Ability of Chemomechanical Preparation with Either Rotary Instruments or Self-adjusting File to Disinfect Oval-shaped Root Canals

José F. Siqueira, Jr, PhD, Flávio R.F. Alves, PhD, Bernardo M. Almeida, DDS, Julio C. Macibado de Oliveira, PhD, and Isabela N. Rochas, PhD

Abstract

Introduction: Oval-shaped root canals might represent a great challenge for proper disinfection. This study compared the capability of a newly developed instrument, the self-adjusting file (SAF), and rotary nickel-titanium (NiTi) instrumentation to eliminate Enterococcus faecalis populations from long oval root canals of extracted human teeth. As a secondary purpose, the ability of a modification in sampling technique to recover bacteria lodged in recesses of oval canals was evaluated.

Methods: Long oval canals from mandibular incisors and maxillary second premolars were infected with E. faecalis (ATCC 29212) for 30 days and then randomly distributed into 2 experimental groups. In group 1, canals were prepared up to a 40/04 rotary BioRaCe instrument by using irrigation with NaviTip needles; in group 2, canals were prepared by using the SAF system with continuous irrigation. NaOCl and ethylenediaminetetraacetic acid were used as irrigants. Bacteriologic samples were taken before (S1) and after preparation (S2a and S2b).

Results: Reduction in the bacterial populations was highly significant in both groups ($P < .001$). Preparation of long oval canals with the SAF was significantly more effective than rotary NiTi instrumentation in reducing intracanal E. faecalis counts ($P = .01$). Frequency of positive cultures in S2 samples was 11 of 20 (55%) for rotary instrumentation and 4 of 20 (20%) for SAF instrumentation ($P = .048$). S2b samples (modified method) yielded more positive samples than S2a (12/40 vs 5/40), but this difference reached no statistical significance ($P > .05$).

Conclusions: The SAF system was significantly more effective than rotary NiTi instrumentation used with syringe/needle irrigation in disinfecting long oval root canals in vitro. A modified sampling technique might be necessary for oval canals. (J Endod 2010;36:1860–1865)

Key Words

Endodontic treatment, Enterococcus faecalis, nickel-titanium instruments, root canal infection, self-adjusting file, sodium hypochlorite

The ultimate microbiological goals of chemomechanical preparation are to completely eradicating intracanal bacterial populations or at least reduce them to levels that are compatible with periradicular tissue healing (1). Bacteria persisting after chemomechanical procedures at levels detectable by culture-dependent techniques have been shown to influence negatively the endodontic treatment outcome (2, 3). Therefore, efforts should be directed toward the development of chemomechanical strategies that maximize root canal disinfection before filing.

Anatomic complexities might represent physical constraints that pose a serious challenge to adequate root canal disinfection. An example includes the cross-sectional root canal configuration, which has been classified as round, oval, long oval, flattened, or irregular (4). Whereas oval canals have been described as those exhibiting a maximum cross-sectional diameter of up to 2 times greater than the minimum diameter, long oval canals have a maximum diameter of 2–4 times greater than the minimum diameter (4). The overall prevalence of long oval root canals in the apical third is about 25%, with mandibular incisors and maxillary second premolars showing an even increased prevalence of this canal configuration (>50%) (5). In canals with these anatomical conditions, hand and rotary instruments working in reaming motion have been reported to leave untouched fins or recesses (6–11). In addition to harboring remnants of pulp tissue or bacterial biofilms, such recesses might also be packed with dentin chips generated and pushed therein by rotating instruments (12). Packed debris can interfere with the quality of obturation (13) and, in infected root canals, can harbor bacteria to serve as a potential source of persistent infection (14).

The self-adjusting file (SAF) (ReDent-Nova, Ra’anana, Israel) has been designed with the purpose of sidestepping some of the limitations of rotary nickel-titanium (NiTi) instruments (15). The SAF is a hollow and flexible instrument designed as a compressible thin-walled pointed cylinder composed of 120-μm-thick NiTi lattice (15). When inserted into the root canal, the instrument is claimed to adapt itself to the canal shape, both longitudinally and cross-sectionally, providing a three-dimensional adaptation (15). The surface of the lattice threads is lightly abrasive to promote a uniform removal of dentin during a back-and-forth grinding motion. The SAF is operated with reciprocating vibrating handpieces, and its hollow design allows for continuous delivery of irrigants throughout the procedure by a special rinsing unit.

A recent micro–computed tomography study showed that the percent of root canal area affected by the SAF method is larger than that affected by popular rotary instrumentation systems (16). Consequently, less unprepared areas that might potentially harbor bacterial biofilm remnants are observed (16). Another study reported that SAF operation with continuous irrigation resulted in root canal walls that were free of debris in all specimens and almost completely free of smear layer (17). The SAF system has the potential to be particularly advantageous in promoting disinfection of oval-shaped canals. However, there is no study that has examined the disinfecting ability of this novel system.
Therefore, the present study was undertaken to investigate the ability of the newly developed SAF system to eliminate viable *Enterococcus faecalis* populations from long oval root canals of extracted human teeth as compared with rotary NiTi instrumentation with syringe and needle irrigation. Furthermore, because common sampling methods with paper points have limitations in attaining a good representative sample from the root canal (18, 19), which might be especially aggravated in oval-shaped canals, a secondary purpose of this study was to evaluate the ability of a modification in the sampling technique to recover bacteria lodged in fins or recesses of long oval canals.

**Materials and Methods**

**Specimen Selection and Preparation**

This study included 44 teeth (single-rooted and single-canalled mandibular incisors and maxillary second premolars) with long oval root canals, which were selected from a collection of teeth that had been extracted for reasons not related to this study. Each tooth was radiographed from both buccolingual and mesiodistal projections, and only those teeth whose root canals presented a >2.5:1 ratio between the buccolingual and mesiodistal dimensions at a level 5 mm from the root apex were selected. Pairs of teeth were selected on the basis of similar radiographic root canal morphology, and each tooth from each pair was randomly assigned to either rotary instrumentation group or the SAF group. The study protocol was approved by the ethics committee of the Estácio de Saé University.

Conventional access cavities were prepared by using round burs and Endo-Z burs (Dentsply-Maillefer, Ballaigues, Switzerland). All root canals were instrumented at the apical foramen up to a hand #25 K-type file in alternated rotation motions under continuous irrigation with running water. Smear layer was removed by using ethylenediaminetetraacetic acid (EDTA) for 3 minutes, followed by 2.5% NaOCl irrigation. After inactivation of residual NaOCl with 10% sodium thiosulfate, the teeth were immersed in trypticase soy broth (TSB) (Difco, Detroit, MI), ultrasonicated for 1 minute to release entrapped air and allow penetration of culture media into root canal irregularities, and then sterilized in autoclave for 20 minutes at 121°C. Each flask contained 10 teeth immersed in 200 mL of TSB. The experiment was planned so that 10 specimens could be prepared and the respective bacteriologic samples processed per day.

**Bacterial Biofilm Formation**

*E. faecalis* strain ATCC 29212 was used to infect the root canals. A suspension was prepared by adding 1 mL of a pure culture of *E. faecalis*, grown in TSB for 24 hours, to 5 mL of fresh TSB. One milliliter of this suspension was used to inoculate each of the flasks. *E. faecalis* was allowed to grow for 30 days at 37°C under gentle shaking. Culture media were replenished every week.

Fifty teeth were used in the antibacterial experiment, and 4 teeth were subjected to scanning electron microscopy to confirm bacterial colonization and biofilm formation. These 4 teeth were fixed in 10% buffered formalin, longitudinally split, and then dried in ascending ethanol concentrations. They were then dehydrated to their critical point in CO2 and sputter-coated with gold under vacuum. Specimens were examined by using a scanning electron microscope (JSM-5800LV; JEOL, Tokyo, Japan).

The teeth that were subjected to further study had the excess culture medium dripped off, and their external root surface was wiped with sterile gauze. The apical foramen was sealed with a fast set epoxy resin to prevent bacterial leakage and a closed-end channel that produces the vapor lock effect (20). To make both handling and identification easier, teeth were mounted vertically up to the cervical region in blocks made of a silicone impression material (President Jet; Coltene 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 10% sodium thiosulfate. For working length (WL) determination, a #20 K-file was introduced in the canal until it reached the apical foramen. The initial (S1) sample was then taken from each canal.

**Rotary NiTi Instrumentation Group**

BioRaCe instruments (FKG Dentaire, La Chaux-de-Fonds, Switzerland) were used in this group as described by Debelian and Trope (21). Twenty root canals were prepared at the WL by using the BR2 instrument (25/04, size/taper) up to the BR5 instrument (40/04), with 2.5% NaOCl as the irrigant. Irrigation was performed with disposable 5-mL syringes and 30-gauge NaviTip needles (Ultradent, South Jordan, UT) taken up to 3 mm short of the WL. After preparation was complete, the canal was rinsed with 5 mL of 17% EDTA, followed by 5 mL of 2.5% NaOCl. The total volume of NaOCl was 15 mL per canal (Fig. 1). The average total time NaOCl remained in the canal was 10.7 minutes (range, 8–13 minutes). The average WL in this group was 19.9 mm (range, 17–23 mm). Chemomechanical procedures were conducted by an operator who had been specifically trained with BioRaCe instruments.

**SAF Group**

The SAF system (Fig. 24, B) was used with the instrument operated by an in-and-out vibrating handpiece (GENTLEpower; KaVo, Biberach a. d. Riß, Germany) combined with a RDT3 head (ReDent-Nova) at a frequency of 5000 movements per minute and amplitude of 0.4 mm. The SAF instrument was inserted in the canal and operated with in-and-out motion to WL for a total of 5 minutes. Continuous irrigation with 2.5% NaOCl or 17% EDTA was applied by using a special irrigation device (VATEA; ReDent-Nova). This device was connected to the irrigation hub on the file and allowed irrigants to be delivered at a flow rate of 5 mL/min. During the first 2 minutes, NaOCl was used, followed by 1 minute of EDTA and then another 2 minutes of NaOCl. The total volume of NaOCl was 20 mL per canal (Fig. 1). The average total time NaOCl remained in the canal in this group was 5.8 minutes (range, 5.5–8 minutes). The average WL in this group was 20.1 mm (range, 16–22 mm). Chemomechanical procedures in this group were performed by an operator who had been specifically trained with the SAF instrument.

After preparation in both groups, each root canal was washed with 1 mL of 10% sodium thiosulfate to inactivate NaOCl, dried, and refilled with sodium thiosulfate, which remained in the canal for 5 minutes. Postpreparation (S2) samples were taken.

**Sampling Procedures Processing**

Root canals were sampled before (S1) and after (S2) chemomechanical procedures.

**S1 Sample.** The root canal was gently rinsed with 1 mL of sterile saline solution to remove unattached cells, and an initial sample was taken by the sequential use of 3–5 paper points placed to the WL. Each paper point remained in the canal for 1 minute. Paper points were transferred to tubes containing 1 mL of sterile 0.85% saline solution and immediately processed.

**S2 Samples.** Initially, the root canal flooded with 10% sodium thiosulfate was sampled by agitating the fluid in the canal with a sterile #35 or #40 gutta-percha point by using a pumping motion and then absorbing the contents with sterile paper points until the canal was...
These samples were called S2a. Another S2 sample was taken following a slight modification from the method described by Metzger et al (22). The root canal was refilled with sodium thiosulfate, and then a sterile precurved stainless steel hand #20 K-file was inserted in the canal up to the WL. The curvature applied to the instrument was gentle and involved approximately the last 3 mm near the instrument’s tip. The precurved instrument was turned so that its tip faced the buccal recess and then moved 3 times with a pulling motion. This motion was repeated after turning the file so that its tip now faced the lingual recess. This approach was intended to disrupt and dislodge the remaining bacteria.

**Figure 1.** Flowchart of the experimental procedures.

**Figure 2.** (A) The SAF. (B) Higher view of the SAF tip. (C) Scanning electron micrograph showing heavy colonization of the root canal wall by *E. faecalis* ATCC 29212 (original magnification, ×10,000). (D) Representative electrophoretic gel showing PCR products of the predicted size for *E. faecalis* to confirm identification in positive samples. (E) Representative specimen from the rotary NiTi instrumentation group showing a recess that remained apparently untouched (arrow). Cross section at 5 mm short of the root apex. (F) Representative specimen from the SAF group showing an apparently uniform preparation of the oval-shaped canal at 5 mm from the apex. (This figure is available in color online at www.aae.org/joe/.)
biofilm remnants and dentinal debris packed or unaffected in the
recesses. Root canal contents were then absorbed with sterile paper
points until the canal was dry. This sample was called S2b. Paper points
used for taking S2a and S2b samples were transferred to tubes contain-
ing 1 mL of sterile saline and immediately processed.

Sample processing involved agitation in vortex for 1 minute, fol-
lowed by 10-fold serial dilutions in saline. Afterwards, aliquots of
100 μL were plated onto Mitis-Salivarius agar plates (Difco) and in-
cubated at 37°C for 48 hours. The colony-forming units (CFUs) grown
were counted and then transformed into actual counts on the basis
of the known dilution factors. Two parameters were evaluated per
sample, qualitative (positive versus negative culture) and quantitative
(number of CFUs).

To confirm identification of *E. faecalis* in all culture-positive
samples, species-specific polymerase chain reaction (PCR) was per-
formed as described previously (23). PCR amplicons were separated
d by electrophoresis in 1.5% agarose gel in Tris-borate-EDTA buffer,
and positive reactions were determined by the presence of the predicted
310-base pair amplicon.

**Statistical Analysis**

The Mann-Whitney test was used for intragroup analysis
comparing the reduction in the number of CFU counts from S1 to
S2a, S2b, or S2ab. S2ab was considered as the overall S2 results, and
data were mounted by using only the highest counts of either S2a or
S2b for each tooth (ie, the worst S2 results per tooth). Because compari-
sions of baseline samples (S1) between groups by using the Mann-
Whitney test revealed no significant differences, comparison between
the efficacy of rotary NiTi instruments and the SAF to disinfect oval-
shaped canals was performed by using S2a, S2b, or S2ab data in the
Mann-Whitney test. Because S2ab data were the worst case scenario,
they were chosen for final presentation and discussion. The incidence
of negative cultures was compared between the 2 groups by using the
two-tailed Fisher exact test. S2a and S2b samples were compared by
using the overall data (n = 40 per S2 sample) in quantitative (Mann-
Whitney test) or qualitative (χ² test with Yates correction) analyses.
Significance level for all analyses was always set at *P* < .05.

**Results**

Scanning electron microscopy analysis of 4 specimens revealed
that the root canal walls were densely colonized by *E. faecalis* cells
forming biofilm-like structures (Fig. 2C). Successful colonization of
the root canal was further confirmed by bacterial growth in all S1
samples. PCR confirmed identification of *E. faecalis* in all positive
samples (Fig. 2D).

**Table 1. Counts of *E. faecalis* CFUs before (S1) and after (S2a and S2b) Chemomechanical Procedures by Using Either Rotary NiTi Instrumentation with BioRaCe Instruments or the SAF System**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean</th>
<th>Median</th>
<th>Range</th>
<th>S2a</th>
<th>Mean</th>
<th>Median</th>
<th>Range</th>
<th>S2b</th>
<th>Mean</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotary</td>
<td>2.05×10⁴</td>
<td>1.00 × 10⁴</td>
<td>6.28 × 10³</td>
<td>9.62 × 10³</td>
<td>1.70 × 10⁴</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAF</td>
<td>6.28×10⁵</td>
<td>8.00×10⁵</td>
<td>8.52×10³</td>
<td>9.62×10³</td>
<td>1.70×10⁴</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

S1: sample taken by paper points after pumping root canal contents with gutta-percha points. S2a: sample taken by paper points after removing procedural hand #20 file from buccal and lingual canal recesses. SAF: self-adjusting file.

The incidence of negative cultures was compared between the 2 groups by using the two-tailed Fisher exact test. S2a and S2b samples were compared by using the overall data (n = 40 per S2 sample) in quantitative (Mann-Whitney test) or qualitative (χ² test with Yates correction) analyses. Significance level for all analyses was always set at *P* < .05.
### TABLE 2. Incidence of Positive Cultures after (S2a and S2b) Chemomechanical Preparation by Using Either Rotary NiTi Instrumentation with BioRaCe Instruments or the SAF System

<table>
<thead>
<tr>
<th>Group</th>
<th>S2a</th>
<th>S2b</th>
<th>S2ab*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotary instruments</td>
<td>4/20 (20)†</td>
<td>8/20 (40)</td>
<td>11/20 (55)</td>
</tr>
<tr>
<td>SAF</td>
<td>1/20 (5)</td>
<td>4/20 (20)</td>
<td>4/20 (20)</td>
</tr>
</tbody>
</table>

S2a, sample taken by paper points after pumping root canal contents with gutta-percha point; S2b, sample taken by paper points after moving precurved hand #20 file into buccal and lingual canal recesses; SAF, self-adjusting file.

*S2ab data entail the highest bacterial counts of either S2a or S2b for each tooth.

Number of cases with positive culture/number of cases examined (%).

(20%) for SAF instrumentation (Table 2). This 35% difference was statistically significant, although it should be considered that the P value was very close to the significance level (P = .048).

S2b samples exhibited more positive samples than S2a (12/40 versus 5/40), but the results were not statistically significant after qualitative (χ², P = .1) and quantitative (Mann-Whitney, P = .08) analyses. Ten specimens revealed positive results in S2b samples but not in S2a, whereas the opposite occurred in 3 specimens.

### Discussion

The present *in vitro* study was conducted to evaluate the ability of chemomechanical preparation with either rotary NiTi instruments or the SAF in reducing *E. faecalis* populations within long oval root canals. Intragroup analysis indicated that both methods succeeded in promoting a significantly high reduction in intracanal bacterial populations. This is in line with several previous reports on the antibacterial efficacy of chemomechanical procedures (24–28). When the 2 groups were compared (intergroup analysis), quantitative data (CFU counts) revealed that the SAF method was significantly more effective than rotary NiTi instrumentation. Data regarding the incidence of negative and positive cultures (qualitative analysis) revealed that whereas in the SAF group 80% of the samples were rendered free of detectable levels of *E. faecalis*, instrumentation with rotary NiTi instruments resulted in only 45% of culture-negative samples. Although this difference was apparently very high and reached statistical significance, it is worth pointing out that the P value in the two-tailed Fisher exact test was close to the limit of significance. This is highly likely to be related to the sample size (number of specimens analyzed).

Because there were samples showing positive results either in S2a or in S2b and different quantitative findings were observed for these 2 sampling procedures, the worst results of S2 for each tooth were used in the comparison between the 2 instrumentation techniques. For instance, if a given S2a sample was negative and the corresponding S2b sample was positive or if S2a yielded lower CFU counts than S2b, the value of S2b was used for comparing the techniques. The same was true for the opposite condition. This was done because two S2 samples were taken for each root canal, regardless of the sampling technique used, and ultimately they reflected the bacteriologic conditions of the canal after preparation.

The results with rotary instrumentation do not come as a surprise, because it has already been widely demonstrated that rotary and hand-operated instruments in reaming motion do not prepare all root canal walls, especially in oval-shaped canals, where recesses are commonly left untouched (6–11, 29). A recent study compared the prepared surface areas of oval-shaped canals by using different instrumentation techniques and revealed that the mean unaffected areas ranged from about 60%–80% for the total canal length (10). At the apical portion of the canal, the mean of untouched areas ranged from 65%–75% (10). Another recent study comparing the cleaning effects of 3 instrumentation techniques in oval-shaped canals reported that none of the techniques resulted in completely prepared and cleaned canals (11).

In the present study, a 40/04 BioRaCe instrument was used to carve a relatively large apical preparation as recommended for mandibular incisors and maxillary second premolars (4, 30). Irrigation was performed with small-sized needles taken close to the WL, which has been shown to enhance antibacterial effectiveness (25, 31). Even so, detectable bacteria were still observed in about one half of the postpreparation samples. Because the number of positive cultures in S2b was higher than in S2a samples, it is reasonable to surmise that remaining bacteria were mostly located in buccal and/or lingual root canal recesses, which remained untouched as a result of the physical limitations of conventional instruments (Fig. 2F). It is also possible to speculate that the time NaOCl remained in the canal was not sufficient for this substance to penetrate into these narrow recesses in sufficient concentration, volume, and flow rate to disrupt biofilm structures adhered to the unaffected walls. It is still worth pointing out that the total time NaOCl remained within the root canal in the rotary instrumentation group was longer than in the SAF group.

The SAF system uses a hollow vibrating instrument, which allows for continuous irrigation with NaOCl or EDTA throughout preparation. Irrigants are exchanged and claimed to be taken to the apical root canal at the same time its abrasive blades are pressed against the walls to promote root canal enlargement. When compared with NiTi instrumentation, it has been reported that the SAF leaves less unprepared areas (16). The present results apparently confirm the superiority of the SAF system to prepare long oval canals. The higher antibacterial efficacy of the SAF method might be related to the instrument’s ability to affect a higher surface area of the canal walls (including the larger diameter of the oval canal) (Fig. 2F), to the continuous delivery of fresh antibacterial irrigants throughout preparation, or both. Better instrumentation of this is also expected to result in better access of irrigants deep within these areas, contributing to elimination of bacterial biofilms. Nonetheless, it should be noticed that although the overall time of NaOCl permanence in the canal was shorter for the SAF group, the total volume was larger (20 versus 15 mL). Whether this affected the results cannot be established at this time.

Common sampling methods have limitations because of the physical difficulties of paper points to reach irregularities and other regions of the root canal system (18, 19). Consequently, this approach might fail to detect viable bacteria in the deepest part of recesses. To allow for a more predictable sampling of narrow canal recesses, a modification in the technique was made by introducing and moving the tip of a slightly precurved hand instrument along the buccal and lingual aspects of the oval canal. Although the results displayed no statistical significance, much more positive samples were observed when using this approach (12 positive samples for S2b and 5 for S2a). Therefore, it might be advisable to use this modified approach to sample oval-shaped canal in microbiological studies.

Because of the inherent limitations of *in vitro* studies, data interpretation and extrapolation to the clinical setting should be made with caution. In addition to the obvious limitations of using extracted teeth in an optimized laboratory environment, it is important to consider that only the main canal was sampled, and no efforts were made toward collecting dentinal shavings or sampling other areas of the root canal system. Thus, because the present study reports exclusively on the ability of instrumentation to eliminate bacterial populations in the main canal, no inference can be made as to disinfection of the whole root canal system. Also, the bacterial markers used in this study consisted of a pure culture of a bacterial species growing under optimal conditions.
of nutrients and with no influence from competitors. This is a condition that is rarely, if ever, found in the clinical setting, where mixed bacterial communities are usually present and arguably enduring famine conditions.

In conclusion, the SAF cleaning-shaping-irrigation system was significantly more effective than rotary NiTi instrumentation used with syringe and needle irrigation in eliminating viable _E. faecalis_ cells from long oval root canals in vitro. Also, a modification of the sampling technique might be considered to improve bacterial recovery in oval-shaped canals.

Acknowledgments

The authors thank ReDent-Nova for providing the SAF instruments used in this study, Dr Zvi Metzger for insightful discussions and suggestions during the development of the experimental protocol, Mr Fernando Magalhães for his valuable technical assistance, and Dr Raviv Zary for preparing all root canals in the SAF group. This study was financially supported in part by Henry Schein tinance, and Dr Raviv Zary for preparing all root canals in the SAF syringe and needle irrigation in eliminating viable communities are usually present and arguably enduring famine condition, that is rarely, if ever, found in the clinical setting, where mixed bacterial Governmental Institutions.

The authors deny any conflicts of interest.

References