Comparison of the *In Vivo* Antimicrobial Effectiveness of Sodium Hypochlorite and Chlorhexidine Used as Root Canal Irrigants: A Molecular Microbiology Study

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Abstract

Introduction: The purpose of this clinical study was to compare the antimicrobial effects of 2.5% sodium hypochlorite (NaOCl) and 0.12% chlorhexidine digluconate (CHX) when used as irrigants during treatment of teeth with apical periodontitis. **Methods:** Forty-seven single-rooted single-canal teeth with necrotic pulps and asymptomatic apical periodontitis were selected for this study according to stringent inclusion/exclusion criteria. Bacterial samples were taken at the baseline (S1) and after (S2) chemomechanical preparation using 2.5% NaOCl ($n = 30$) or 0.12% CHX ($n = 17$) as the irrigant. Bacterial, archaeal, and fungal presence was evaluated by broad-range polymerase chain reaction (PCR), whereas bacterial identifications were performed by a closed-ended reverse-capture checkerboard approach targeting 28 candidate endodontic pathogens. **Results:** All S1 samples were PCR positive for bacterial presence but negative for both archaea and fungi. Both NaOCl- and CHX-based protocols were significantly effective in reducing the bacterial levels and number of taxa. No significant differences were observed between them in all tested parameters including the incidence of negative PCR results in S2 (40% for NaOCl vs 47% for CHX, $p = 0.8$), reduction in the number of taxa per canal ($p = 0.3$), and reduction in the bacterial levels ($p = 0.07$). The most prevalent taxa in S2 samples from the NaOCl group were *Propionibacterium acnes*, *Streptococcus* species, *Porphyromonas endodontalis*, and *Selenomonas sputigena*. In the CHX group, the most prevalent taxa in S2 were *Dialister invisus*, *Actinomyces israelii*, *Prevotella baroniae*, *Propionibacterium acidifaciens*, and *Streptococcus* species. **Conclusions:** Treatment protocols using irrigation with either NaOCl or CHX succeded in significantly reducing the the number of bacterial taxa and their levels in infected root canals, with no significant difference between these substances. *(J Endod 2011;37:143–150)*

Key Words

Apical periodontitis, checkerboard DNA-DNA hybridization, chlorhexidine, endodontic treatment, polymerase chain reaction, sodium hypochlorite

Apical periodontitis is an infectious diseased caused by intraradicular microbial biofilms (1). Consequently, the outcome of the endodontic treatment depends on successful microbial elimination from the infected root canal system so as to achieve a host manageable bioburden (2). During treatment, chemomechanical preparation plays a critical role in disinfection by causing a drastic reduction in the bacterial populations located in the main root canal. In addition to the mechanical effects of instrumentation and irrigation procedures, the use of an antimicrobial substance for irrigation is indicated because it significantly enhances bacterial elimination (3–5).

Although many substances have been suggested for root canal irrigation, sodium hypochlorite (NaOCl) remains the most widely used irrigant solution because of its pronounced antimicrobial activity and the ability to dissolve organic matter (6). Chlorhexidine (CHX) has been proposed as a potential substitute for NaOCl given its optimum effects against endodontic bacteria (7, 8). Studies comparing the antimicrobial effectiveness of NaOCl and CHX have generated conflicting results. Some studies found that NaOCl is more effective (9, 10), others reported that CHX is more effective (11, 12), and others observed no significant difference between them (13–15). As for lipopolysaccharide (LPS) elimination from the root canal, a study reported that neither 2.5% NaOCl nor 2% CHX gel totally eliminated this virulence factor of gram-negative bacteria in any of the teeth evaluated, suggesting a low detoxifying activity for both substances (16).

Even though several *in vivo* studies have investigated the antibacterial effects of endodontic procedures, only a few have identified the bacterial taxa enduring treatment procedures (2). Most of these studies have been based on culturing techniques, which are largely known to have relevant limitations, most notably the low sensitivity and the inability to detect viable as-yet-uncultivated bacteria (17). Both limitations can underestimate the bacterial taxa occurring in endodontic infections and persisting after treatment. Culture-independent molecular microbiology methods can sidestep these shortcomings of culture methods because they exhibit increased sensitivity and specificity as well as the ability to reliably identify culture-difficult and even as-yet-uncultivated bacteria (17). Thus far, no molecular study has been used to compare the bacterial taxa identifications after chemomechanical procedures using either NaOCl or CHX as the irrigant.

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Although bacteria are the main microorganisms found in primary endodontic infections (17), there are some reports of the presence of archaea (18) and fungi (19) in primarily infected root canals. To the best of our knowledge, no study has consistently investigated the effects of intracanal procedures against these microorganisms using sensitive molecular techniques.

The purpose of this clinical study was to compare the antimicrobial efficacy of 2.5% NaOCl and 0.12% CHX when used as irrigants during the chemomechanical preparation of infected root canals associated with apical periodontitis lesions. Bacterial, archaeal, and fungal presence was evaluated by broad-range polymerase chain reaction (PCR), whereas bacterial identifications were performed by a closed-ended reverse-capture checkerboard DNA-DNA hybridization approach targeting 28 candidate endodontic pathogens.

Materials and Methods

Subjects

Fifty patients attending the endodontic clinic at the School of Dentistry, Estácio de Sá University, Rio de Janeiro, RJ, Brazil, for evaluation and treatment of apical periodontitis were included in this study. Teeth were selected based on stringent inclusion/exclusion criteria. Each patient contributed a single-rooted single-canal tooth. Only teeth with intact pulp chamber walls, necrotic pulps as confirmed by negative response to sensitivity pulp tests, and clinical and radiographic evidence of asymptomatic apical periodontitis lesions were included. The size of the apical periodontitis lesions ranged from 2 × 3 mm to 12 × 15 mm, and attempts were made to evenly distribute teeth with different lesion sizes between the two experimental groups. Exclusion criteria included teeth from patients who received antibiotic therapy within the previous 3 months, teeth with gross carious lesions, teeth with fractures of the root or crown, teeth that had received previous endodontic treatment, symptomatic teeth, and cases showing periodontal pockets deeper than 4 mm. Patients included in the study reported no significant systemic condition. Approval for the study protocol was obtained from the Ethics Committee of the Estácio de Sá University.

Treatment and Sampling Procedures

An aseptic technique was used throughout the endodontic treatment. Before rubber dam isolation, each tooth had supragingival biofilms removed by scaling and cleansing with pumice. Caries and/or coronal restorations were removed with sterile high-speed and low-speed burs. After rubber dam application, dental floss was securely tied around the neck of the tooth. The operative field including the tooth, clamp, and surroundings were cleaned with 3% hydrogen peroxide until no further bubbling of the peroxide occurred. All surfaces were then disinfected by vigorous swabbing with 2.5% NaOCl. After completing the access with another sterile bur under sterile saline irrigation, the operative field, including the pulp chamber, was once again cleaned and disinfected the same way as described previously. NaOCl was neutralized with 5% sodium thiosulphate, and sterility control samples were taken from the tooth surface with sterile paper points. For inclusion of the tooth in the study, these control samples had to be uniformly negative after PCR with universal primers 8f and 1492r. Based on this criterion, three teeth from the CHX group had to be excluded from the study.

The first root canal sample (S1) was taken as follows. The canal was filled with sterile saline solution with care to not overflow, and a sterile #15 K-file was introduced to a level approximately 1-mm short of the root apex, based on diagnostic radiographs, and a gentle filing motion was applied. Three sterile paper points were consecutively placed in the canal to the same level and used to soak up the fluid in the canal. Each paper point was left in the canal for at least 1 minute. Paper points were transferred aseptically to cryotubes containing Tris-EDTA buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA, pH = 7.6) and immediately frozen at −20°C.

Chemomechanical preparation was completed at the same appointment in all cases. The alternated rotation motion technique was used to prepare all canals (4, 20). Briefly, the coronal two thirds of the root canals were enlarged with Gates-Glidden burs. The working length was established 1-mm short of the root apex, and the patency length coincided with the radiographic root edge. This was established with an electronic apex locator (Novapex; Forum Technologies, Rishon le-Zion, Israel) and confirmed by radiographs. Apical preparation was completed to the working length with hand nickel-titanium files (Nitiflex; Dentsply-Maillefer, Ballaigues, Switzerland) in a back-and-forth alternating rotation motion. Master apical files ranged from #50 to #70, depending on both root anatomy and initial diameter of the root canal. Whenever instruments larger than #60 were required, stainless steel Flexofile instruments (Dentsply-Maillefer) were used. Apical patency was confirmed with a small file (#15 or #20 NitiFlex) throughout the procedures after each larger file size. Preparation was completed using stepback of 1-mm increments. In 30 root canals, the irrigant used was 2.5% NaOCl solution, whereas a 0.12% CHX solution was used in the other 20 canals (three were excluded later because of contamination of the sterility controls). A 27-G needle was used to deliver 2 mL of the test solutions after each instrument size.

Each canal was dried using sterile paper points and then flushed with 5 mL of either 5% sodium thiosulfate or a mixture of 0.07% lecithin, 0.5% Tween 80, and 5% sodium thiosulfate to neutralize any residual NaOCl or CHX, respectively. Subsequently, the root canal walls were gently filed, and a postinstrumentation sample (S2) was taken from the canal using sterile paper points as described previously. Afterward, the smear layer was removed, the canals were medicated with a calcium hydroxide paste for 1 week, and then they were filled by the lateral compaction technique.

DNA Extraction

Clinical samples were brought to room temperature, and then DNA was extracted by using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA) following the protocol recommended by the manufacturer. DNA from a panel of several oral bacterial species was also prepared to serve as controls (21).

Broad-range PCR for Bacteria, Archaea, and Fungi

Aliquots of extracted DNA were used in 16S rRNA gene-based PCR protocols using universal primers for members of the domains bacteria (22) or archaea (23, 24) and in a 18S rRNA gene-based PCR assay with universal primers for fungi (domain eukarya) (25) (Table 1). PCR reactions were performed in 50 μL of reaction mixture containing 1 μmol/L concentrations of each primer, 5 μL of 10× PCR buffer (Fermentas, Ontario, Canada), 3 mmol/L $MgCl_2$, 1.25 U *Taq* DNA polymerase (Fermentas), and 0.2 mmol/L each deoxyribonucleoside triphosphate (Biotools, Madrid, Spain). Positive and negative controls were included in each batch of samples analyzed. Positive controls consisted of DNA extracted from *Porphyromonas gingivalis* (ATCC 33277), *Methanobrevibacter arboriphilus* (DSMZ 744), and *Candida albicans* (ATCC 10231). Negative controls consisted of sterile ultrapure water instead of sample.

PCR amplifications were performed in a DNA thermocycler (Mastercycler Personal; Eppendorff, Hamburg, Germany). Cycling conditions were as follows: for archaea, initial denaturation at 94°C/2 min, 36 cycles at 94°C/30 s, 58°C/30 s, and 72°C/1 min, and final extension