Influence of the Apical Preparation Size and the Irrigant Type on Bacterial Reduction in Root Canal–treated Teeth with Apical Periodontitis

Renata Costa Val Rodrigues, PhD,[*†] *Homan Zandi, DDS,*[‡] *Anne Karin Kristoffersen, PhD,*[§] *Morten Enersen, PhD,*[§] *Ibrahimu Mdala, PhD,*[‖] *Dag Ørstavik, PhD,*[‡] *Isabela N. Rôças, PhD,*[*] *and José F. Siqueira, Jr, PhD*[*]

Abstract

Introduction: This clinical study evaluated the influence of the apical preparation size using nickel-titanium rotary instrumentation and the effect of a disinfectant on bacterial reduction in root canal–treated teeth with apical periodontitis. **Methods:** Forty-three teeth with posttreatment apical periodontitis were selected for retreatment. Teeth were randomly divided into 2 groups according to the irrigant used (2.5% sodium hypochlorite [NaOCl], $n = 22$; saline, $n = 21$). Canals were prepared with the Twisted File Adaptive (TFA) system (SybronEndo, Orange, CA). Bacteriological samples were taken before preparation (S1), after using the first instrument (S2), and then after the third instrument of the TFA system (S3). In the saline group, an additional sample was taken after final irrigation with 1% NaOCl (S4). DNA was extracted from the clinical samples and subjected to quantitative real-time polymerase chain reaction to evaluate the levels of total bacteria and streptococci. **Results:** S1 from all teeth were positive for bacteria. Preparation to the first and third instruments from the TFA system showed a highly significant intracanal bacterial reduction regardless of the irrigant ($P < .01$). Apical enlargement to the third instrument caused a significantly higher decrease in bacterial counts than the first instrument ($P < .01$). Intergroup comparison revealed no significant difference between NaOCl and saline after the first instrument ($P > .05$). NaOCl was significantly better than saline after using the largest instrument in the series ($P < .01$). **Conclusions:** Irrespective of the type of irrigant, an increase in the apical preparation size significantly enhanced root canal disinfection. The disinfecting benefit of NaOCl over saline was significant at large apical preparation sizes. *(J Endod 2017;■:1–6)*

Key Words

Chemomechanical preparation, endodontic retreatment, post-treatment apical periodontitis, sodium hypochlorite, Twisted File Adaptive

Significance

The outcome of endodontic retreatment is dependent on the effective control of root canal infection. This clinical study showed that the larger the apical preparation size, the higher the bacterial reduction in infected canals. The antibacterial effects of NaOCl were mostly observed after apical enlargement.

The major goal of treatment/retreatment of infected root canals of teeth with apical periodontitis is to eliminate bacterial populations as much as possible (1). Chemomechanical preparation of the root canal is a combination of the mechanical effects of instrumentation and irrigation with chemical effects of irrigants to achieve root canal cleaning, shaping, and disinfection. Mechanical preparation using irrigants with no antimicrobial effects can significantly reduce the intracanal bacterial counts (2–4). However, the use of antimicrobial irrigants has been shown to significantly improve disinfection during root canal preparation (5–7).

The apical width of preparation can be regarded as an important aspect of the treatment of infected root canals. *In vitro* studies have revealed that the larger the apical preparation size of infected canals, the greater the intracanal bacterial reduction (3, 8, 9). However, clinical studies using culture (2, 4, 7, 10, 11) or microscopy (12) have shown inconsistent results regarding the antibacterial benefits of apical enlargement. A recent systematic review concluded that more evidence-based clinical research is needed on the subject (13). Moreover, thus far, no clinical study has evaluated the antibacterial effects of apical enlargement during retreatment of teeth with apical periodontitis.

Intraradicular bacterial infection is the main cause of posttreatment apical periodontitis (14–16). Although no specific species has been recognized as a risk factor for posttreatment disease, several studies have shown that *Streptococcus* species are among the most prevalent bacterial taxa identified in postpreparation samples (16–18) and retreatment cases (14, 15, 19, 20). Their high prevalence and dominance in the canals of teeth with posttreatment apical periodontitis (14–16) suggest that streptococci can play an important role in persistent infections associated with this disease.

From the *Molecular Microbiology Laboratory, Department of Endodontics, Faculty of Dentistry, Estácio de Sá University; †Department of Endodontics, Faculty of Dentistry, Veiga de Almeida University, Rio de Janeiro, RJ, Brazil; and ‡Department of Endodontics, Institute of Clinical Dentistry, §Department of Oral Biology, Faculty of Dentistry, and ‖Department of General Practice, University of Oslo, Oslo, Norway.

Address requests for reprints to Dr Renata Costa Val Rodrigues, Faculty of Dentistry, Estácio de Sá University, Av Alfredo Baltazar da Silveira, 580/cobertura, Recreio, Rio de Janeiro, RJ, Brazil 22790-710. E-mail address: recostaval@gmail.com

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This *in vivo* study was conducted to evaluate the effects of apical preparation size using rotary nickel-titanium instruments and irrigation with either sodium hypochlorite (NaOCl) or saline on intracanal bacterial reduction during retreatment of teeth with posttreatment apical periodontitis. A highly sensitive quantitative real-time polymerase chain reaction (qPCR) assay was used to quantify total bacteria and *Streptococcus* species levels before and after preparation steps.

Material and Methods

Case Selection

Approval for the study protocol was obtained from the Ethics Committee of the Estácio de Sá University, Rio de Janeiro, RJ, Brazil, and The Regional Ethics Committee, University of Oslo, Oslo, Norway. Forty-three patients (28 women and 15 men; mean age = 42 years; range, 24–61 years) presenting to the endodontic clinic at 2 dental schools (Estácio de Sá University and University of Oslo) were included in this study for retreatment of teeth with posttreatment apical periodontitis. Only single-rooted teeth (39 patients) and roots with a single canal from multirooted teeth (4 patients) were included in the study. All teeth showed radiograph evidence of posttreatment apical periodontitis and root canal fillings no more than 4 mm short of the apex. The initial treatments were performed at least 4 years previously. No symptoms were present. All teeth had adequate coronal restorations as determined clinically and radiographically and no evidence of exposure of the root canal filling material to the oral cavity. Patients showed no significant systemic disease. Exclusion criteria included teeth with periodontal pockets deeper than 4 mm, teeth with severe crown destruction that prevented proper rubber dam isolation, and teeth with intraradicular posts.

Sample Taking and Treatment Procedures

A strictly aseptic technique for sample collection during endodontic retreatment was performed. After an oral rinse with 0.12% chlorhexidine, supragingival plaque biofilms were removed by scaling and cleansing with pumice. Caries and/or coronal restorations were removed with sterile high-speed and low-speed burs. The tooth was isolated with a rubber dam, and the operative field, which included the tooth, clamp, and surrounding dam, was cleaned using 3% hydrogen peroxide and disinfected with 2.5% NaOCl. After completing the access preparation with sterile burs under sterile saline irrigation, the operative field, this time also including the pulp chamber, was once again cleaned and disinfected as described previously. The residual NaOCl was neutralized with 10% sodium thiosulfate, and sterility control samples were taken by scrubbing sterile paper points on the internal surface of the cavosurface angle of the access cavity as described previously (16, 21). This included the area of the access cavity walls where the paper points used later for taking root canal samples might accidentally touch. The paper points used for sterility controls 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.

Gutta-percha fillings were removed using D-Race DR1 (size 30/.10 at 1000 rpm) and DR2 (size 25/.04 at 600 rpm) instruments (FKG Dentaire, La Chaux-de-Fonds, Switzerland). At this point, irrigation was performed with sterile saline solution, and no solvent was used. The working length (WL) was established 1 mm short of the apical foramen with the aid of an electronic apex locator (Novapex; Forum Technologies, Rishon Le-Zion, Israel) and confirmed by radiographs. Next, the canal was left filled with saline, and a small hand instrument was placed at the WL and used to gently file the canal walls. An initial microbiologic sample (S1) was taken from the root canal with sterile paper points consecutively placed at the WL. Each paper point was left in the canal for about 1 minute to absorb the canal content. Care was taken to avoid touching the access cavity walls with the paper points used for sampling. Paper points were transferred to cryotubes containing RNAlater (Ambion, Austin, TX), stored at −4°C for 12 hours, and then frozen at −20°C.

For inclusion of a tooth in the study, sterility control samples had to be negative, and S1 samples had to be positive for bacterial presence in the qPCR assay described later. All 43 patients with 1 tooth each satisfied these criteria and were included in the study. Teeth were randomly distributed into 2 groups according to the irrigant used (2.5% NaOCl or sterile saline solution). Chemomechanical procedures were completed at the same appointment in all cases using the Twisted File Adaptive system (TFA; SybronEndo, Orange, CA) for instrumentation. The sequence of instruments was selected according to the root canal anatomy and the manufacturer's directions. Canals from single-rooted teeth were prepared using the medium/large pack (2-color bands) of TFA instruments (25/.08, 35/.06, and 50/.04), and molar canals were treated using the small pack (1-color band) of TFA instruments (20/.04, 25/.06, and 35/.04).

NaOCl Group

Twenty-two root canals were irrigated with 2.5% NaOCl during preparation. The initial instrumentation with the DR2 instrument was performed at the WL, and the canal was rinsed with 5 mL 2.5% NaOCl. TFA instruments were operated in the Elements Motor (SybronEndo) and used up to the WL. The first instrument in the kit was used, and then the canal was irrigated with 6 mL 2.5% NaOCl, dried using sterile paper points, and flushed with 1 mL 10% sodium thiosulfate for 1 minute to inactivate NaOCl. Next, a sample (S2) was taken from the canals as described for S1. The canal was irrigated with NaOCl, and the second TFA instrument was used. After apical preparation with the third TFA instrument, the canal was irrigated with NaOCl, dried, and flushed with sodium thiosulfate, and another sample (S3) was taken. The total volume of 2.5% NaOCl up to S3 was 23 mL. The irrigant was delivered using disposable syringes and NaviTip needles (Ultradent, South Jordan, UT) inserted up to 3 mm short of the WL. Twenty canals in this group were enlarged to size 50/.04, and the other 2 canals were instrumented to size 35/.04.

Saline Group

Twenty-one teeth had their root canals prepared as described for the NaOCl group but using saline (0.9% NaCl) as the irrigant and at the same final volume as NaOCl. After S3 sample taking, irrigation with 10 mL 1% NaOCl was performed, the canal was dried and flushed with 1 mL 10% sodium thiosulfate for 1 minute, and an additional sample (S4) was taken. Nineteen canals in this group were instrumented to size 50/.04, and the other 2 were prepared to size 35/.04.

After preparation in both groups, EDTA was used for smear layer removal, and the canal was medicated with a calcium hydroxide paste. One week later, the canal was filled with gutta-percha and sealer, and the tooth was coronally restored.

DNA Extraction and qPCR Analysis

Clinical samples were thawed to room temperature, and DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA) following the protocol recommended by the manufacturer.

To quantify the total bacterial load and levels of *Streptococcus* species before and after preparation steps, 16S ribosomal RNA gene–targeted qPCR was performed with Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) on an ABI 7500 Real-time PCR instrument (Applied Biosystems) in a total reaction volume of 20 μL as described previously (14). The universal bacterial primers

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