



# Apical extrusion of bacteria following the use of reciprocating single-file and rotary multi-file instrumentation systems in oval root canals

Extrusão apical de bactérias após o uso de sistemas alternativos de instrumentação de arquivo único e rotativo de arquivo múltiplo em canais radiculares ovais

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## ABSTRACT

**Objective:** All instrumentation techniques and instruments are associated with apical extrusion during chemomechanical preparation, and this causes postoperative pain and flare-up. However, it is controversial whether reciprocal systems or rotary systems cause more apical extrusion. The objective of this in vitro study was to determine the differences in the amounts of apically extruded bacteria (AEB) associated with nickel-titanium rotary and reciprocating systems when used in oval-shaped root canals. **Material and Methods:** Seventy human mandibular premolar teeth with oval-shaped canals were randomly assigned to four experimental groups (15 teeth in each group) and one control group (10 teeth). The root canals were contaminated with *Enterococcus faecalis* and instrumented using two full-sequence rotary instruments (ProTaper Universal [PTU] and ProTaper Next [PTN]) and two reciprocating single-file instruments (Reciproc [R] and WaveOne [WO]). A 0.9% NaCl solution was used as an irrigant, and the bacterial extrusion was quantified as the number of colony-forming units for each sample. The results were statistically analyzed using the Kruskal-Wallis one-way analysis of variance and the Mann-Whitney U test, and the statistical significance level was set at  $p < 0.05$ . **Results:** The R system was associated with the highest amount of bacterial extrusion ( $p < 0.05$ ). The PTU system caused more bacterial extrusion than the PTN and WO systems ( $p < 0.05$ ). There was no significant difference between the PTN and WO systems ( $p > 0.05$ ). **Conclusions:** All instrumentation techniques caused apical bacterial extrusion. The instrument design and preparation techniques affect the number of extruded bacteria.

## KEYWORDS

Apical extrusion; Bacteria; Endodontics; Root canal preparation.

## RESUMO

**Objetivo:** Todas as técnicas e instrumentos de instrumentação estão associados à extrusão apical durante o preparo quimomecânico, o que causa dor e surto no pós-operatório. No entanto, é controverso se sistemas recíprocos ou rotativos causam extrusão mais apical. O objetivo deste estudo in vitro foi determinar as diferenças na quantidade de bactérias apicalmente extrudadas (AEB) associadas aos sistemas rotativo e alternativo de níquel-titânio quando usadas em canais radiculares em forma oval. **Material e Métodos:** Setenta dentes pré-molares inferiores humanos com canais em forma oval foram divididos aleatoriamente em quatro grupos experimentais (15 dentes em cada grupo) e um grupo controle (10 dentes). Os canais radiculares foram contaminados com *Enterococcus faecalis* e instrumentados usando dois instrumentos rotativos de seqüência completa (ProTaper Universal [PTU] e ProTaper Next [PTN]) e dois instrumentos alternativos de arquivo único (Reciproc [R] e WaveOne [WO]). Uma solução de NaCl a 0,9% foi usada como irrigante e a extrusão bacteriana foi quantificada como o número de unidades formadoras de colônias para cada amostra. Os resultados foram analisados estatisticamente usando a análise de variância unidirecional de Kruskal-Wallis e o teste U de Mann-Whitney, e o nível de significância estatística foi estabelecido em  $p < 0,05$ . **Resultados:** O sistema R foi associado à maior quantidade de extrusão bacteriana ( $p < 0,05$ ). O sistema PTU causou mais extrusão bacteriana que os sistemas PTN e WO ( $p < 0,05$ ). Não houve diferença significativa entre os sistemas PTN e WO ( $p > 0,05$ ). **Conclusões:** Todas as técnicas de instrumentação causaram extrusão bacteriana apical. O desenho do instrumento e as técnicas de preparação afetam o número de bactérias extrudadas.

## PALAVRAS-CHAVE

Extrusão apical; Bactérias; Endodontia; Preparação do canal radicular.

## INTRODUCTION

During the chemomechanical root canal preparation of a tooth, microorganisms and their by-products may be extruded into the periapical tissues [1]. Although the extrusion amount may vary, all endodontic instruments and preparation techniques cause apical extrusion. During root canal preparation, even a small amount of infected debris can cause periradicular inflammation when the root canal system contains virulent clonal pathogenic bacterial species, and they are extruded into the periradicular tissues [2].

The severity of the host inflammatory response to the bacterial extrusion is associated with virulence (qualitative factor) and bacterial counts (quantitative factor) [2]. Nevertheless, in clinical conditions, it is not possible to control the qualitative factors because they depend on the composition of the intracanal bacterial communities. However, clinicians can control the quantitative factors to minimize apical extrusion by determining the treatment procedures [3].

Reciprocating systems are produced to reduce the number of steps and files needed during root canal treatment, while still providing sufficient root canal preparation and cleaning [4]. Although reciprocating single-file systems are able to cut significant amounts of dentin in short periods of time, and they have expedited the mechanical enlargement of root canals, it has been suggested that these systems tend to push more debris, bacteria, and irrigation solution into the periapical region than conventional instrumentation systems [5-8]. This is thought to be due to the fact that conventional full-sequence instrumentation systems provide a slower and gradual mechanical enlargement in root canals while reciprocating instruments provide faster mechanical preparations by only using one, effective instrument in a short period of time during endodontic treatment [9,10]. Moreover, it has been reported that the rotary motion tends to direct debris towards the canal orifice, collecting the debris into the flutes of the instruments, thus prevent their compaction in the root canal [11]. However, it is still controversial

whether reciprocating or conventional rotary systems cause more apical extrusion during the chemomechanical preparation of root canals. Some studies have reported that reciprocating single-file systems cause less extrusion than conventional rotary systems [10,12,13], while others have found that there is no difference between them [14,15].

The ProTaper Universal (PTU) (Dentsply Maillefer, Ballaigues, Switzerland) system is composed of a conventional nickel-titanium (NiTi) alloy, and it has a convex triangular cross-section, a variable progressive taper, and a non-cutting safety tip [16]. It is claimed that instruments with such a cross-sectional design are cut dentine more effectively [17]. ProTaper Next (PTN) (Dentsply Maillefer, Ballaigues, Switzerland) is a novel NiTi rotary system that uses an M-Wire NiTi alloy to enhance flexibility and cyclic fatigue resistance. This system has progressive and regressive percentage tapers, and an off-centered rectangular design for superior strength [18,19]. Its offset design is claimed to maximize the amount of augering debris that can be removed from the root canal in comparison to a file with a centered mass and axis of rotation [20].

The Reciproc (R) (VDW, Munich, Germany) and WaveOne (WO; Dentsply Maillefer, Ballaigues, Switzerland) single-file NiTi system is also manufactured using an M-Wire NiTi alloy, and it is claimed to be able to completely prepare root canals with only one instrument [21]. The R system has an S-shaped cross-sectional design with sharp cutting edges, whereas the WaveOne (WO) system is characterized by a triangular or modified triangular cross-section resulting in a lower cutting efficiency and smaller chip space [5].

In oval-shaped root canal systems, some areas may remain untouched by the instruments during instrumentation, which may adversely affect the proper cleaning and shaping of the root canals and impact the outcome of the treatment [22,23]. To date, little information is available regarding the amount of apically extruded bacteria (AEB) when preparing oval-

shaped root canals when using reciprocating instruments in comparison to full-sequence rotary systems during endodontic treatment. Based on the above-mentioned information, the purpose of this in vitro study was to compare the number of AEB generated with two full-sequence rotary systems and two single-file reciprocating instrument systems in oval-shaped root canals. The null hypothesis of this study was that there would be no significant differences in terms of the number of AEB among the four different systems being compared.

## MATERIAL AND METHODS

The study was approved by the Clinical Research Ethics Committee of Dumlupinar University (decision date: 21.02.2018, ID number: 2018-03/1). Power analysis was performed and calculation of the sample size indicated that a minimum of 48 samples would be required to observe the differences between the four systems, with an alpha risk of 0.05, a power of 0.8, and an effect size of 0.5 [7,13]. More samples were included to increase the power of the study.

### Selection and preparation of the teeth

A total of 150 extracted human single-rooted mandibular premolar teeth with complete root formation and similar working lengths (approximately 21 mm) and an initial apical diameter corresponding to a size 15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) were randomly selected.

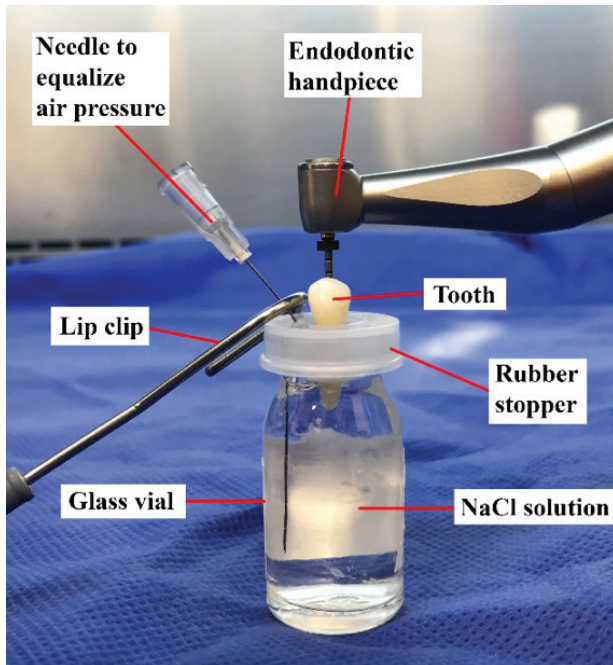
Digital periapical radiographs (Scan-X Duo, Air Techniques, New York, NY, USA) were taken in the mesiodistal and buccolingual directions, and only oval-shaped root canals were selected (long/short cross-section diameter ratios of  $\geq 2.5$ , at 5 mm from the apex) [24]. Moreover, it was determined that these teeth had single canals with mature apices and curvatures ranging between 0 and 10 degrees [25]. Teeth with calcified canals, large apical foramina, and multiple canals were excluded

from this research [26,27]. Seventy-three teeth met all the above-mentioned criteria, and 70 teeth were used for this study.

The debris and soft tissue remnants were removed from the root surfaces, and the teeth were kept in a physiological saline (0.9% NaCl; Polifarma İlaç, San. Tic. A.S., Istanbul, Turkey) solution before use. The endodontic access cavities were prepared with size 2 round diamond bur (Endo Access Bur; Dentsply Maillefer, Ballaigues, Switzerland) under profuse water cooling. Then, using a fine barbed broach (Dentsply Maillefer, Ballaigues, Switzerland), the pulp remnants were extirpated. To contaminate the root canals with *Enterococcus faecalis* (*E. faecalis*) (ATCC 29212), the access chambers were used as a reservoir [26,27].

### Test apparatus

For each tooth, an aperture (2.3 mm in diameter) was punctured through the middle of the rubber stopper of a glass vial with a heated instrument. Two layers of nail varnish were applied to all of the root surfaces to prevent bacterial microleakage. Then, each tooth was pressed into that aperture up to the level of the cemento-enamel junction. The rubber stopper was then placed in the mouth of the glass vial. A 27-gauge curved needle (Ayset, Adana, Turkey) was used to vent the vial during insertion in order to equalize the air pressure inside and outside the vial (Figure 1). While using an electronic apex locator (Root ZX; J. Morita Corp., Tokyo, Japan) to determine the electronic working length (WL), the needle was also used as an electrode [27,28]. Then, the entire system model was sterilized at 121°C in an autoclave at a pressure of 15 pounds for 20 minutes. To standardize the foramina and their patency, a sterilized size 10 K-file (Dentsply Maillefer, Ballaigues, Switzerland) was placed 1 mm beyond the foramen, and a hole was created in the nail varnish [26].



**Figure 1** - The experimental model system.

### Bacteria preparation and contamination

A pure culture of *E. faecalis* was used to contaminate the root canals. A suspension was prepared by adding 1 mL of a pure culture of *E. faecalis*, inoculated in 7 mL of brain heart infusion (BHI) broth (Difco, Detroit, MI, USA), and incubated for 24 h at 37 °C. Then, the turbidity of the suspension was adjusted to 0.5 McFarland standard to ensure that the number of bacteria was  $1.5 \times 10^8$  colony-forming units (CFU) mL<sup>-1</sup>. To standardize the size of the foramen and the apical patency in the nail varnish, a sterile size 15 K-file was placed 1 mm beyond the apical foramen [27]. A size 10 K-file with 10  $\mu$ L of the suspension was used to push the *E. faecalis* through the canals in a class I laminar airflow cabinet. Then, the teeth were incubated at 37°C for 30 days. During the incubation period, to prevent dehydration of the samples, new sterile BHI medium was used daily to maintain the biofilms in the root canals [10]. Finally, each of the vials was filled with a 0.9% NaCl solution, and four experimental groups (15 teeth in each group) and one control group (10 teeth) were created.

### Root canal preparation

All of the root canal preparations were performed in a class I laminar airflow cabinet under aseptic conditions by one operator. Using an electronic apex locator, 1 mm short of the 'apex' reading (0.0) was defined as the WL for each of the teeth. A new sterilized instrument set was used to prepare all the teeth. All four types of the instruments were used with a 6:1 reduction handpiece (Sirona, Bensheim, Germany) powered by a torque-limited electric motor (Silver Reciproc Motor; VDW, Munich, Germany) according to the manufacturer's instructions.

Since any extrusion of an antimicrobial agent into the vial could kill the extruded bacteria, 0.9% NaCl was used for irrigation because it has no antibacterial effect. Irrigation during root canal preparation was performed using a total volume of 7 mL 0.9% NaCl solution for each root canal because of the different numbers of the files used in study groups. Final irrigation was performed using 3 mL of 0.9% NaCl solution. A total of 10 mL 0.9% NaCl solution was used per canal during the experiment and applied with a 27-G side-vented tip needle (Endo-Eze; Ultradent, South Jordan, UT, USA) [10,13,26,27]. In all the groups, the root canal was irrigated and patency was confirmed using a size 10 K-file for each tooth [13,26].

### The PTU group

PTU system files were used with a gentle in-and-out motion, continuous rotary movement speed of 300 rpm, and 2 Ncm of torque. The files were used in the following sequence: SX (19.04, 1/2 of the WL), S1 (18.02, 2/3 of the WL), S2 (20.04, 2/3 of the WL), F1 (20.07, full WL), F2 (25.08, full WL), F3 (30.09, full WL), and F4 (40.06, full WL). The instruments were regularly cleaned to remove debris from the flutes. The SX file was used with a brushing outstroke motion until resistance was felt in the root canal. The file was then withdrawn, cleaned, and inspected before being reused. The root canal was irrigated and patency was



confirmed using a size 10 K-file until the SX file reached half of the WL. The same procedures were repeated with all subsequent files [13,26].

### The PTN group

PTN system files were used at 300 rpm, with 2 Ncm of torque. The files were used in the following sequence: X1 (17.04, full WL), X2 (25.06, full WL), X3 (30.07, full WL), and X4 (40.06, full WL). The X1 file was used with a brushing outstroke motion until resistance was felt in the root canal. The file was then withdrawn, cleaned, and inspected before being reused. The root canal was irrigated and patency was confirmed using a size10 K-file. These procedures were repeated until the X1 file reached the WL. The same procedures were performed with subsequent files [13].

### The WO group

WO large files (40.08) were used with gentle in-and-out pecking movements at amplitudes that did not exceed 3–4 mm. After three pecking motions, the instruments were withdrawn, then cleaned and inspected before being reused. The root canal was irrigated and patency was confirmed using a size10 K-file. Each third of the root length was instrumented with three in-and-out pecking movements. These procedures were repeated until the file reached the WL. Gentle apical pressure was combined with a brushing motion against the lateral walls of the root canal. The instruments were used with the reciprocating working motion generated by the motor [7,13].

### The R group

In the R group, the root canals were prepared using R40 (40.06) reciprocating instruments. The R files were used in the same way as the files in the WO group [7,13].

### Control group

Instrumentation was not used in this group [26].

Following the root canal preparation, 0.01 mL of the NaCl solution was taken from each of the vials in the control and experimental

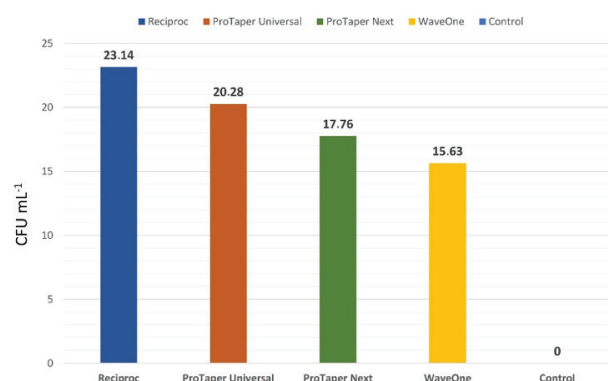
groups. This suspension was cultured on BHI agar, which was incubated at 37 °C for 24 h. Using the classical bacterial counting method, the bacterial colonies were counted after 24 h of incubation, and the results were given as a number of colony-forming units (CFUs) [26,29].

### Statistical analysis

All of the statistical tests were performed using SPSS software (SPSS Inc, Chicago, IL, USA). The data were analyzed statistically using the Kruskal-Wallis one-way analysis of variance and the Mann-Whitney U test. The comparisons were considered to be statistically significant at values of  $p < 0.05$ .

## RESULTS

The means and standard deviations of the extruded bacteria in the experimental and control groups are presented in Table I. Extrusion was not observed in the control group. The mean values in the R group were statistically significantly higher than those in the three other experimental groups ( $p < 0.05$ ). Likewise, the mean values in the PTU group were statistically significantly higher than those in the PTN and WO groups ( $p < 0.05$ ). However, the difference between the PTN and WO groups was not statistically significant ( $p > 0.05$ ) (Figure 2).



**Figure 2** - The mean values of the extruded bacteria for each group.

**Table 1** - The mean numbers and standard deviations of the extruded bacteria in all the groups.

| Groups             | N  | Mean (CFU mL <sup>-1</sup> ) | SD   |
|--------------------|----|------------------------------|------|
| Reciproc           | 15 | 23.14 <sup>a</sup>           | 3.53 |
| ProTaper Universal | 15 | 20.28 <sup>b</sup>           | 2.40 |
| ProTaper Next      | 15 | 17.76 <sup>c</sup>           | 2.95 |
| WaveOne            | 15 | 15.63 <sup>c</sup>           | 2.55 |
| Control            | 10 | 0.00 <sup>d</sup>            | 0.00 |

p < 0.05; SD: standard deviation. Values with the same letters were not statistically different.

## DISCUSSION

This study aimed to evaluate apical extrusion of intracanal bacteria during root canal instrumentation associated with two reciprocating single-file systems and two full-sequence rotary instrumentation systems in oval-shaped root canals. Therefore, R, WO, PTU, and PTN systems were used for root canal instrumentation and the apical bacterial extrusion caused by these systems was tested and compared. According to the results of this study, the R system resulted in more AEB than the other experimental systems, and the PTU system resulted in more apical extrusion than the WO and PTN systems. Therefore, the null hypothesis was rejected.

In our study, a standardized tooth model was used to increase the possibility that the number of AEB was a consequence of the instrumentation [28]. For standardization, ISO size 40 was selected as the apical diameter of the master apical files in all of the groups. Because a 0.9% NaCl solution does not exhibit any antibacterial effects, it was selected for the irrigation to ensure that the bacterial elimination and extrusion were only caused by the mechanical effects of the instruments [26,27].

It has been suggested that *E. faecalis* is resistant to intracanal medicaments [30], and it can survive without the support of other bacteria in the root canal [31]. Additionally, this bacterium has been observed at high rates in root canal failures [32]. Therefore, *E. faecalis* was the preferred bacteriological marker for our study.

The R files extruded significantly more

intracanal bacteria than all of the other files. This is in accordance with a previous study by Bürklein and Schäfer [7], who reported greater debris extrusion with the R system in comparison to the PTU and WO systems. However, Tinoco et al. [10] found no significant difference between the two reciprocating systems (R and WO) in terms of AEB. This difference might be explained by the differences in the types of teeth used in the study and the apical diameters of the master apical instruments. Tinoco et al. [10] assessed mandibular incisors (up to size 25); our study evaluated mandibular premolar teeth (up to size 40).

The R files have sharp cutting edges and an S-shaped cross-sectional design; however, PTN instruments have an off-center rectangular design and active tips [5,33]. PTU and WO instruments have a triangular or modified triangular cross-section, resulting in a smaller chip space and lower cutting efficiency [5]. An increased cutting ability may increase debris transportation toward the apex when used in combination with a reciprocal motion [7]. When comparing the two reciprocating systems, it is important to emphasize that WO instruments have a larger core mass than R instruments. This geometric feature translates to a reduced flute depth, which is related to their ability to remove debris coronally [10]. The differences in the number of bacteria extruded from apical foramen may be caused by the preparation techniques, geometric features, and/or the cross-sectional designs of these systems.

In our study, the PTU system caused more extrusion than the PTN system. In PTU and PTN systems, variable taper percentages are present on a single file, which is a common design feature between these two systems. At the apical 3 mm, the F2, F3, and F4 files of the PTU system have tapers of 0.08, 0.09, and 0.06, respectively. The X2, X3, and X4 files of the PTN system have tapers of 0.06, 0.07, and 0.06, respectively [13]. The larger tapers at the apical 3 mm of the file cause more aggressive preparation of the root canals during endodontic treatment, which may explain the greater bacterial extrusion associated with the PTU system in comparison to the PTN system. Additionally, the number of files required

for treatment is greater in the PTU system (seven) than the PTN system (four files), which may be another reason for the bacterial extrusion differences between these two systems [13]. The results of our study are in agreement with the findings in previous studies, which reported that the PTU system caused greater extrusion than the PTN system [13,14,34,35].

In our study, the PTU system caused more extrusion than the WO files. The rotary file's continuous forward motion facilitates exit of debris up the flute of the file. However, the reciprocating file's backward motion might cause build-up of the debris in the protrusions and isthmus areas [36,37]. Robinson et al. [38] compared the cleaning efficacy of rotary (PTU) and reciprocating files (WO). They suggested that reciprocating files (WO) build up debris into isthmus and protrusions because their backward motion causes a burnishing-type effect. This could partially explain the difference between these two groups in terms of why the WO instruments apically extruded less bacteria. Additionally, similar results were obtained by other studies that observed that the PTU system caused more extrusion than the WO system [13,35,39].

In our study, there was no significant difference in the amount of AEB between the PTN and WO systems. Silva et al. [13] and Ozsu et al. [35] also reported that the difference between the PTN and WO systems was not statistically significant in terms of the apical debris extrusion. Our results are in accordance with the results of these studies.

In our study 0.9% NaCl solution was used for irrigation instead of sodium hypochlorite in order to maintain the vitality of the bacteria to determine the amount of AEB. In this way, it was ensured that the extrusion of the bacteria depended on the mechanical action of the instruments [27]. If an antibacterial irrigation solution is used during root canal treatments, the number of viable bacteria may be reduced, and different results could be obtained.

The results reported in our study may be different than those obtained in a clinical situation.

Because the periapical tissues act as a natural barrier during root canal treatment in clinical situations, the bacterial extrusion may be limited.

## CONCLUSIONS

Within the limitations of our study, all the instrumentation techniques, either the reciprocating single-file or rotary-file systems, caused apical intracanal bacterial extrusion during root canal instrumentation in oval-shaped root canals. The PTN and WO systems were associated with less apical bacterial extrusion than the R and PTU systems. The root canal preparation techniques and cross-sectional designs of the instruments are related to the amount of AEB.

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