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ORIGINAL ARTICLE

Apical extrusion of *Enterococcus faecalis* in different canal geometries during the use of nickel titanium systems with different motion types

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bacterial extrusion;
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Abstract *Background/purpose:* Extrusion of intracanal bacteria leads to treatment failures. Compare the apical extrusion of intracanal bacteria (*Enterococcus faecalis*) during canal preparation with three different instrumentation techniques [RECIPROC, One Shape (OS), and Twisted-File Adaptive (TFA)] with different motion types.
Materials and methods: Ninety teeth with different canal morphologies were divided into three main groups, each including 30 teeth (10 mandibular incisors, 10 mandibular premolars, and 10 curved roots). Roots were resected until 13-mm working length was obtained and fixed to glass vials filled with brain–heart infusion broth. Each canal was filled with *E. faecalis* suspension. The three main groups were further grouped into three subgroups. Each group was further subgrouped into three, with each subgroup including 10 roots from each type of teeth (10 incisors/subgroup, 10 premolars/subgroup, and 10 curved canals/subgroup). These subgroups were prepared with one of RECIPROC, OS, or TFA. Bacterial colonies extruded into each vial were incubated in brain–heart infusion agar at 37°C for 5 days and counted using a colony counter as the number of colony-forming units per milliliter. Statistical analyses were performed using one-way analysis of variance, *post hoc* Tukey honest significant difference, and Kruskal–Wallis tests.
Results: Apically extruded bacteria were not statistically different from each other ($P > 0.05$). The amount of apically extruded bacteria was statistically similar for both different instruments in the same type of tooth ($P > 0.05$) and same instrument in different types of teeth ($P > 0.05$).

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Conclusion: Neither the motion type of instrument nor the canal morphology affected the degree of bacterial extrusion.

Introduction

Elimination of bacteria is the main objective of root canal treatment. This objective can be achieved with biomechanical preparation. During canal preparation, extrusion of debris, tissue remnants, and presence of microorganisms beyond the apical foramen may result in inflammation of the periapical tissues, postoperative pain, and delay of periapical healing.[1,2] Er et al[3] pointed out that both the amount and the virulence of apically extruded bacteria may affect the intensity of these undesired consequences. Microbiota and the preparation technique used are two important aspects in terms of apical extrusion because the degree of apical extrusion of bacteria along with debris is related to the preparation technique, and to the physical and mechanical behaviors of the instrument used.[4] The main microbial species causing the failure of root canal treatment include *Enterococcus faecalis*, *Propionibacterium alactolyticus*, and *Propionibacterium propionicum*.[5] In particular, *E. faecalis* is the most commonly isolated species from post-treatment diseases.[6]

In the light of previous studies, it is known that use of all rotary instruments may result in the apical extrusion of bacteria during canal preparation.[3,6–9] Recently, advanced single- or multifile rotary instruments with different kinematics were examined in terms of their potential in the apical extrusion of bacteria.[2,10] RECIPROC (VDW, Munich, Germany) is one of these single-file systems working with reciprocal motion, and One Shape (OS; MICRO-MEGA, Besançon, France) is another single-file system working with continuous rotation. Twisted-File Adaptive (TFA; SybronEndo, Orange, CA, USA) is a multifile system working with the combination of both reciprocation and continuous rotation. In the study by Türker et al,[10] the degree of apical extrusion was similar for OS and TFA. Furthermore, Burklein et al[11] reported that reciprocating single-file instruments with greater tapers advance debris and bacteria beyond the apical foramen more than other types of systems.

However, all the aforementioned findings were obtained as the outcomes of studies including only one type of tooth. This study aimed to compare the apically extruded bacteria with RECIPROC, OS, and TFA systems. However, different from the previous studies, this study aimed to compare the apically extruded bacteria with these instruments during the preparation of teeth with different canal morphologies including mandibular incisors with narrower straight canals, mandibular premolars with larger straight canals, and mesiobuccal roots of maxillary first molars, which are curved compared with the others. The authors of this study questioned (1) whether different motion styles will affect the degree of apically extruded bacteria, (2) which type of tooth will represent the most extrusion during the use of each rotary system, and (3) whether the apically extruded bacteria will differ for different files during the instrumentation of the same type of tooth. The null hypotheses were (1) mandibular incisors and curved canals require more forcing during preparation due to their narrower morphology, and thus result in more extrusion; and (2) reciprocal motion pushes more debris, thus resulting in more bacterial extrusion.

Materials and methods

Selection of teeth samples and preparation of test apparatus

This study was approved by the Ethical Committee of Gaziantep University (Gaziantep, Turkey). A total of 90 freshly extracted teeth with complete root formation and free of any resorption or cracks were included in this study. The first 30 teeth were mandibular incisors with narrow canals with similar dimensions, whereas the second 30 teeth were mandibular premolars with oval canals. The remaining 30 were the mesiobuccal roots of the maxillary first molars with a curvature of 25–35°. Periapical radiographs were taken from the mesiodistal and buccolingual directions to confirm that all teeth have only one canal and are free of any canal blockage. All teeth were decoronated. A Size 15 K file (Sybron Endo, Scafati, Italy) was inserted into canals until it was visible at the apex. The file was then withdrawn 1 mm for working length. All samples were shortened until a 13-mm working length was obtained for each sample. Two coats of nail varnish were applied to the external surface of roots to avoid any bacterial leakage through lateral canals. A Size 10 K file was inserted until it passed through 1 mm beyond the apex to remove the varnish of apical foramen and to allow bacterial extrusion. Roots were tightly inserted through the rubber stoppers of vials until the coronal 3 mm of the samples was outside the vials. A 24-gauge needle was inserted into the rubber stoppers to equalize the pressure. The vials were filled with brain–heart infusion broth (Becton Dickinson, Franklin Lakes, NJ, USA; Figure 1). The opening between the samples and rubber stopper was sealed using a self-curing acrylic (Imicryl, Istanbul, Turkey). Vials were placed in five test apparatuses (Figure 2). These test apparatuses were autoclaved in ethylene oxide gas (STERIS, Mentor, OH, USA) for a 12-hour cycle at 2.4 bar and 55°C using an anprolene ethylene oxide gas sterilizer (3M, Two Harbors, MN, USA).

For contamination of root canals, a pure culture of *E. faecalis* American Type Culture Collection 29212 was used. A suspension was prepared by adding 1 mL pure culture of

Figure 1 A sample embedded and fixed into a glass vial filled with brain—heart infusion broth through the rubber stopper of the vial. Needle was inserted into the rubber stopper to equalize the pressure.

E. faecalis into the brain—heart infusion broth. The suspension was adjusted to a McFarland standard number 0.5 to ensure that the concentration of bacteria was 1.5×10^8 colony-forming units/mL. Each canal was filled with *E. faecalis* suspension and a Size 15 K file was advanced into canals to spread the suspension throughout the canal. After the contamination of all samples, the entire test apparatus was incubated at 37°C for 30 days.

Root canal preparation

All roots were divided into three main groups, each including 30 mandibular incisors, 30 mandibular premolars, and 30 curved mesiobuccal roots of the maxillary first molars. Each group was further subgrouped into three, with each subgroup including 10 roots from each type of teeth. Samples in the RECIPROC (VDW, Munich, Germany) group were divided into three subgroups including mandibular incisors (Group RI; $n = 10$), mandibular premolars (Group RP; $n = 10$), and curved canals (Group RC; $n = 10$). Canals were prepared with a Size 25 RECIPROC file—0.08 tapered in the apical 3 mm and 0.06 tapered in the remaining portion—adjusted to engine-driven motor (VDW Gold, Munich, Germany) using the settings for RECIPROC files.

Samples in the OS (MICRO-MEGA) group were divided into three subgroups including mandibular incisors (Group OSI; $n = 10$), mandibular premolars (Group OSP; $n = 10$), and curved canals (Group OSC; $n = 10$). Canal preparation was performed with a Size 25 file—0.06 tapered OS file adjusted to engine-driven motor (VDW Gold) using the settings for OS files (400 rpm; 2.5 N·cm).

Samples in the TFA (SybronEndo, Orange, CA, USA) group were divided into three subgroups including mandibular incisors (Group TFI; $n = 10$), mandibular premolars (Group TFP; $n = 10$), and curved canals (Group TFC; $n = 10$). Canals were prepared with a Size SM2 TFA file, which corresponds to 25/0.06. The instruments were operated with Elements Adaptive Motion Technology (SybronEndo, Orange, CA, USA), working with a combination of rotation and reciprocal motion.

During the use of each type of instrument, at the point where resistance occurred, files were removed and flutes were cleaned. Instrumentation continued until the working length was established. In each turn, 2 mL of 0.9% NaCl solution was used for irrigation. A total of 10 mL NaCl solution was used for the irrigation of each root. The instrument used was replaced with a new one after the preparation of two samples.

Positive control group

For the positive control group, three samples were infected but their canals were not prepared. After 30 days, bacterial viability was confirmed by taking smear from the canal with an absorbent paper point. This smear was examined under a light microscope (Figure 3) to verify the presence of bacteria.

Negative control group

For the negative control group, three samples from each type of teeth were not infected. Root canals of these teeth were prepared with all types of files. The flowchart of the group classification is presented in Figure 4.

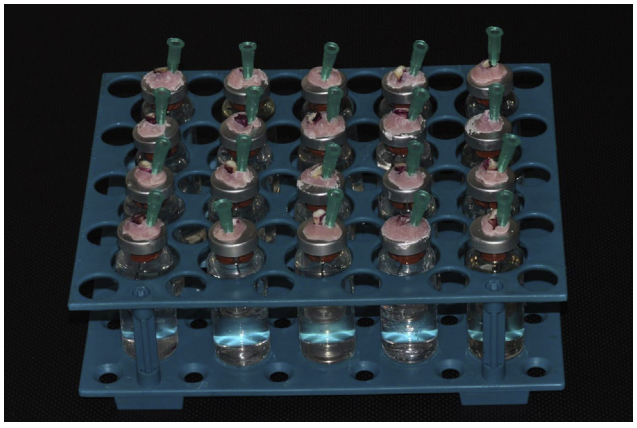

Figure 2 A sample of the test apparatus.

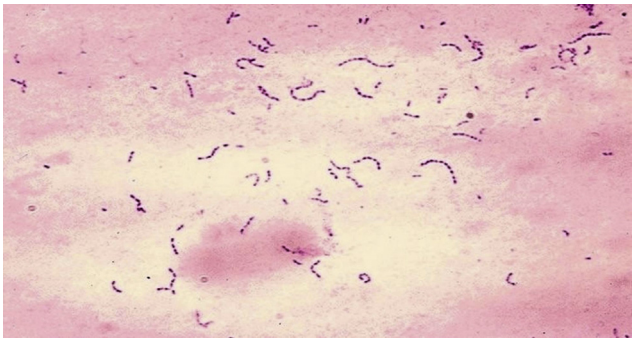

Figure 3 One of microscopic images of the positive control group.

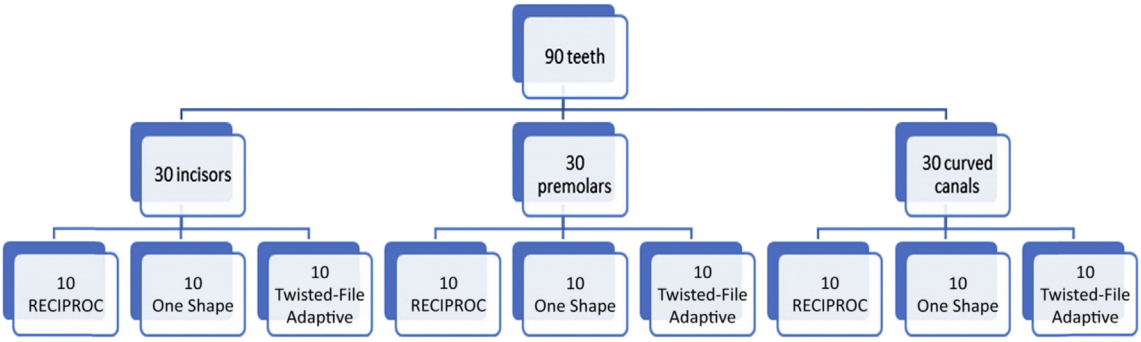

Figure 4 Flowchart of the experiment.

Determination and statistical analysis of the amount of apically extruded bacteria

After the completion of preparation, 0.1 mL of broth was derived from each vial and incubated in brain–heart infusion agar at 37°C for 5 days. Bacterial colonies were counted using a colony counter as the number of colony-forming units per milliliter. All statistical analyses were performed with one-way analysis of variance and *post hoc* Tukey honest significant difference tests for main groups. For subgroups, the data represented nonparametric distribution according to the Shapiro–Wilk test and statistical analysis was performed with the Kruskal–Wallis test. SPSS software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

Results

No bacteria were detected in the negative control group, whereas bacteria were detected in the positive control group. The mean amount of apically extruded bacteria, their standard deviations, and P values for both main groups and subgroups are presented in Tables 1 and 2. In the overall evaluation, there was no significant difference among the main groups ($P > 0.05$). According to the comparison of subgroups, the amount of apically extruded bacteria was statistically similar for both different instruments in the same type of tooth and same instrument in different types of teeth ($P > 0.05$).

Table 1 The mean amount of apically extruded bacteria for each main group and their standard deviations.

	N	Mean apically extruded bacteria in colony-forming units	Standard deviation	P
RECIPROC	30	0.933	1.946	
One Shape	30	0.900	1.748	
Twisted-File Adaptive	30	0.566	1.832	
Total	90	0.800	1.831	
Negative control	3	No colony-forming units		0.697

Discussion

Enterococcus faecalis was included in this study because it is the main bacterial species associated with persistent endodontic infections and treatment failures due to its ability to survive alone without any symbiotic support.[12] The test model in this study was similar to those described previously,[3,7–9] which offered standardization and avoidance of the disruption of results due to other environmental factors. By adopting this model, we were able to associate bacterial extrusion only with the instrumentation technique and tooth type. Another point that should be considered is the choice of irrigation solution. Although NaCl was used for the vitality of bacteria in this study to determine the amount of apically extruded bacteria and NaOCl might have resulted in lesser amount of bacterial extrusion, complete elimination of bacteria is not possible for the majority of the treatments.[9] It should be noted that a small amount of bacterial extrusion occurs if the virulence of bacteria—such as *E. faecalis*—is high, which subsequently initiates periapical inflammation.[5] Therefore, bacterial extrusion potential of instrumentation techniques and minimizing the bacterial extrusion are critically important. For this purpose, several studies were performed to determine the most reliable instrumentation technique.[3,6,9,10] These studies included only one type of teeth—especially the teeth with straight canals. However, to the best of our knowledge, there are no studies comparing both instruments and canal morphology. The authors of this study aimed to (1) compare the apical extrusion of intracanal bacteria with three different instrumentation techniques with different motion types,

Table 2 Mean amount of apically extruded bacteria for subgroups, their standard deviations, and P values.

The type of teeth	The type of instrument			P
	RECIPROC	One Shape	Twisted-File Adaptive	
Incisor	1.400 ± 2.118	0.600 ± 1.264	0.200 ± 0.421	0.115
Premolar	0.300 ± 0.948	0.400 ± 0.699	0.400 ± 0.516	0.446
Curved canal	1.100 ± 2.469	1.700 ± 2.584	1.100 ± 3.142	0.628
P	0.106	0.624	0.585	

which are gaining popularity in recent years due to their advantage of time saving and working safety, as previous studies revealed that single-file systems did not result in more extrusion compared with multifile systems[7,10]; (2) evaluate whether the extrusion potential of any of the instruments studied may differ according to the type of tooth included. In this way, the authors tried to determine the most suitable instrumentation technique for different types of teeth. However, the study results revealed that neither the type of the instrument nor the morphology of the root canal affected the amount of apically extruded bacteria. The null hypothesis was thus rejected. These results are in accordance with the results of Türker et al[10] who found no significant difference in the bacterial extrusion by TFA and OS instruments. Kustarci et al[9] also revealed that engine-driven instruments (K3, RaCe, FlexMaster) represented the same degree of bacterial extrusion. In the study by Mohammadi and Khademi,[13] use of Mtwo and FlexMaster resulted in statistically similar amounts of bacterial extrusion. The results of the aforementioned studies and that of this study imply that correct application of instruments and avoiding excessive forcing and removing bacteria along with debris with sufficient irrigation throughout canal orifices are more important in terms of bacterial extrusion rather than the motion type, cross-sectional geometry, and metallurgic properties of the instruments. Different from the previous studies, this study further found that canal morphology did not affect the degree of apical extrusion for different types of instruments.

In the study by Burklein et al,[11] it was proposed that reciprocating instruments are more susceptible to push more bacteria beyond the apex compared with other instruments and instrumentation techniques. Tinoco et al[7] reported that this may be related to the aggressive movement of reciprocating motion, which advances into the canals by removing great amounts of debris. The authors of this study hypothesized that RECIPROC files may push more debris, particularly in the mandibular incisors and curved canal. However, the results of this study did not show any difference for RECIPROC in terms of bacterial extrusion compared with OS—rotational motion—and TFA—combination of rotation and reciprocation. The study by Teixeira et al[2] revealed that using R-40 alone did not influence the degree of bacterial extrusion compared with the use of R-25. Their results and those of this study can be explained with the instrument design of RECIPROC files, which directs bacteria along with debris toward the canal orifice, thereby avoiding larger amounts of bacterial extrusion. This is also true for rotational motion as reported by Beeson et al.[14] Reddy and Hicks[15] stated that engine-driven instruments pack the debris into the flutes and direct them toward the canal orifice. This may explain why all of the instrumentation techniques in this study showed similar degrees of bacterial extrusion regardless of the type of tooth.

Er et al[3] stated that preflaring the coronal portion of canal reduces the amount of apically extruded bacteria. Thus, if any resistance is experienced while using single-file systems, the procedure should be stopped immediately and irrigation + cleaning of flutes should be carried out. Ghivari et al[6] reported that engine-driven instruments result in lesser amount of bacterial extrusion because they work with a fixed rotational speed (torque) and they contact with the apical zone for a lesser period compared with hand techniques. This was also verified by Türker et al[10] who found that OS extruded less bacteria compared with ProTaper NEXT (PTN). They claimed that this is related to the number of files, which is more for PTN. These three aspects pointed out by previous researchers may be related to the results of this study. In this study, all instruments were removed from root canal when resistance is experienced. Following the cleaning of flutes and irrigation, preparation was continued. All instruments were applied with their standard rotational and torque values. In addition, all preparations were performed with single-file systems. As a result, the amount of bacterial extrusion was not different between the instruments, even in curved canals and mandibular incisors with narrow canals. Thus, although it was previously reported that several factors including instrument size, taper, and technique may affect the amount of bacterial extrusion, according to the results of this study, application of instruments with correct rotational speed (torque), preflaring/irrigation of the coronal portion before apex is reached, cleansing the flutes, and using as fewer number of instruments as possible seem to outweigh the design and working type of instrument in terms of bacterial extrusion regardless of the canal morphology and curvature.

All study groups resulted in the extrusion of *E. faecalis*. There was no statistical difference among the study groups. Neither the motion style nor the canal morphology affected the degree of extrusion. The study results show that effective chemomechanical root canal preparation is more important than the instrument used or the morphology of canal in terms of bacterial extrusion.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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