	Mean	175400000.000	241800.000	99.861
	Std. Deviation	12781931.692	73783.166	0.047
	CEC			
	Mean	176100000.000	394400.000	99.775
	Std. Deviation	10692157.043	114238.736	0.067
	Total			
	Mean	175750000.000	318100.000	99.817
	Std. Deviation	11474800.767	122018.937	0.071
Total	**TEC**			
	Mean	172200000.000	243533.333	99.858
	Std. Deviation	11241548.550	75425.567	0.046
	CEC			
	Mean	174066666.667	400133.333	99.769
	Std. Deviation	12005554.270	101609.032	0.062

Discussion

The results showed a significant reduction in microbial load in all three rotary subgroups, with no significant difference among them, irrespective of access cavity design. Similarly, Barbosa et al. (33) reported a reduction in microbial load following the application of Reciproc Blue R25 and Reciproc Blue R40 files. Some other studies also confirmed the optimal efficacy of Reciproc Blue R25 for reduction of microbial load (34, 35). The present results revealed no significant difference between the Reciproc R25 (with reciprocal movement), OneShape (with reciprocal movement) and Only One File (with rotary movement) irrespective of access cavity design in reduction of microbial load. To the best of the authors' knowledge, reduction of microbial load by Only One File system has not been previously evaluated in the literature. A systematic review by Küçükkaya Eren et al. (34) concluded that both reciprocal (23.32%-88.47%) and rotary (23.33%-89.86%) systems decrease the microbial load in the root canals; however, none of them can completely disinfect the root canal system. In another systematic review, Siddique et al. (36) reported comparable disinfecting efficacy of both reciprocal and rotary systems in the root canals. Cavalli et al. (37) demonstrated that Reciproc and Mtwo rotary systems had similar efficacy in reduction of endotoxins and cultivable bacteria in the root canals. However, they could not completely disinfect the entire root canal system. It has been confirmed that single-file reciprocal and rotary systems have similar efficacy for reduction of microbial load (35, 38-42). Marinho et al. (41) demonstrated that Reciproc, Mtwo, ProTaper, and RaCe rotary files significantly decreased the microbial load but no significant difference was noted in level of endotoxins. Their results were in agreement with the present findings.

Single-file reciprocal systems accelerate and facilitate the process of root canal instrumentation (35, 43). The only concern in this regard is about their efficacy to optimally disinfect the root canal due to the short contact time of instruments with dentinal walls. Moreover, root canal preparation in a shorter time may be associated with the use of smaller volume of root canal disinfecting solutions or their shorter contact time with the root canal walls. However, previous studies have shown comparable shaping ability of reciprocal and rotary systems by using a full range of instruments (34, 43, 44).

The present results showed significantly greater reduction in microbial load in TEC compared with CEC design, irrespective of type of rotary instrument used. According to the results, access cavity design can significantly affect the quality of cleaning and shaping and microbial load reduction in the root canal system. Therefore, more uninstrumented areas may remain in complex canals (such as curved canals) with CEC design due to absence of a straight path (26, 27). On the other hand, CEC design negatively affects the angle of entry of instruments into the canal and may limit their range of movements (26). Since the residual bacteria can compromise the outcome of treatment, some strategies may be required to improve the quality of root canal disinfection during or after chemomechanical preparation of the root canal system in CEC design. This finding was in agreement with the results of Andac et al, who reported significantly smaller reduction in bacterial count in CEC design, compared with TEC after instrumentation with ProTaper Gold and 2Shape rotary systems (27). Also, Vieira et al. demonstrated significantly smaller bacterial reduction in CEC group compared with TEC after instrumentation of incisor teeth with curved canals with XP-Endo Shaper system (26).

In contrast to the present results, Tüfenkçi et al. (21) evaluated the efficacy of root canal instrumentation with Reciproc and ProTaper Next systems for reduction of E. faecalis count in the mesiobuccal canal of mandibular molars with TEC and CEC designs. They found no significant difference between the two designs. Difference between their results and the present findings may be attributed to the use of different files and type of canals. Barbosa et al. (33) assessed the efficacy of root canal instrumentation for reduction of microbial load in mandibular molars with TEC and CEC designs using Reciproc Blue R25 and R40. They showed the highest amount of microbial load in R40/CEC group, which was in line with the present findings. However, they found no significant difference between CEC and TEC designs in use of R25 files, which was different from the present findings. This difference may be due to their different irrigation protocol since they used 0.5% NaOCl as irrigant after using each file. They also performed a final rinse with 2 mL of 0.5% NaOCl, 2 mL of 17% EDTA, and 2 mL of 0.5% NaOCl. However, only saline was used for irrigation in the present study.

Duration of incubation of root canals with E. faecalis microbial suspension varies from 24 hours to 30 days in the literature (26, 45-47). Longer incubation period allows deeper penetration of microorganisms into dentinal tubules, which further complicates their removal and better simulates the clinical condition (27). Thus, the teeth were incubated with E. faecalis for 28 days in the present study to ensure deep contamination of dentinal tubules, which was a strength of this study. Also, to assess the effect of interventions, colony counting was performed in the present study, which is simple and efficient. Although molecular methods for DNA detection may be more accurate, they may also detect non-vital microorganisms, which is a drawback (21, 34, 48). Use of paper points for sample collection has the limitation of taking a sample only from the canal content, and not the microorganisms penetrated into the tubules (18, 26, 34). Thus, in the present study, a hand file was introduced into the canal with in-and-out movement to allow better collection of microorganisms from all parts of the root canal system. Moreover, use of sterile distilled water as root canal irrigant has the advantage of not killing the microorganisms. Since the purpose of this study was to compare the mechanical effects of the three filing systems on reduction of E. faecalis count, antimicrobial irrigants were not used (25, 27, 38). Overall, the present results showed that CEC design significantly compromised efficient root canal disinfection. Since treatment of apical periodontitis entirely depends on infection control (26), excessive preservation of dentin has the potential to negatively affect the outcome of endodontic treatment of infected teeth.

Further studies with different irrigating solutions are required to assess the efficacy of different rotary instruments and the interaction effect of access cavity design, instrument type, and type of irrigant on multi-species microbial biofilm in the root canal system.

These results indicate that TEC design resulted in significantly greater reduction of E. faecalis in the root canal system but the three single-file rotary systems showed no significant difference in this respect in any cavity design.

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