Clinical Comparison of the Effectiveness of Single-file Reciprocating Systems and Rotary Systems for Removal of Endotoxins and Cultivable Bacteria from Primarily Infected Root Canals

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Abstract

Introduction: This clinical study was conducted to compare the effectiveness of single-file reciprocating systems and rotary systems in removing endotoxins and cultivable bacteria from primarily infected root canals. **Methods:** Forty-eight primarily infected root canals were selected and randomly divided into 4 groups: WaveOne (Dentsply Maillefer, Ballaigues, Switzerland) (n = 12); Reciproc (VDW, Munich, Germany) (n = 12), ProTaper (Dentsply Maillefer) (n = 12), and Mtwo (VDW) (n = 12). Samples were collected before and after chemomechanical preparation. The irrigation was performed by using 2.5% sodium hypochlorite. A chromogenic limulus amebocyte lysate assay test was used to quantify endotoxins. Culture techniques were used to determine bacterial colony-forming unit counts. **Results:** In the baseline samples (ie, samples collected before chemomechanical preparation), endotoxins and cultivable bacteria were recovered from 100% of the root canal samples. No differences were found in the median percentage values of endotoxin reduction achieved with reciprocating systems (ie, WaveOne [95.15%] and Reciproc [96.21%]) and with rotary systems (ie, ProTaper [97.98%] and Mtwo [96.34%]) ($P < .05$). Both single-file reciprocating systems (ie, WaveOne [99.45%] and Reciproc [99.93%]) and rotary systems (ProTaper [99.85%] and Mtwo [99.41%]) were effective in reducing the cultivable bacteria (all $P < .05$). Moreover, the culture analysis revealed no differences in bacterial load reduction ($P > .05$). **Conclusions:** Both single-file reciprocating systems (ie, WaveOne and Reciproc instruments) and rotary systems (ie, ProTaper and Mtwo instruments) showed similar effectiveness in reducing endotoxins and cultivable bacteria from primarily infected root canals, but they were not able to eliminate them from all root canals analyzed. *(J Endod 2014;40:625–629)*

Key Words

Bacteria, disinfection, endodontics, endotoxin, root canal

One of the main goals of root canal treatment is to reduce the amount of bacteria as well as their byproducts, all contributing to the perpetuation of apical periodontitis (1–3). Lipopolysaccharides, one of the most important byproducts present on the outer layer of the membrane of gram-negative bacterial species (4–6), have been detected in 100% of the root canals with primary endodontic infection (1, 7, 8) with high levels closely related to severe inflammatory responses (7–10).

Although practitioners commonly use manual instrumentation, the use of nickel-titanium (NiTi) rotary files has become a standard technique because of their more rapid procedures (2, 3, 11, 12), more centered preparations (11–13), and less apical extrusion of debris (14, 15). Although ProTaper (Dentsply Maillefer, Ballaigues, Switzerland) and Mtwo (VDW, Munich, Germany) rotary systems have provided significant bacterial/endotoxin reductions (1, 3, 16, 17), no instrument can optimally make root canal systems free of bacteria (16, 18–21) and endotoxins (1, 7, 10, 22).

A new concept has recently proposed the use of a single-file system to shape the root canal completely from start to finish (2, 3, 23, 24), particularly the Reciproc (VDW) and WaveOne (Dentsply Maillefer) systems, which are 2 M-wire reciprocating systems (24). However, evidence on their cleaning and disinfecting abilities is only incipient.

Previous *in vitro* studies have evaluated the ability of single-file systems in shaping root canals regarding anatomy preservation (25), debris removal (26), apical extrusion of debris (27), cyclic fatigue resistance (23, 27, 28), cleaning effectiveness (24), and bacterial reduction/elimination (2, 3). However, no clinical study has compared the effectiveness of single-file reciprocating systems and rotary instrumentation in removing endotoxins from primarily infected root canals. Therefore, this clinical study was conducted to compare the effectiveness of single-file reciprocating systems

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and rotary systems in removing endotoxins and cultivable bacteria from primarily infected root canals.

Materials and Methods

Forty-eight patients requiring primary endodontic treatment were included in the present study. A detailed dental history was obtained from each patient. Those who had received antibiotic treatment during the last 3 months or who had any general disease were excluded. The Human Research Ethics Committee of the São José dos Campos Dental School approved the research protocol describing the sample collection for this investigation, and all volunteer patients signed an informed consent form.

All the selected teeth were single rooted with a primary endodontic infection showing the presence of 1 root canal and the absence of periodontal pockets deeper than 4 mm. None of the patients reported spontaneous pain. Teeth that could not be isolated with a rubber dam were excluded. The following clinical/radiographic features were found in root canals with primary endodontic infections investigated: pain on palpation (12/48), tenderness to percussion (16/48), and a radiolucent area greater than 3 mm in size (36/48).

Files, instruments, and all materials used in this study were treated with Co^{60} gamma radiation (20 kGy for 6 hours) for sterilization and the elimination of pre-existing endotoxins (EMBRARAD; Empresa Brasileira de Radiação, Cotia, SP, Brazil). The method used for disinfection of the operative field was previously described elsewhere (1, 7). Briefly, the teeth were isolated with a rubber dam. The crown and surrounding structures were disinfected with 30% hydrogen peroxide (volume/volume for 30 seconds) followed by 2.5% sodium hypochlorite (NaOCl) for the same period of time and then inactivated with 5% sodium thiosulfate. The sterility of the external surfaces of the crown was checked by taking a swab sample from the crown surface and streaking it onto blood agar plates, which were then incubated both aerobically and anaerobically.

A 2-stage access cavity preparation was made without the use of water spray but under manual irrigation with sterile/apyrogenic saline solution and using a sterile/apyrogenic high-speed diamond bur. The first stage was performed to promote a major removal of contaminants, including carious lesions and restoration. In the second stage, before entering the pulp chamber, the access cavity was disinfected according to the protocol described previously. Sterility of the internal surface of the access cavity was checked as previously described, and all procedures were performed aseptically. The first endotoxin sampling was taken by introducing sterile/apyrogenic paper points (size #15, Dentsply Maillefer) into the full length of the canal, which was determined radiographically and retained in position for 60 seconds for sampling. Immediately afterward, the sample was placed in a pyrogen-free glass and immediately suspended in 1 mL limulus amebocyte lysate (LAL) water according to the endotoxin dosage by using a kinetic chromogenic LAL (Lonza, Walkersville, MD) assay. This sampling procedure was repeated with 3 paper points that were pooled in a sterile tube containing 1 mL Viability Medium Göteborg Agar III (VMGA III) transport medium (29) for microbial cultivation.

After accessing the pulp chamber and subsequent first endotoxin sampling, teeth were randomly divided into 4 groups: WaveOne (n = 12), Reciproc (n = 12), ProTaper (n = 12), and Mtwo (n = 12). After the first sampling, the root canal length was determined from the preoperative radiograph and confirmed using an apex locator (Novapex; Forum Technologies, Rishon le-Zion, Israel). The root canals were then prepared according to the group selection.

All instruments were set into permanent rotation with a 6:1 contra-angle handpiece (Sirona, Bensheim, Germany) powered by a torque-limited electric motor (VDW.Silver Reciproc motor, VDW). For each Mtwo and ProTaper file, individual torque limit and rotational speed programmed in the file library of the motor were used, whereas Reciproc and WaveOne were used in a reciprocating working motion generated by the motor. The preparation sequences were as follows.

Group WaveOne

The WaveOne instruments were used according to the manufacturer's instructions. A size #25 WaveOne file with a 0.08 taper (Dentsply Maillefer) was used in a reciprocating motion. The instrument was used in an in-an-out pecking motion of about 3 mm in amplitude with apical pressure. After 3 pecking motions, the instrument was removed from the canal and cleaned. Next, a size #15 K-type file was taken to the working length (WL) to check whether the canal was patent. These procedures were repeated until the WaveOne instrument reached the WL (−1 mm).

Group Reciproc

The Reciproc R40 instruments were used according to the manufacturer's instructions. The Reciproc R40 instrument was introduced into the canal until resistance was felt and then activated in a reciprocating motion. The instrument was used in an in-an-out pecking motion of about 3 mm in amplitude with apical pressure. After 3 pecking motions, the instrument was removed from the canal and cleaned. Next, a size #15 K-type file was taken to the WL (−1 mm) to check whether the canal was patent. These procedures were repeated until the Reciproc instrument reached the WL.

Group ProTaper

ProTaper instruments were used according to the manufacturer's instructions in a gentle in-and-out motion. Afterward, the instrument was removed from the canal and cleaned. Next, a size #15 K-type file was taken to the WL (−1 mm) to check whether the canal was patent. The instrumentation sequence was as follows: SX instrument at two thirds of the WL, S1 instrument at the WL (−1 mm) (taper = 0.02–0.11, size #17), S2 instrument at the WL (−1 mm) (taper = 0.04–0.115, size #20), F1 at the WL (−1 mm) (taper = 0.055–0.07, size #20), F2 instrument at the WL (−1 mm) (taper = 0.055–0.08, size #25), and F3 instrument at the WL (taper = 0.05–0.09, size #30).

Group Mtwo

All Mtwo instruments were used to the full length of the canals (single length technique) according to the manufacturer's instructions in a gentle in-and-out motion. Next, the instrument was removed from the canal and cleaned. Next, a size #15 K-type file was taken to the WL (−1 mm) to check whether the canal was patent. The instrumentation sequence was as follows: a 0.04 taper size 10 instrument, a 0.05 taper size #15 instrument, a 0.06 taper size #20 instrument, a 0.06 taper size #25 instrument, and a 0.05 taper size #30 instrument.

Irrigation was performed with disposable syringes and 30-G NaviTip needles (Ultradent, South Jordan, UT) by using 5 mL 2.5% NaOCl solution between the pecking sequences (groups 1 and 2) and between files (groups 3 and 4). Before the second sampling after instrumentation, NaOCl was inactivated with 5 mL sterile 0.5% sodium thiosulfate during a 1-minute period, which was then removed with 5 mL sterile/apyrogenic water.

Before the second sampling (s2) after instrumentation, NaOCl was inactivated with 5 mL sterile 0.5% sodium thiosulfate during a 1-minute period, which was then removed with 5 mL sterile/apyrogenic water. Next, a new sampling procedure was performed as described previously at s1.

Determination of Endotoxin Concentration (Kinetic Chromogenic LAL Assay)

The kinetic chromogenic LAL assay (Lonza) used for quantification of endotoxins was previously reported by the author (7). Briefly, for the test, 100 mL apyrogenic water (reaction blank), the 5 standard endotoxin solutions (0.005–50 endotoxin units [EUs]/mL), root canal samples, and positive controls (each root canal sample contaminated with a known concentration of endotoxin [10 EUs/mL]) were added to a 96-well apyrogenic plate. The tests were performed in quadruplicate. The plate was incubated at 37°C ± 1°C for 10 minutes in a Kinetic-Quantitative Chromogenic Lysate (KQCL) (Lonza) reader, which was coupled to a microcomputer by means of the WinKQCL software. Next, 100 mL chromogenic reagent was added to each well. After the beginning of the kinetic test, the software continuously monitored absorbance at 405 nm in each microplate well and automatically calculated the log/log linear correlation between the reaction time of each standard solution and the corresponding endotoxin concentration.

Determination of Cultivable Bacterial Counts (Culturing Procedure)

The method used for culture procedures in the present study was previously reported by the author (7, 10). Briefly, the transport media containing the root canal samples were thoroughly shaken for 60 seconds (Vortex; Marconi, Piracicaba, São Paulo, Brazil). Serial 10-fold dilutions were made up to 10^{-4} in tubes containing fastidious anaerobe broth (FAB; Lab M, Bury, UK). Fifty microliters of the serial dilutions were plated onto 5% defibrinated sheep blood fastidious anaerobe agar (FAA, Lab M) by using sterile plastic spreaders to culture nonselectively obligate anaerobes and facultative anaerobes. The plates were incubated at 37°C in anaerobic atmosphere for up to 14 days. After this period, colony-forming units (CFUs) were visually quantified for each plate.

Statistical Analysis

The data collected (CFUs and endotoxin concentrations) were statistically analyzed by using SPSS for Windows (SPSS Inc, Chicago, IL). The Kolmogorov-Smirnov test showed that the distributions of the studied variables deviated from the normality. The Wilcoxon test was used when significant differences were found between different sampling times. A comparison between the root canal treatment groups (ie, WaveOne, Reciproc, ProTaper, and Mtwo) was performed by using the Kruskal-Wallis test. The significance level was always set at 5% ($P < .05$).

Results

The sterilized samples taken from external and internal surfaces of the crown and its surrounding structures were tested before and after entering the pulp chamber, showing no microbial growth and endotoxin. Table 1 provides an overview of the endotoxin concentrations (EUs/mL) and the amount of cultivable bacteria (CFUs/mL). The percentage values of bacterial and endotoxin reductions found in all groups tested at different sampling times are shown in Figure 1. The standard curve for the detection of endotoxins fulfilled the criteria of linearity (r = 1). Endotoxins were detected in 100% (48/48) of the root canal samples collected before (s1) and after root canal instrumentation (s2). No differences were found in the median percentage values of endotoxin reduction achieved between single-file reciprocating systems (WaveOne [95.15%] and Reciproc [96.21%]) and rotary systems (ProTaper [97.98%] and Mtwo [96.34%], $P < .05$). In the baseline samples (s1), cultivable bacteria were recovered from 100% of the root canals tested (48/48). Both single-file reciprocating systems (WaveOne [99.45%] and Reciproc [99.93%]) and rotary systems (ProTaper [99.85%] and Mtwo [99.41%]) showed similar effectiveness in reducing bacterial load (all $P < .05$) (Fig. 1). At s2, bacterial negative culture was detected in 4 of 12 (33%) for WaveOne, 5 of 12 (42%) for Reciproc, 4 of 12 (33%) for ProTaper, and 6 of 12 (50%) for Mtwo. Overall, the culture analysis revealed no differences in the median percentage values of bacterial reduction found in single-file reciprocating systems and rotary systems ($P > .05$).

Discussion

The results obtained from the present study have revealed that both single-file reciprocating systems and rotary systems are effective in reducing endotoxins and bacterial load from primarily infected root canals with no statistical differences between them. However, both single-file reciprocating systems and rotary systems were not able to eliminate them completely.

It is known that primary endodontic disease is a polymicrobial infection holding different gram-negative bacterial species (30). These species, present on the outer layers of the membrane, have a cell constituent called lipopolysaccharides, also known as endotoxins, which can exert a potent inflammatory effect against periapical tissues (6) when released during disintegration, multiplication, or death of bacteria (6). Because endotoxins can egress into periapical tissue, contributing to the initiation/perpetuation of an inflammatory process, the main goal of endodontic treatment should not only rely on the bacterial elimination but also on the reduction/elimination of endotoxins.

In the present study, the bacterial culture analysis revealed the presence of bacteria in all initial samples, with the number of CFU values per canal ranging from 1.1×10^3 to 6.1×10^7, which is comparable with previous studies (7, 10, 22, 31).

The kinetic chromogenic LAL assay indicated the presence of endotoxins in all root canal samples analyzed, with a median value ranging from 103 to 126.5 EUs/mL, which supports its role in endodontic

TABLE 1. Effectiveness of Single-file Reciprocating Systems and Rotary Systems for the Removal of Endotoxins (EUs/mL) and Cultivable Bacteria (CFUs/mL) from Primarily Infected Root Canals

	Endotoxins (EUs/mL)		Cultivable bacteria (CFUs/mL)	
Instrumentation groups	Before treatment (s1)	After treatment (s2)	Before treatment (s1)	After treatment (s2)
WaveOne	112 (7.96–394)	1.79 (0–6.37)	1.4×10^5 (1.2×10^3–2.3×10^7)	2.3×10^2 (0–3.1×10^2)
Reciproc	126.5 (9.64–298)	2.06 (0–5.29)	1.7×10^5 (1.1×10^3–6.1×10^7)	1.2×10^2 (0–2.1×10^2)
ProTaper	103 (4.73–235)	1.63 (0–7.98)	1.6×10^5 (1.5×10^3–7.2×10^6)	3.4×10^2 (0–4.1×10^2)
MTwo	117 (15.3–303)	2.37 (0–15.1)	1.5×10^7 (6.2×10^3–4.3×10^7)	8.4×10^2 (0–9.6×10^2)

CFUs, colony-forming units; EUs, endotoxin units.

Median and range values of endotoxins and cultivable bacteria found in primarily infected root canals.

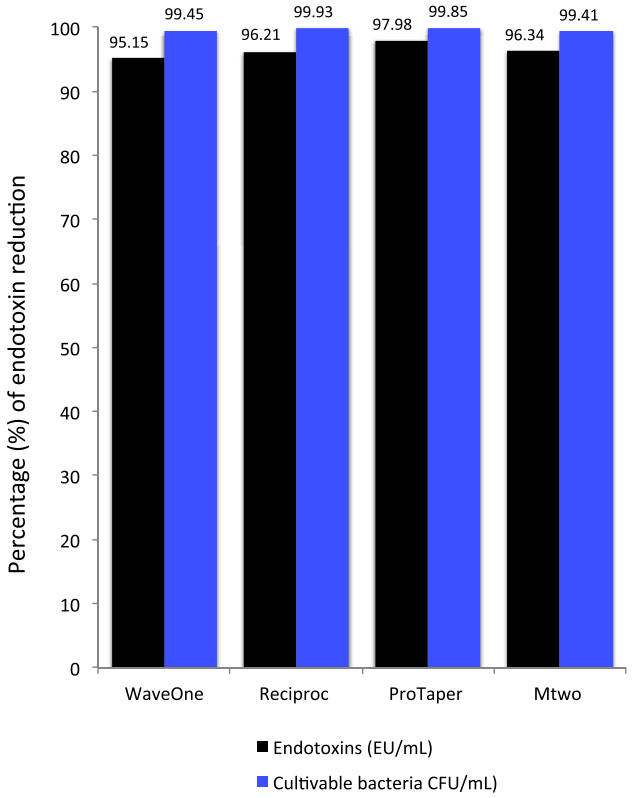

Figure 1. Distribution of the percentage values of endotoxins and cultivable bacteria reduction found in primarily infected root canals after chemomechanical preparation using single-file systems and rotary systems.

infection (1, 7, 10). These values are likely the median values encountered by previous studies using the kinetic chromogenic LAL test (7, 32) but inconsistent with others using the endpoint-Quantitative Chromogenic Lysate (QCL) test (10, 33). According to Martinho et al (32), the kinetic chromogenic LAL test used in the present study is one of the LAL tests that best fits for the analysis of endotoxins in root canal infection; it is more precise and allows better reproducibility compared with the endpoint-QCL assay.

Single-file techniques have been used for root canal preparation because of its convenience; a single file is required to shape the root canal completely from start to finish, requiring a shorter period of time to prepare curved canals. New instruments were launched based on opinion and convenience rather than proven effectiveness (2, 3, 23, 24). However, up to now, evidence on their cleaning and disinfecting abilities is limited to *in vitro* studies (2, 3, 34) but is not reported in clinical practice. Hence, the present study compared the disinfection ability of these single files in disinfecting primarily infected root canals to well-known rotary full-sequence NiTi systems (ProTaper and Mtwo rotary files) of the same manufacturers.

The Reciproc and WaveOne files used in the present study are made of a special NiTi-alloy called M-Wire, created by means of an innovative thermal treatment process (35). The benefits of this M-Wire alloy are increased flexibility and improved resistance to cyclic fatigue of the instruments (36). The Reciproc and WaveOne files are used in a reciprocal motion, which requires special automated devices.

Currently, the culture analysis revealed that both single-file reciprocating instrumentation (WaveOne [99.45%] and Reciproc R25 file [99.93%]) and rotary instrumentation techniques (ProTaper [99.85%] and Mtwo [99.41%]) showed a similar effectiveness in reducing bacterial load. These results were corroborated by Machado et al (16), who compared *in vitro* the reciprocating and rotary systems by a culture-dependent method and reported a bacterial reduction of more than 94%, irrespective of the file system tested. Alves et al (2) showed, by an *in vitro* study using either quantitative polymerase chain reaction or culture, a highly significant reduction in intracanal bacterial counts by using single-file techniques.

Regarding the endotoxin reduction, the data obtained in the present study indicated that both single-file reciprocating systems and rotary systems were effective in reducing more than 95% of endotoxin contents from primarily infected root canals found in the baseline samples with no statistical differences between them. Particularly, the use of Mtwo files together with irrigation with 2.5% NaOCl was able to achieve an endotoxin reduction of 96.34%. Likewise, Martinho et al (1) reported an effectiveness of 98.06% in the endotoxin reduction when using Mtwo files associated with 2.5% NaOCl. Up to now, it is worth to point out that no previous study had evaluated the effectiveness of single-file reciprocating files, particularly WaveOne and Reciproc R25, in reducing endotoxins present in root canal infection.

Regardless of the instrumentation technique selected, the data obtained in the present study indicated that bacteria were still detected in 33%–50% of the root canals analyzed. The limited ability of root canal procedures in eliminating bacteria has been reported by previous studies in which cultivable bacteria and endotoxins could still be recovered in approximately 20%–60% of the samples (2, 10, 17, 31). Moreover, our results indicated that endotoxins were detected in 100% of the root canal samples, which is in agreement with previous investigations (1, 7, 10, 32).

It is important to highlight that some differences can be found between the file systems selected. This has long been discussed, and controversy exists on whether the size of apical enlargement can significantly influence the outcome of root canal disinfection (3, 24). It is worth to point out that the reciprocating instruments selected (WaveOne and Reciproc R25 files) have a tip diameter equivalent to a #25 K-type file. Conversely, in the Mtwo and ProTaper groups, the final instruments used for canal preparation had a tip diameter of up to #30 K-type file (Mtwo size #30 with a 0.05 taper and ProTaper F3). In addition, the WaveOne file 25.08 has a continuously decreasing taper from the tip to the shaft (0.0. 0.65, 0.60, 0.55) characterized by different cross-sectional designs over the entire length of the file (at the tip region where the cross-section presents radial lands), whereas in the middle part and near the shaft, the cross-sectional design changes from a modified triangular convex cross-section with radial lands to a neutral rake angle with a triangular convex cross-section analogue to the ProTaper F2 file near the shaft (24). In contrast, the Reciproc files have a continuous taper along the first 3 mm of their working part followed by a decreasing taper to the shaft; the S-shaped cross-section is used along the working part of the instrument (24).

Despite the different apical preparation diameter of the systems tested as well as the differences in the design features of the file systems evaluated in the present study, our results showed no differences in the median percentage values of bacterial endotoxin reduction after analysis of the culture and endotoxin content. Moreover, none of the file systems tested was effective in completely eliminating bacteria and endotoxins from all root canals. Therefore, it is not unreasonable to assume that the negligible difference found between Reciproc/WaveOne and Mtwo/ProTaper instruments regarding the apical preparation diameter, including their design features, did not affect the outcome of the root canal bacterial and endotoxin disinfection. Such findings are in agreement with previous *in vitro* studies that showed no statistical significant differences when the effectiveness of different instrumentation systems was compared (2, 3, 17).

Overall, the present investigation showed that although in the groups of WaveOne and Reciproc systems only 1 file was fully used for root canal preparation, there was no impact on the root canal disinfection, particularly against endotoxins as well as on cultivable bacteria present in primarily infected root canals compared with full-sequence ProTaper and Mtwo systems. In conclusion, this clinical study showed that both single-file reciprocating systems (WaveOne and Reciproc instruments) and rotary systems (ProTaper and Mtwo instruments) showed similar effectiveness in reducing endotoxins and cultivable bacteria from primarily infected root canals, but they were not able to eliminate them from all the root canals analyzed.

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The authors deny any conflicts of interest related to this study.

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