Adjunctive Steps for Disinfection of the Mandibular Molar Root Canal System: A Correlative Bacteriologic, Micro–Computed Tomography, and Cryopulverization Approach

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Abstract

Introduction: This study evaluated the disinfecting ability of chemomechanical preparation with rotary nickel-titanium instruments, followed by 2 distinct adjunctive procedures in the root canals of extracted mandibular molars by means of a correlative analytical approach. **Methods:** Twenty-two extracted mandibular molars were selected and anatomically matched between groups on the basis of micro–computed tomographic analysis. In the first phase of the experiment, root canals were contaminated with *Enterococcus faecalis* and subjected to chemomechanical preparation with BT RaCe instruments and 2.5% NaOCl irrigation. Then either XP-Endo Finisher instrument or passive ultrasonic irrigation was used to supplement disinfection. Micro–computed tomography was used to show whether the percentage of unprepared areas correlated to bacterial counts. In the second phase, the same teeth were contaminated once again, and the adjunctive procedures were used. Samples from the isthmus area of mesial roots and the apical 5-mm fragment of distal roots were obtained by cryopulverization. Samples taken before and after treatment steps in both phases were evaluated by quantitative polymerase chain reaction and statistically analyzed. **Results:** In phase 1, preparation in both groups resulted in substantial decrease of bacterial counts ($P < .001$). The adjunctive approaches led to a further small bacterial reduction, which was significant for XP-Endo Finisher ($P < .05$). No significant differences were observed between groups for persisting bacterial counts. Correlative analysis revealed no statistically significant relationship between bacterial reduction and the percentage of unprepared areas ($P > .05$). In phase 2, both methods had significant antibacterial effects in the main canal, but none of them could predictably disinfect the isthmus/recess areas. **Conclusions:** Both XP-Endo Finisher and passive ultrasonic irrigation exhibited antibacterial effectiveness, but only the former caused a significant reduction in the bacterial counts after chemomechanical preparation. None of them were effective in predictably disinfecting the isthmus/recess areas. *(J Endod 2016;■:1–6)*

Key Words

Adjunctive approaches, bacterial reduction, cryopulverization, endodontic treatment, micro-computed tomography, passive ultrasonic irrigation

Significance

Adjunctive procedures have been recommended to be used after chemomechanical preparation to enhance disinfection of the root canal system. The effects of XP-Endo Finisher and passive ultrasonic irrigation (PUI) were evaluated in the root canal systems of extracted mandibular molars. Only XP-Endo Finisher significantly reduced the intracanal bacterial counts after chemomechanical preparation. However, disinfection of the isthmus/recess areas by the 2 approaches was unpredictable.

Irrespective of the instrumentation techniques, instruments, and irrigants, a thorough cleaning, disinfection, and shaping of the root canal have not been commonly achieved, especially in teeth with curved canals or unusual anatomies (1–3). Studies that used high-resolution micro–computed tomographic imaging technology (micro-CT) have shown that 11%–48% of the main root canal areas remain untouched after instrumentation (4–7). These areas may be colonized by biofilms (8) that have the potential to remain unaffected and put the treatment outcome at risk. Moreover, certain anatomic complexities of the root canal system, such as ramifications, recesses, and isthmi, are not commonly reached by instruments and irrigants. Bacteria located in these areas can persist and lead to persistent apical periodontitis (9). Actually, clinical bacteriological studies have demonstrated that bacteria are still detected in about 30%–60% of the canals after chemomechanical preparation (10–14). Bacteria persisting in the canal are the most important risk factor for post-treatment apical periodontitis (13, 15).

Efforts have been expended toward developing adjunctive approaches to enhance root canal disinfection (16). This involves approaches that drive irrigants to

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0099-2399/$ - see front matter

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http://dx.doi.org/10.1016/j.joen.2016.08.003

difficult-to-reach areas or allow for the instrument to reach and mechanically debride unaffected regions. One of these adjunctive approaches is passive ultrasonic irrigation (PUI), which comprises the ultrasonic activation of an irrigant. Data from *in vitro* and *in vivo* studies evaluating the benefits in terms of antibacterial effects of the adjunctive PUI approach with NaOCl have been inconclusive (10, 17–20).

The XP-Endo Finisher (FKG Dentaire, La Chaux-de-Fonds, Switzerland) instrument was recently introduced with the promise of enhancing root canal cleaning and disinfection. This is a size 25 nontapered instrument made of nickel-titanium (NiTi) MaxWire alloy (Martensite-Austenite Electropolish FleX). At room temperature the instrument is straight in its martensite phase, but at body temperature it changes to the austenite phase and develops a spoon shape; when rotated and moved up and down in the canal, this shape makes the instrument expand and contract to touch the canal walls and shake the irrigant solution (21). A recent study showed that XP-Endo Finisher and PUI succeeded in rendering the mesial root canal system with significantly lower levels of hard-tissue debris compared with conventional irrigation and a modified Self-Adjusting File system protocol (22). So far, only 1 study has investigated the antibacterial benefits of using XP-Endo Finisher and reported better results than conventional irrigation (23). No study has evaluated the antibacterial effects of this instrument after chemomechanical procedures.

The present study was intended to evaluate the disinfecting and shaping ability of chemomechanical preparation with rotary NiTi instruments, followed by 2 distinct adjunctive approaches in the root canals of extracted mandibular molars by means of a correlative bacteriologic and micro-CT analysis. A cryopulverization approach was used to evaluate the bacteriologic conditions of the isthmus and recess areas after the use of either XP-Endo Finisher instrument or PUI adjunctive procedures.

Materials and Methods

Tooth Selection and Preparation

The study protocol was approved by the Ethics Committee of the Estácio de Sá University, Rio de Janeiro, RJ, Brazil. Twenty-two extracted mandibular molars with 2 independent canals joined apically by an isthmus in the mesial root (Vertucci type II) and a single distal canal (Vertucci type I) were selected from a collection of 185 mandibular molars on the basis of radiographs taken in both buccolingual and mesiodistal directions, exploration with small files after access preparation, and micro-CT imaging by using a SkyScan 1174v2 scanner (Bruker-microCT, Kontich, Belgium) operated at 50 Kv, 800 mA, isotropic resolution of 19.86 μm, and 180° rotation around the vertical axis with a rotation step of 1.0 by using a 0.5-mm-thick aluminum filter.

Images of each specimen were reconstructed with a ring artifact correction of 5, a beam hardening correction of 15%, and smoothing of 5 (NRecon v.1.6.9.16; Bruker-microCT). CTAn v.1.14.4 software (Bruker-microCT) was used for 3-dimensional (3D) evaluation of the root canal regarding volume and surface area, and CTVol v.2.2.1 software (Bruker-microCT) was used for visualization and qualitative evaluation of the root canal system configuration. The specimens were pair-matched on the basis of the morphologic and anatomic aspects of the mesial and distal root canal systems, assessed by micro-CT, and 1 specimen from each pair was randomly assigned to 1 of the 2 experimental groups.

The root canals were explored with #15 hand K-files until the instrument tip reached the apical foramen, as visualized by a stereomicroscope. This measure was recorded as the patency length, and the canals were initially enlarged up to this point by using the BioRaCe BR2 (25/04) instrument (FKG Dentaire) operated in the VDW Gold motor (VDW, Munich, Germany) at 300 rpm, 1.5 N • cm, to standardize the initial canal diameter and create room for bacterial contamination. Smear layer was removed by using 17% EDTA and 2.5% NaOCl irrigation. NaOCl was inactivated with 5% sodium thiosulfate. The teeth were scanned again in micro-CT by using the previously mentioned parameters, and the obtained data sets were used as baseline for comparison with post-preparation images.

Phase 1

For contamination, the root canals were filled with trypticase soy broth (Difco, Detroit, MI) by using Navitip (Ultradent Products Inc, South Jordan, UT) needles until the broth flowed through the apical foramen. The teeth were placed in a flask containing 50 mL trypticase soy broth and ultrasonicated for 1 minute to release entrapped air and allow for penetration of the culture medium into root canal irregularities. Next, the teeth were sterilized in an autoclave. A fresh culture of *Enterococcus faecalis* ATCC 29212 grown for 24 hours at 37°C was used as inoculum for root canal contamination. The teeth were incubated for 30 days at 37°C under gentle shaking, and the culture medium was replenished every week. Later, all contaminated teeth had the excess culture medium dripped off, and their external root surfaces were wiped with sterile gauze. Two teeth were fixed in 10% buffered formalin and processed for scanning electron microscopy (SEM) to confirm bacterial colonization as described elsewhere (24).

The apical foramina of each root were sealed with Topdam (FGM, Joinville, SC, Brazil) to prevent apical bacterial leakage and create a closed-end system. Before root canal preparation, the outer root surfaces were cleaned with 3% hydrogen peroxide and disinfected with 2.5% NaOCl, followed by inactivation of the latter with 5% sodium thiosulfate. Teeth were mounted vertically up to the cervical region in blocks made of silicone impression material (President Jet; Coltène AG, Cuyahoga Falls, OH). The tooth crown, including the pulp chamber walls, and the silicone surface were disinfected with 2.5% NaOCl, followed by inactivation of this substance with 5% sodium thiosulfate. Samples were taken from the root canal by using paper points before (P1S1) and after chemomechanical preparation (P1S2) and after the adjunctive approach (P1S3) (Fig. 1*A*). The root canal was rinsed with 1 mL sterile 0.85% saline solution to remove unattached cells, and 3–5 sterile paper points were used sequentially at the working length (WL), which was established at 0.5 mm of the patency length. Each paper point remained in the canal for 1 minute. Paper points were transferred to tubes containing 1 mL Tris-EDTA buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA, pH 7.6) and frozen at −20°C. In the mesial root, samples were taken from each canal, but they were pooled for further bacteriologic analyses because the 2 canals merged into 1 at the apical portion.

Canals were prepared at the WL by using the BT RaCe system (FKG Dentaire), operated in the VDW Gold motor at 600 rpm, 1.5 N • cm, up to the BT3 instrument. Irrigation was carried out by using 2.5% NaOCl delivered by Navitip needles taken up to 2 mm short of the WL (Fig. 1*A*). During instrumentation of the mesial canals, the orifice of the distal canal was sealed with Topdam (and vice-versa) to avoid leakage of irrigants into it. After apical preparation, the canal was irrigated with NaOCl, EDTA (for smear layer removal), and then NaOCl again (Fig. 1*A*). After inactivation of NaOCl with 5% sodium thiosulfate, P1S2 sample was taken as described above, and teeth from each group were subjected to either PUI or XP-Endo Finisher adjunctive procedures as follows.

PUI. Root canals were irrigated with 2 mL 2.5% NaOCl, which was ultrasonically activated in the canal for 1 minute by using the EndoUltra device (Vista Dental Products, Racine, WI), with the probe tip placed 1 mm short of the WL. The canals were irrigated with 2 mL EDTA, which

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