Effectiveness in the Removal of Endotoxins and Microbiological Profile in Primary Endodontic Infections Using 3 Different Instrumentation Systems: A Randomized Clinical Study

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Abstract

Introduction: This clinical study was conducted to correlate the microbiological profile and levels of endotoxins found in primary endodontic infection with the presence of clinical features and to evaluate the removal of microorganisms and endotoxins using rotary, reciprocating, and hybrid systems for biomechanical preparation. **Methods:** Thirty single root canals with primary endodontic infection were evaluated with signs and symptoms and were randomly divided into 3 groups according to the instrumentation system used ($n = 10$) as follows: rotary Mtwo instruments (VDW, Munich, Germany) with 8 files, the reciprocating Reciproc system (VDW) with a single file, and Genius hybrid instruments with 3 files (1 rotary and 2 reciprocating files) with irrigation using 24 mL 2.5% sodium hypochlorite. Samples were collected before (S1) and after instrumentation (S2) before being submitted to microbiological culture (colony-forming units/mL) and the checkerboard DNA-DNA hybridization test. Endotoxins were quantified using the limulus amebocyte lysate assay. **Results:** Microbiological culture showed statistical differences in the reduction of colony-forming units/mL with all systems tested ($P < .05$), but no statistical difference was found among the groups. The most frequently detected species were *Capnocytophaga ochracea* (53%) and *Fusobacterium nucleatum* (53%) at S1 and *F. nucleatum* (50%) and *Leptotrichia buccalis* (50%) at S2. As for the reduction of endotoxins at S2, Mtwo presented the best results (95.05%) followed by the Genius (91.85%) and Reciproc (64.68%) groups, but no statistical difference was found among the groups. Previous pain, tenderness to percussion, and presence of a sinus tract were associated with specific microorganisms ($P < .05$). **Conclusions:** Signs and symptoms were correlated with microorganisms. Endodontic treatment was effective in reducing bacteria and endotoxins but was not capable of completely removing them from the root canal. *(J Endod 2017;■:1–9)*

Key Words

Checkerboard DNA-DNA hybridization, instrumentation, primary endodontic infection

Significance

Several instrumentation systems have been developed with the aim of better cleaning and shaping of root canals in a shorter time. Three instrumentation systems were tested: rotatory, reciprocating, and hybrid, which combines rotation and reciprocity. Their effectiveness against microorganisms and endotoxins within root canals of symptomatic and asymptomatic teeth with primary endodontic infection was evaluated.

In endodontic infections, the aim of endodontic treatment is to decrease the number of bacterial cells and their products from the root canal and prevent new microorganisms from reaching the periapical region, thus promoting ideal conditions for healing. Therefore, biomechanical preparation is an important step of endodontic treatment, which is performed by using instruments and irrigants for cleaning and shaping the root canal (1).

The microbiota of root canal infections is highly diversified, including gram-positive, gram-negative aerobic and mainly anaerobic microorganisms (2, 3). Gram-negative bacteria have several virulence factors such as proteases, fimbria, and lipopolysaccharides (LPS) (4). LPS, best known as an endotoxin, stimulates bone resorption by acting on the synthesis and release of cytokines, which in turn activates osteoclasts, thus being directly related to periapical lesions (5, 6). Thus, the removal of microorganisms and their by-products must be achieved by the action of the instruments onto the walls of the infected dentin, which leads to the mechanical displacement of the intracanal biofilm. Auxiliary chemical substances must also be used because they have an antimicrobial action that contributes to the removal of contaminated dentin, in

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addition to acting on the microorganisms and their by-products present inside the dentinal tubules (7).

Instrumentation systems using nickel-titanium (NiTi) rotary files have evolved over time, leading to new designs and faster and easier techniques, not only preserving the original shape of the root canal but also minimizing the risk of errors (8). Several instrumentation systems can be used to achieve this goal. The Mtwo rotary system (VDW, Munich, Germany), which consists of a series of NiTi instruments used in continuous rotation motion, includes a large file to prevent the accumulation of debris in the apical region (9). The reciprocating single file was introduced to increase fracture resistance because it uses thermo-treated files made of standard NiTi alloy (10). The Reciproc files (VDW) were developed based on reciprocating motions at 150° counterclockwise rotations to cut dentin and at 30° clockwise rotations to release the file from the canal wall (11). The Genius hybrid system (Ultradent, South Jordan, UT) has been developed recently to associate rotary and reciprocating techniques. This system is composed of 1 rotating file used to enlarge the canal entrance, whereas 2 reciprocating files are used to prepare the root canal with 170° counterclockwise rotations and 50° clockwise rotations. To our knowledge, however, there is no *in vivo* study in the literature investigating the benefits, especially the antimicrobial activity on microorganisms and endotoxins, of hybrid systems associated to sodium hypochlorite (NaOCl) compared with rotary and reciprocating systems.

Thus, the present randomized clinical study aimed to correlate the microbiological profile and levels of endotoxin found in primary endodontic infection with the presence of clinical signs and symptoms as well as to evaluate the removal of microorganisms and endotoxins using 3 different techniques for biomechanical preparation, namely, rotary Mtwo, reciprocating Reciproc, and hybrid Genius systems.

Materials and Methods

Patient Selection

Thirty patients attending the endodontic clinic at the São José dos Campos Dental School (São Paulo State University), São José dos Campos, São Paulo, Brazil, with a diagnosis of pulp necrosis and radiographically visibly periradicular lesions (symptomatic and asymptomatic) were included in the present study. Criteria for inclusion were as follows: only single-rooted teeth with primary endodontic infection confirmed by a negative response to sensibility tests and radiographic evidence of apical periodontitis. Patients with periodontal pockets deeper than 4 mm, previous endodontic treatment, antibiotic and antifungal therapy in the past 3 months, and teeth that could not be isolated with a rubber dam were excluded from this study. Clinical signs and symptoms such as previous pain, tenderness to percussion and palpation, presence of a sinus tract, and exudate were recorded. The local research ethics committee approved the protocol describing the sample collection for this investigation, and all the voluntary patients signed an informed consent form.

Sample Collection

All the steps of this dental intervention were performed under aseptic conditions. Files, instruments, and all the materials used in this study were treated with Co-60 gamma radiation (20 kGy for 6 hours) for sterilization and elimination of preexisting endotoxins (CBE; Empresa Brasileira de Radiação, Cotia, SP, Brazil). Patients were anesthetized, the teeth were isolated with a rubber dam, and the crown and surrounding structures were disinfected using sterile swabs moistened with 30% H_2O_2 (v/v) for 30 seconds followed by 5.25% NaOCl for the same period of time and 5% sodium thiosulfate for inactivation (6).

Two-stage access cavity preparation was performed 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 after isolation with a rubber dam. All procedures were performed aseptically.

Immediately before biomechanical preparation, an initial sample (S1) was collected from the root canal to serve as the baseline. For endotoxin sampling, sterile/apyrogenic paper points (size #15; Dentsply Maillefer, Ballaigues, Switzerland) were introduced into the full length of the canal, which was determined radiographically, and retained in position for 60 seconds. Immediately after, the sample was placed in a pyrogen-free glass container and immediately suspended in 1 mL limulus amebocyte lysate (LAL) water according to the endotoxin dosage using the kinetic chromogenic LAL (Lonza, Walkersville, MD) assay. This sampling procedure was repeated with 3 paper points, which were then pooled in a sterile tube containing 1 mL VMGA III transport medium (12) for microbial analysis.

After the first sampling (S1), the biomechanical instrumentation was performed with 2.5% NaOCl. The working length (WL) was determined by using an apex locator (RomiApex A-15; Romidan Dental Solution, Kiryat-Ono, Israel) and confirmed radiographically using the digital RX (Micro Imagem, Indaiatuba, SP, Brazil) set 1 mm short of the apical foramen. A #15 hand K-file was used to initially enlarge the canal. The teeth were randomly divided into 3 groups ($n = 10$) according to the instrument system used for root canal preparation.

Mtwo Rotary System Group. The first group was instrumented using Mtwo files (Romibras LTDA, Rio de Janeiro, Brazil) adapted to an electric motor (VDW) in rotary movement. The files were used as follows: 0.04 taper size #10 instrument, 0.05 taper size #15 instrument, 0.06 taper size #20 instrument, 0.06 taper size #25 instrument, 0.07 taper size #25 instrument, 0.05 taper size #30 instrument, 0.04 taper size #35 instrument, and 0.04 taper size #40 instrument, which correspond to kits 701 and 702. The instrumentation was performed in a gentle in-and-out motion, taking the file to the WL. Irrigation was performed with 3 mL 2.5% NaOCl solution between each file, totaling 24 mL at the end of the instrumentation.

Reciproc Reciprocating System Group. The second group was instrumented with 1 single file from the Reciproc System (VDW) adapted to an electric motor (VDW) in reciprocation movement. The file was used as follows: 0.06 taper size #40 instrument. Instrumentation was performed according to the crown-down technique (ie, coronal, medium, and apical) with irrigation with 8 mL 2.5% NaOCl solution for each third, totaling 24 mL at the end of the instrumentation.

Genius Hybrid System Group. The third group was instrumented using the Genius hybrid system (Ultradent) adapted to an electric motor (EVOS, Ultradent) as follows: 0.08 taper size #30 instrument to amplify the entrance of the canal in rotary motion followed by 0.04 taper size #25 instrument and 0.04 taper size #40 instrument at the WL in a reciprocation movement. Irrigation was performed with 8 mL 2.5% NaOCl solution between each file, totaling 24 mL irrigation solution.

Foraminal cleaning was performed in all teeth with a #30 K-file along the tooth length at the end of biomechanical preparation. Then, each root canal was irrigated with 5 mL 5% sodium thiosulfate, and the final irrigation was performed with 10 mL sterile physiological saline. Next, the second sampling (S2) was performed as previously described, with the samples being submitted to endotoxin analysis, microbiological culture, and the checkerboard test.