Does adding an instrument after root preparation with Reciproc® R25 increase bacterial reduction?

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Abstract

Aim: The aim of this study was to compare the bacterial reduction achieved with reciprocating instruments such as Reciproc® R25 (VDW GMBH, Munich, Germany) and Reciproc® R25 combined with rotary file Mtwo 40.04 (VDW GMBH) during root canal preparation.

Materials and Methods: Fifty mesiobuccal root canals of maxillary molars were contaminated with Enterococcus faecalis broth culture and then an initial bacterial sample was collected from the root canal with paper cones and plated on brain–heart infusion agar. The root canals were divided into four groups as follows: Reciproc® R25 (n = 20) and Reciproc® R25 combined with Mtwo 40.04 instrument (n = 20). The negative controls consisted of five uncontaminated root canals and the positive control consisted of five contaminated roots that were not subjected to any decontamination procedure. Irrigation was performed using sodium chloride. After instrumentation, samples were collected with paper cones and the rate of bacterial reduction was calculated. Microbiological testing (colony-forming units [CFUs]) was performed to quantify the decontamination obtained by the proposed protocols. Statistical analysis was performed by paired t-test and analysis of variance test.

Results: Both techniques significantly reduced the number of bacteria in the root canal (P < 0.05), with no significant difference among them (P > 0.05).

Conclusion: The addition of the instrument Mtwo 40.04 after the root preparation with the instrument R25 does not improve its decontamination capability.

Keywords: Apical enlargement; bacterial reduction; reciprocating motion; root canal preparation; rotary instruments

INTRODUCTION

Root canal instrumentation is considered one of the most important stages during root canal treatment. The purpose of this step is to remove the infected soft and hard tissues from the root canal to create access for the delivery of irrigating solutions and medications, thus promoting an adequate disinfection of root canals. Traditionally, canals are enlarged and shaped using a series of instruments with increasing tip diameters. Although practitioners commonly use manual instrumentation, rotary multi-instruments of nickel-titanium (NiTi) have become widely available in the recent years, mainly because of their superelasticity, which facilitates efficiency and reduces iatrogenicity during canal preparation.

The Reciproc® system (VDW, Munich, Germany) is designed to complete root canal preparation with only one standardized instrument. These files are made up of a special NiTi-alloy called M-Wire that is created by an innovative thermal treatment process. The benefits of this M-Wire NiTi are increased flexibility of the instruments and improved resistance to cyclic fatigue along the canal preparation. These files are used in a reciprocal motion and are available in different sizes: 25, taper 08; 40, taper 06; and 50, taper 05. It has been shown that
instruments subjected to reciprocation have increased resistance to fatigue and longer useful life when compared with instruments used in continuous rotation motion.\textsuperscript{8,9} However, evidence of their cleaning and disinfecting abilities is incipient.

Although no instrumentation technique ensures the elimination of all debris and bacteria, especially the apical third, it has been shown that the ability to eliminate bacteria from the root canal is proportional to the number of instruments used, i.e., the larger the number of instruments used, the greater the decontamination of the root canal.\textsuperscript{[10-12]} Thus, there is still controversy regarding the optimal size of apical root canal enlargement to maximize cleaning efficiency of the root canal. It has been recommended that the root canal be enlarged to at least three sizes beyond the initial file to bind to the apical third of the canal.\textsuperscript{[13]} Khademi \textit{et al}.\textsuperscript{[14]} concluded that the minimum instrumentation size needed for the penetration of irrigants to the apical third of the root canal was a \#30 file. For mesiobuccal root canals of maxillary molars, the use of a 0.25 mm R25 instrument is indicated.\textsuperscript{[15]} Therefore, the requirement of an enlargement of the apical third of three instruments above the anatomic diameter for one adequate cleaning and shaping would not be achieved with an R25 instrument.

Therefore, the aim of this study was to compare the bacterial reduction achieved with reciprocating instruments such as Reciproc\textsuperscript{®} R25 (VDW GMBH, Munich, Germany) and Reciproc\textsuperscript{®} R25 combined with rotary file Mtwo 40.04 (VDW GMBH) during root canal preparation. The null hypothesis tested was that using an additional instrument after root canal preparation with an R25 increases bacterial reduction.

\textbf{MATERIALS AND METHODS}

\textbf{Specimen preparation}

Approval for this study was obtained from the local Ethical Committee (protocol no. 570.397). Fifty mesiobuccal root canals of maxillary molars were used. The teeth were stored in 0.9% saline solution until use. Preoperative radiographs were taken to ensure that the teeth did not have fractures, lateral canals, or calcifications. None of the teeth had received prior root canal treatment. The crowns were sectioned transversely at the level of the cementoenamel junction using a water-cooled diamond disc. The root length was standardized to 13 mm. Apical patency was determined by inserting a size 08 K-file (Dentsply Maillefer, Ballaigues, Switzerland) into the root canal until the tip was visible at the apical foramen. Only narrow canals with initial apical diameters no longer than a size 10 K-file (Dentsply Maillefer) were included. All specimens were instrumented with files \#10 and \#15 calibrated 1 mm below the length of work under irrigation with distilled water. The root canals were filled with 17% ethylenediaminetetraacetic acid (EDTA) for 3 min to remove the smear layer and washed with 5 mL distilled water.

Cyanoacrylate was then applied to the external surface of the roots and the root apex was sealed with composite resin (3M ESPE, St. Paul, MN, USA). Upon completion of this phase, 50 microtubes (Odeme, Joaçaba, Santa Catarina, Brazil) were filled with laboratory silicone Zetalabor (Zhermack Spa, Badia Polynesia, Rovigo, Italy), with the roots oriented in the cervical portion upward in the silicone. All the samples were sterilized in an autoclave for 30 min at 120°C. At the end of the sterilization, the roots were set in a microbial rack to begin contamination.

\textbf{Control of sterilization}

One tooth from each group was randomly selected and subjected to sterilization control. A sterile paper point was placed in contact with the root canal walls for 15 s and individually transported to a plastic microtube containing 1 mL 0.9% saline solution (Basso, Caxias do Sul, RS, Brazil). The material was homogenized, and an aliquot of 100 μL saline solution was cultivated on blood agar after 5 min. The samples were incubated at 37°C for 48 h in order to verify bacterial growth. Those samples that were selected for control of sterilization did not show any bacterial growth and were discarded; the remaining sixty teeth were randomly distributed between six boxes.

\textbf{Contamination of samples}

For this experiment, \textit{Enterococcus faecalis} (American Type Collection 19433), obtained and prepared at the Microbiology Laboratory of Biological Sciences Institute at University of Passo Fundo, was used. The bacteria were cultivated on brain–heart infusion broth (BHI) for 24 h at 37°C in microbiological incubators.

Forty-five samples were contaminated with 100 μl of \textit{E. faecalis}. The negative controls consisted of five uncontaminated root canals.

In each of the 45 previously sterilized samples, 100 mL \textit{E. faecalis} culture was inoculated into the root canal. After this procedure, the sterile BHI was added to the root canal so that it was completely filled with the culture medium. The \textit{E. faecalis} culture was maintained for 30 days in order to promote bacterial growth, with the BHI being renewed every 48 h. All procedures were performed under aseptic conditions in a laminar flow hood. Once a week, an aliquot of BHI from a randomly selected tooth from each group was subjected to Gram staining and cultured on blood agar followed by catalase and esculin tests to verify the absence of contamination with other microorganisms.
Experimental groups
After contamination, the roots were divided randomly into two experimental groups, according to the instrumentation technique used, as follows:

- Negative control (n = 5): sterilized roots that were not contaminated
- Positive control (n = 5): contaminated roots that were not subjected to any disinfection procedure
- Reciproc® R25 (tip size 25, 0.08 taper) (VDW) (n = 20): The file was gently inserted into the cervical third with an in-and-out pecking motion of low amplitude. After three in-and-out movements, when more pressure was needed to make the instrument advance further into the canal, the file was removed to clean the flutes. The canal was then copiously irrigated with 2 mL of sodium chloride. After, the file was reused in the same manner along the middle third followed by irrigation. This protocol was repeated until the length work was reached by the R25 instrument. Finally, the file was inserted up to the full extension of the root canal with a brushing motion against the lateral walls of the root canal and a final flush with 2 mL of sodium chloride was performed. The file was activated in a reciprocating movement for an electric engine Reciproc® Silver (VDW GMBH) using the settings predefined by the manufacturer.
- Reciproc® R25 + Mtwo tip size 40 and 0.04 taper (VDW GMBH) (n = 20): the preparation of the canals with R25 was performed in an identical manner to the previous group. However, the Mtwo 40.04 rotary file was added to the preparation with torque and speed predefined by the manufacturer. A brushing action was performed against the walls. The irrigation procedure was similar to the one used on the previous group.

Irrigation during instrumentation was performed with 10 mL of sodium chloride using a syringe and 27-G needle NaviTip (Ultradent Products, South Jordan, UT), which was inserted into the root canal without binding, using an in-and-out motion for better flow. One milliliter of EDTA 17% was used for 1 min to remove the smear layer and after 1 min, the final irrigation was performed with 1 mL of sodium chloride.

Microbiological analysis
Before mechanical-chemical preparation was performed, the root canal was filled with sterile saline solution and this solution was agitated with a #15 file (Dentsply Mailiefer) for 30 s to promote contact of the solution with the walls of the accessory canal. Then, a #15 sterile absorbent paper cone was inserted and shaken against the canal walls for 30 s and transferred to a microtube containing 1000 μL of sterile saline solution at a concentration of 0.9%. This solution was homogenized and serial dilutions were performed up to $10^3$. A 75 μL volume of the solution and its serial dilutions were used for seeding. The drop technique was performed, whereby five drops of 15 μL of each concentration were placed into Petri plates containing plate count agar and incubated for 48 h. After the incubation period, colony-forming units (UFCs) were counted to evaluate initial contamination. To measure decontamination protocol efficacy, the same procedures were performed, with only one alteration: instead of the #15 cone, the #25 cone was used in the R25 group and the #40 cone was used in the Reciproc® R25 combined with the Mtwo 40.04 instrument group.

Counting of each Petri plate was performed three times by the same previously trained observer. A second observer performed the same procedure to confirm the UFCs counts.

Statistical analysis
Each bacterial count was log-transformed for statistical analysis. The paired t-test was used for intragroup analysis, and analysis of variance was used for intergroup analysis. The statistical significance level was established at $P < 0.05$ and data were analyzed using StatPlus AnalystSoft Inc., version 6.0 (Vancouver, BC, Canada).

RESULTS

The negative control did not show any bacterial growth, which indicated that aseptic conditions were maintained during the experiment. The positive control showed one contamination of $4.0 \times 10^3 \pm 3.5 \times 10^3$ [Table 1]. Both techniques significantly reduced the number of bacteria in the root canal ($P < 0.05$), with no significant difference among them ($P > 0.05$). However, neither one was able to promote a complete decontamination of the root canal system.

DISCUSSION

The elimination of microorganisms from the root canal is the main goal of endodontic therapy because their presence is related to the failure rate.[16] In particular, *E. faecalis* has been the focus of endodontic research because of its high resistance to root disinfection procedures,[17] and a low amount of nutrients is sufficient for their viability.[18] In the present study, root preparation with Reciproc® R25 and Reciproc® R25 combined with rotary file Mtwo 40.04 exhibited similar bacterial elimination. Therefore, from a microbiological perspective, the use of one file after the use of R25 did not improve root canal

| Groups | Before preparation | After preparation | Bacterial reduction (%) |
|--------|-------------------|------------------|------------------------|
| R25    | $3.9 \times 10^3 \pm 3.3 \times 10^3$ | $6.6 \times 10^3 \pm 6 \times 10^3$ | 59 |
| R25 + Mtwo | $3.7 \times 10^3 \pm 2.7 \times 10^3$ | $4.1 \times 10^3 \pm 2.3 \times 10^3$ | 74 |
| 40.04  |                   |                  |                        |

UFCs: Colony-forming units
decontamination. Thus, the null hypothesis tested was accepted. Our results are in agreement with other studies that showed that the use of different tip sizes results in similar bacterial counts.\[5,19,20\]

The Reciproc\textsuperscript{®} R25 prepares the root canal to size 25 at the tip with a taper of 0.08 in the first apical millimeters. This elevated taper combined with a pecking motion can create a canal preparation larger than the instrument’s tip size. In this study, the Mtwo instrument used after Reciproc\textsuperscript{®} R25 was of size 40 with a 0.04 taper. Although Mtwo instruments present a double-cutting edge with S-shaped geometry and the nearly identical Reciproc\textsuperscript{®} file may have similar properties,\[9\] this instrument with cutting tip 40 did not significantly increase bacterial reduction after the use of Reciproc\textsuperscript{®} R25. Jeon et al.\[21\] conclude that although the two reciprocating file systems studied did not show statistically significant differences in apical preparation size, a greater number of pecking times at the working length may result in an apical size that is larger than the actual file size. Therefore, clinicians must be careful during the main cone selection and analyze their fit before obturation.

NiTi instruments have reduced the clinical incidences of iatrogenic preparation and have reduced the root preparation time compared with hand instruments. However, these instruments include disadvantages, such as fractures associated with repeated cyclic fatigue and torsional loading and the necessity of using several instruments to enlarge the canal to an optimal apical size during crown-down preparation.\[22\] Due to these disadvantages, Yared\[23\] introduced reciprocating movement to generate a simpler and safer method of instrumentation for use in root canals. In addition to being used with a single instrument, the reciprocating motion has been shown to decrease the impact of cyclic fatigue compared with continuous NiTi rotational motion.\[9\] This can be due to the behavioral features of this movement. The reciprocating movement relieves stress on the instrument by special counterclockwise (cutting action) and clockwise (release of the instrument) movements and therefore reduces the risk of cyclic fatigue caused by tension and compression on instrument.\[9,23\] In general, reciprocating root canal preparation is an evolution of the balanced force technique that allows shaping with a crown-down technique.\[6,24\]

In the present study, bacterial count reduction rates were 59% for R25 and 74% for R25 + Mtwo 40.04. This reduction can be considered low. However, sodium chloride was used as an endodontic irrigant because our main objective was to evaluate the effectiveness of the technique without the influence of an antimicrobial substance. This fact reaffirms that mechanical preparation alone is not enough to eradicate bacteria and that it is necessary to combine it with the use of an auxiliary chemical substance (either sodium hypochlorite [NaOCl] or chlorhexidine gel) to achieve an effective disinfection protocol.\[25\] However, further studies should be conducted to evaluate the effectiveness of single files with different auxiliary chemical substances, such as NaOCl, chlorhexidine, and natural substances, such as grape seed extract.

CONCLUSION

Thus, it is concluded that the addition of the instrument Mtwo 40.04 after root preparation with the instrument R25 does not improve its decontamination capability.

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Conflicts of interest

There are no conflicts of interest.

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