Clinical Antibacterial Effectiveness of Root Canal Preparation with Reciprocating Single-instrument or Continuously Rotating Multi-instrument Systems

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Abstract

Introduction: This *in vivo* study compared the antibacterial effectiveness of a reciprocating single-instrument system (Reciproc; VDW, Munich, Germany) and a rotary multi-instrument system (BioRaCe; FKG Dentaire, La Chaux-de-Fonds, Switzerland) during the preparation of infected root canals of teeth with primary apical periodontitis. **Methods:** Root canals from single-rooted teeth with necrotic pulps and apical periodontitis were instrumented using either Reciproc ($n = 29$) or BioRaCe ($n = 30$) instruments under irrigation with 2.5% sodium hypochlorite. DNA was extracted from samples taken before and after preparation and subjected to quantitative analysis of total bacteria and streptococci by using the quantitative real-time polymerase chain reaction. **Results:** All initial samples were positive for the presence of bacteria, with median numbers of 7.1×10^5 and 1.31×10^5 bacterial cells for the Reciproc and BioRaCe groups, respectively. After preparation with Reciproc and BioRaCe, 16 (55%) and 15 (50%) root canals still had detectable bacteria with median counts of 7.05×10^2 and 6.03×10^1, respectively. Both systems were highly effective in reducing the total bacterial counts ($P < .001$), and there were no significant differences between them ($P > .05$). Streptococci were highly frequent, and both systems succeeded in significantly reducing their levels ($P < .001$). **Conclusions:** Both reciprocating single-instrument and rotary multi-instrument systems were highly effective in reducing the counts of total bacteria and streptococci in root canals of teeth with apical periodontitis. Regardless of the system used, approximately one half of the teeth still had detectable bacteria. *(J Endod 2016;42:25–29)*

Key Words

Apical periodontitis, bacterial reduction, endodontic treatment, molecular microbiology, single-file instrumentation

Apical periodontitis is a disease caused by bacterial infection of the root canal system; consequently, optimal endodontic treatment outcome depends on successful infection control (1, 2). Root canal preparation can be regarded as the most important phase of the endodontic treatment with regard to bacterial elimination (3). Traditionally, canals are enlarged and shaped by using a series of instruments with increasing tip diameters. Rotary multi-instrument systems, which consist of a series of nickel-titanium (NiTi) instruments used in continuous rotation motion, have become widely available and accepted over the last decade.

Recently, reciprocating single-instrument approaches have been proposed for root canal preparation (4), and new systems have been introduced. The Reciproc system (VDW, Munich, Germany) is one of these single-instrument techniques, which uses an instrument made of standard NiTi alloy with M-wire treatment. It is available in 3 sizes (R25, R40, and R50); each one is selected according to the initial root canal diameter. The instrument has a variable taper along its shaft; in the last 3 mm from the tip, the R25, R40, and R50 instruments are 0.08, 0.06, and 0.05 mm/mm tapered, respectively. The Reciproc instrument is operated in reciprocating motion in such a way that 3 cycles allow it to rotate 360°. 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 (5, 6).

Although not expected to exhibit significant differences in the shaping ability, a reason for concern about using single-instrument systems is that the time of preparation may be drastically reduced, and this may adversely affect the cleaning and disinfection effectiveness of the chemomechanical procedures. However, single-instrument techniques have been shown to provide similar cleaning (7–9), shaping (7, 10–12), and disinfecting (11, 13–16) effects comparable with rotary multi-instrument systems. These studies were conducted *in vitro* or *ex vivo*, and their results cannot be directly extrapolated to the clinical condition. Only a few clinical studies have addressed the antibacterial effectiveness of single-instrument systems. Two studies using culture showed that Reciproc was not different from multi-instrument systems in eliminating bacteria from the root canal (17, 18). Because culture-independent molecular microbiology methods are more sensitive than culture and can detect as-yet-uncultivated bacteria, they are expected to provide a more accurate picture of the antimicrobial effects of treatment procedures (19). To the best of our knowledge, only 1 study so far has used culture-independent methods to evaluate the *in vivo* antibacterial effects of a single-instrument system (Self-Adjusting File; ReDent-Nova, Ra'anana, Israel) (20).

The present study evaluated the *in vivo* antibacterial effectiveness of the Reciproc system in comparison with a widely used brand of rotary multi-instrument system, the BioRaCe (FKG Dentaire, La Chaux-de-Fonds, Switzerland), during the preparation of

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infected root canals of teeth with primary apical periodontitis. A reduction of the levels of total bacteria and streptococci was evaluated by means of a culture-independent molecular microbiology assay (quantitative real-time polymerase chain reaction [qPCR]).

Materials and Methods

Case Selection

Sixty patients (40 females and 20 males, mean age = 39 years ranging from 16–85 years) attending the endodontic clinic at the Department of Endodontics, Estácio de Sá University, Rio de Janeiro, Rio de Janeiro, Brazil, with indication for root canal treatment were included in this study. Each patient contributed 1 tooth. Criteria for inclusion were as follows: only teeth with a single root and a single canal presenting with necrotic pulps confirmed by a negative response to sensitivity pulp tests, intact pulp chamber walls, and clinical and radiographic evidence of asymptomatic apical periodontitis. Exclusion criteria included the following: presence of gross carious lesions or root/crown fracture, previous endodontic treatment, presence of symptoms, antibiotic therapy administered within the previous 3 months, and periodontal pockets deeper than 4 mm. The study protocol was approved by the ethics committee of Estácio de Sá University, and informed consent was obtained from all individuals or their parents/guardians.

Sample Taking and Treatment Procedures

Samples were taken from the root canals following strict aseptic measures. Supragingival biofilms were removed by scaling and cleansing with pumice, a rubber dam was applied, and caries and/or coronal restorations were removed under sterile saline irrigation. Before and after preparation of the access cavity, the operative field (dam, clamp, and tooth) was disinfected by using a 2-step disinfection protocol with the sequential use of 6% hydrogen peroxide and 2.5% sodium hypochlorite (NaOCl). Next, 10% sodium thiosulfate was used to inactivate NaOCl, and sterility control samples were taken by using sterile paper points scrubbed against the cavosurface angle of the access cavity. These paper points were transferred aseptically to a cryotube containing Tris-EDTA buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA, pH = 7.6) and immediately frozen at −20°C. Teeth were only included in the study if these sterility control samples were negative in an end-point polymerase chain reaction (PCR) assay using 16S ribosomal RNA (rRNA) gene-based universal bacterial primers.

Immediately before chemomechanical preparation, an initial sample (S1) was taken from the root canal to serve as the baseline. Sterile 10% sodium thiosulfate solution was applied to the pulp chamber without overflowing, and a small hand file was used to carry the solution into the root canal. This instrument was used to gently file the canal walls using circumferential motions to suspend the canal contents in solution. Sterile paper points were consecutively taken up to approximately 1 mm short of the radiographic root apex. Care was taken to avoid touching the paper points on the access cavity walls. Each paper point was left in the canal for about 1 minute to soak up the fluid in the canal. Paper points were transferred to cryotubes containing RNA *later* (Ambion, Austin, TX), stored at −4°C for 12 hours and then frozen at −20°C.

After irrigation with 1 mL 2.5% NaOCl, the working length (WL) was established 1 mm short of the apical foramen by using an electronic apex locator (Novapex; Forum Technologies, Rishon Le-Zion, Israel). A size 20 hand K-type file was used to initially enlarge the canal and establish the apical foramen patency.

Teeth were randomly divided into 2 groups (n = 30) according to the instrument system used for root canal preparation. All canals were completely instrumented in a single visit.

Reciproc Group. The Reciproc R40 or R50 (VDW) instrument was used in reciprocating motion powered by a torque-limited electric motor (VDW Silver, VDW) using the preset adjustments. The instrument was placed in the canal until resistance was felt and then activated. In sequence, the instrument was moved in an apical direction using in-and-out pecking motion, with approximately 3 mm in amplitude, using light apical pressure. After 3 pecking motions, the instrument was removed and cleaned, and the canal was irrigated with 2.5% NaOCl. Patency of the canal was checked by using a size 15 K-type file. These procedures were repeated until the WL was reached by the Reciproc instrument. Irrigation throughout the procedures was performed by using disposable syringes and 30-G NaviTip needles (Ultradent, South Jordan, UT) placed 3 mm short of the WL. Each root canal was instrumented with a single Reciproc instrument, and each instrument was used to prepare only 1 canal. Patency of the apical foramen was checked with a size 20 K-type hand file after preparation.

BioRaCe Group. Root canals were prepared by using the BioRaCe instruments (FKG Dentaire) operated in continuous rotation motion at 300 rpm powered by an electric motor (VDW Silver). Master apical files ranged from BR5 (40/.04) to BR6 (50/.04) depending on both the root anatomy and the preoperative root canal diameter. Patency of the apical foramen was confirmed with a size 20 K-type hand file throughout the procedures. Irrigation was performed as described previously.

Next, in both groups, the smear layer was removed by rinsing the canal with 5 mL 17% EDTA and 5 mL 2.5% NaOCl. A total volume of 15 mL NaOCl was used in both groups. The canal was dried using sterile paper points and then flushed with 1 mL 5% sodium thiosulfate for 1 minute for NaOCl inactivation. A postpreparation sample (S2) was taken from the canals as described for S1.

The root canals were medicated with calcium hydroxide and filled 1 week later by using gutta-percha and sealer in the lateral compaction technique.

DNA Extraction and Quantitative Real-time PCR Analysis

DNA from clinical samples was extracted by using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA) following the manufacturer's directions. The levels of total bacteria and streptococci were quantified before and after chemomechanical preparation by using a 16S rRNA gene-based qPCR with Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) on an ABI 7500 Real-time PCR instrument (Applied Biosystems). A total reaction volume of 20 μL was used. Primers, qPCR conditions, standard curve construction, controls, and data analyses were as described previously (21). All measurements were taken in triplicate for samples, standards, and controls.

Statistical Analysis

Sample size calculation revealed that 21 teeth per group would suffice to show a 5% difference in bacterial counts with a power of 90%. Intragroup bacterial reduction was analyzed by comparing S1 and S2 samples with the Wilcoxon matched pairs test. S1 samples were compared between groups by using the nonparametric Mann-Whitney U test, which showed no statistically significant difference ($P > .05$). Consequently, the absolute counts in S2 were used for intergroup comparisons also using the Mann-Whitney U test. For intergroup analysis of the presence/absence data in S2, the Fisher exact test was used. The significance level for all tests was established at $P < .05$.

Results

One tooth from the Reciproc group showed positive results for bacteria in the sterility control sample and was discarded from the