Effectiveness of Self-Adjusting File, XP-endo Finisher, and passive ultrasonic irrigation in bacterial root canal control

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ABSTRACT

Objective: To evaluate the effectiveness of complementary protocols of sanitization in decontamination of infected root canals. **Methods:** A total of 50 single-rooted maxilary anterior human teeth were selected. The crowns were removed and lengths standardized at 16 mm. The specimens were inoculated with *Enterococcus faecalis* and incubated at 37 °C for 60 days. Thirty teeth were prepared with Bio-Race instruments up to diameter corresponding to #60.02, and then complemented with Self-Adjusting File (SAF); XP-endo Finisher (XPF) and passive ultrasonic irrigation (PUI). Ten samples were used as positive control and ten were not contaminated. Initial and final samples were collected and incubated at 37 °C for a period of 48 hours. Bacterial growth was analyzed in culture, determining the presence or absence of bacteria. Optical density of the culture medium was interpreted by UV spectrophotometry. Specimens were sectioned and prepared for evaluation under SEM. Images of root surfaces were analyzed and classified by scores according to the presence of debris. Kruskal-Wallis test was used for statistical analysis. Level of significance was 5%. **Results:** Mean optical density (μm) of the sanification protocols showed bacterial reduction in all groups. The experimental groups did not present statistically significant differences ($p = 0.196$). Analysis of SEM images revealed no significant difference ($p = 0.414$) between groups scores. **Conclusion:** Complementary sanitization protocols reduced bacterial contamination.

Keywords: Root canal preparation. Root canal irrigants. Root canal therapy.

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Introduction

Treatment of endodontic infection has a greater chance of success when an apropriate root canal preparation and cleaning protocol is performed. Complex internal anatomy allows for areas that are inaccessible to endodontic instruments and irrigating solutions, which may be responsible for persistence of infection.[1,2]

The microenvironment of the root canal favors adhesion of several bacterial species to the dentinal surface and allows for biofilm formation resistant to antimicrobial agents. Identification and knowledge of predominant microorganisms in root canal infections are decisive factors for effective microbial control.[1]

Enterococcus faecalis is a bacterium observed in persistent infections, associated with endodontic therapy failure. This is attributed to its capacity to invade dentinal tubules, develop under unfavorable conditions, and organize itself into biofilm.[2,3]

Use of irrigating solution during root canal preparation seeks to increase elimination of bacteria, remove debris, lubricate root canal walls, and dissolve organic tissue.[4] Different irrigating solutions have been proposed. Sodium hypochlorite (NaOCl) has been the most used one in Endodontics due to its antimicrobial properties and dissolution capacity of organic tissues.[4,5] Ethylenediaminetetraacetic acid (EDTA) has been used as an aid in the removal of inorganic component (smear layer) produced during root canal preparation.

Irrigating solution efficacy depends on its direct contact with microorganisms. It is also important to consider its volume, as well as exposure time and adequate protocol.[4,5] Conventional root canal irrigation employs syringe-adapted needles associated with positive apical pressure. The tip of a needle is positioned from 1 to 2 mm of working length and irrigation is done with high volume and frequent replacement of irrigating solutions.[6] Passive ultrasonic irrigation (PUI) consists of ultrasonic activation of a conventional endodontic instrument[7] or a non-cutting instrument with a diameter less than the prepared canal[8] after complete root canal preparation. The instrument must have free movement without making contact with dentinal walls, with the canal being completely filled by the irrigating solution of choice.[9,10] The effects of instrument vibration are cavitation and formation of acoustic streaming. Cavitation is the phenomenon of bubbles formation that grow until they break, releasing great energy. Acoustic streaming is formed by rapid movement of the solution around the instrument, causing the former to contact root canal walls abruptly.[11] These effects have been pointed out as being responsible for the best results provided by ultrasonic irrigation. However, no well-established use protocol exists.[12]

Other strategies have been proposed to intensify bacterial removal and complement final cleaning and disinfection of the root canal system.[6,13,14]

In order to improve effectiveness of conventional instruments and promote better cleaning of root canal preparation, Self-Adjusting File System (SAF) (ReDent NOVA, Ra'anana, Israel) was introduced into the endodontic armamentarium.[15] SAF was created with the purpose of preparing root canal with a single instrument (diameters of 1.5 and 2.0 mm). Its structure comprises a hollow, compressible, thin-walled body composed of a delicate NiTi trellis covered by an abrasive surface. Its action promotes dentine wear by low amplitude (0.4 mm) vibration movement (3000 to 5000 vibrations per minute) promoted by a specific apparatus. When inserted into the root canal, this instrument adapts to its shape, both longitudinally and transversally, which favors a three-dimensional action.[16,17]

Another instrument currently available on the market for final cleaning is XP-endo Finisher (XPF), produced with Ni-Ti MaxWire- (Martensite-Austenite electropolish-Flex) based alloy. According to the manufacturer, XPF is a file #25 with taper 0, driven by any motor with continuous rotary movement. Due to the small diameter of the core, the instrument has flexibility and resistance to cyclic fatigue. However, it is not an instrument to shape root canal walls, but for touching them. Its shape changes according to temperature conditions. When cooled below 35 °C, it corresponds to the martensite phase, in which it is malleable and can be shaped according to the operator's needs. When the instrument is heated to body temperature (35 °C), it changes to the austenite phase. At this stage, when bent it creates a very particular form of cleaning instrument. This increases the chance of reaching areas of the root canal where conventional instruments have not had access.[18]

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All of these contemporary resources have contributed to accessing the complex system of root canals, with the objective of disaggregating bacterial biofilms. The present study evaluated the effectiveness of SAF, XPF, and passive ultrasonic irrigation systems for bacterial control of root canals.

Material and methods

Preparation of teeth

A total of 50 anterior human single-rooted teeth extracted for different reasons from patients of the Emergency Department of School of Dentistry of Universidade Federal de Goiás were used in the study. Teeth with root canal obliteration and root laceration were excluded from the sample. The study began after approval by the Research Ethics Committee of Universidade Federal de Goiás #1.214.507.

The extracted teeth were packed in vials containing 0.2% thymol solution. Before preparation, the teeth were immersed in 5% NaOCl (Fitofarma, Lt. 20442, Goiânia, GO, Brazil) for 30 minutes. Periapical radiographs of teeth were made in lingual-lingual and proximal-proximal directions with radiographic film (Eastman Kodak Comp., USA) to confirm the presence of a single canal and absence of anatomical variations.

Standard access cavities were prepared in 40 teeth using high-speed diamond bur (1012, KG Sorensen, Cotia, São Paulo, Brazil). Root canal patency was achieved using a K-flex #15 file (Maillefer, Ballaigues, Switzerland). Cervical and middle thirds preparation was performed with BR0 instruments (#25.08) (BioRace™, FKG Dentaire, La Chaux-de-Fonds, Switzerland) and BR1 (#15.05). The apical third was instrumented with BioRace in the following sequence: BR2 (#25/0.04), BR3 (#25.06), BR4 (#35.04), and BR5 (#40.04), coupled to X-Smart Plus endodontic motor (Dentsply Maillefer, Ballaigues, Switzerland) with speed of 500 rpm and torque of 1 Ncm. Each instrument was used in five canals. Root canals irrigation was performed using 3 mL of 2.5% NaOCl at each endodontic instrument change.

After root canal preparation, under continuous air/water jet, the crowns were removed with Endo-Z laminate drill (Maillefer, Ballaigues, Switzerland) at high rotation and 90° angle to the long axis of the tooth. Root lengths were standardized at 16 mm. Root canals were dried with #40 paper cones and filled with 17% EDTA (pH 7.2, Formula and Action, São Paulo, SP, Brazil) for 3 minutes. Teeth were then autoclaved for 30 minutes at 120 °C.

Bacterial contamination

Enterococcus faecalis (ATCC 29212), which was inoculated into 7 mL of BHI agar (BHI; Difco Laboratories, Detroit, MI, USA) and incubated at 37° C for 24 hours, was used in this study. Bacterial cells were suspended in saline solution to an approximate final concentration of 3 x 10^8 cells mL^{-1}, fitted to McFarland's #1 tube.

5 mL of the sterilized BHI was mixed with 5 mL of bacterial suspension (Enterococcus faecalis) and inoculated into root canals for 60 days. This procedure was repeated every 72 hours within 60 days always using pure culture with 24 hours of preparation and adjusted to tube #1 of McFarland. Teeth were kept in a microbiological incubator at 37 °C.

After the period of bacterial contamination, the canals were dried and filled with distilled water. Sterilized #40 paper points were introduced into the root canal and held for 3 minutes for initial microbiological collection. The points were individually transported and immersed in 7 mL of BHI added with neutralizing Tween 80 and sodium thiosulfate in appropriate concentrations, followed by incubation at 37 °C for 48 hours. After bacterial growth was verified, the experimental groups were prepared.

Teeth were randomly divided into three experimental and two control groups: 1. SAF; 2. XPF; 3. PUI; 4. Positive control; and 5. Negative control.

Specimens of Groups 1, 2, and 3 were prepared with BioRaCe (FKG Dentaire, Swiss Dental Products, La Chaux-de-Fonds, Swiss) rotational system coupled to X-Smart Plus endodontic motor (Dentsply Maillefer, Ballaigues), speed of 500 rpm and torque of 1 Ncm, with BR6 instruments (#50.04) and BR7 (#60.02), each instrument being used in five root canals. Irrigation was performed by the conventional technique with 3 mL of NaOCl 2.5% with Ultradent 5 mL syringe and Navitip irrigation cannula (Ultradent, South Jordan, UT), with a diameter of 0.30 mm positioned at 12 mm in the root canal.

In Group 1, SAF (ReDent NOVA, Ra'anana, Israel) of 1.5 mm diameter adapted to a RDT3 contra-angle

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(Redent-Nova Inc., Ra'anana, Israel) was used, which was coupled to the vibrating handpiece (Gentlepower, KaVo, Biberach Riss, Germany) and connected to a drive device. After passive insertion of SAF into the root canal, the motor was driven, and by means of a constant vibration movement (5,000 vibrations per minute) with amplitude of 0.4 mm, the instrument was used in the working length in short manual movement of penetration and retraction, with slight apical pressure for 1 minute. Root canal irrigation was performed with 5 mL NaOCl 2.5%. After preparation, drying was done with the tip of #60 sterilized absorbent paper point. The root canal was then irrigated with 17% EDTA, kept under agitation with K-flex #15 manual file for 3 minutes. After this period, final irrigation was performed with 5 mL of 2.5% NaOCL. The total volume of irrigating solution was calculated from the first sample and the same amount was used throughout the experiment for the other groups.

In Group 2, XPF instrument coupled to X-Smart Plus endodontic motor (Dentsply Maillefer, Ballaigues, Switzerland) with a speed of 800 rpm (FKG Dentaire, Swiss Dental Products, La Chaux) and 1 Ncm torque, was introduced throughout the working length of the root canal. This instrument was operated with rotary movements of slow and gentle penetration and retraction for 1 minute. Irrigation was performed with 5 mL 2.5% sodium hypochlorite. The same previously described procedures were performed.

In Group 3, for irrigation performed after the last instrument (BR7) was used, the solution was agitated by ultrasonic tip E1-Irrisonic (Helse Dental Tecnology, Santa Rosa de Viterbo-SP, Brazil), coupled to EMS Piezon Master 200 Ultrasound (EMS, São Bernardo Do Campo-SP, Brazil), adjusted to power of 10%. The ultrasonic tip was positioned 1 mm short the working length and activated for 30 seconds with care not to touch root canal walls. The canals were dried with absorbent paper points and irrigated with 3 mL of 17% EDTA, which was agited for 30 seconds with the ultrasonic tip and held for 2 minutes and 30 seconds, totaling 3 minutes. Then, root canals were again irrigated with 5 mL of 2.5% NaOCl solution.

Subsequently, all specimens were irrigated with 3 mL of saline solution. Root canals were dried and filled with sterile distilled water. Each sample was collected using three #60 sterilized absorbent paper points(final collection). The points were individually transported and immersed in 7 mL of BHI and neutralized, followed by incubation at 37 °C for 48 hours.

Group 4 (positive control) was used to check for bacterial viability throughout the experiment, whereas negative control (Group 5) was used to confirm sterility of samples.

After microbiological collection, they were transported aseptically to a microbiological incubator at 37 °C and remained there for 48 hours. After evaluation of changes in the culture medium, an inoculum of 0.1 mL from the medium was transfered to new tubes containing 7 mL Letheen broth (Letheen Broth; Difco Laboratories, Detroit, MI, USA). These media were stored as described above. Bacterial growth in samples was analyzed by turbidity of the culture medium with the aid of a UV spectrophotometer (Spectrophotometer Model Nova 1600 UV, Piracicaba, SP, Brazil).

Scanning electron microscopy

SEM examination methods used in this study were previously described.[19]

Longitudinal grooves were made in the long axis of the entire root length of the ten teeth in each group. Sectioning was done in a laminar flow hood with a sterilized chisel and a hammer.

Fragments were fixed in buffered formalin solution for one week. Afterwards, dehydration was carried out in ascending ethanol solution of 70%, 95%, and 99.5%, with two exchanges per solution, totaling 30 minutes in each solution. Drying was done at critical point (AutoSamdri-815, Tousimis Research Corporation, Rockville, Maryland, USA).

Metallographic preparation of teeth was done for analysis under scanning electron microscope (MEV, JED, JSM, 6360LV, Tokyo, Japan) with magnification of 1.600 to have the presence of debris on dentinal surface checked. The root was measured and divided equally into cervical, middle, and apical thirds, which were evaluated separately. Three previously instructed observers analyzed the images for the presence of debris on the dentinal surface of the root using the following classification: Score 1 - Root surface with presence of debris and visible dentinal tubules entrance; Score 2 - Root surface with presence of debris and invasion of dentinal tubules entrance; Score 3 -

Root surface with presence of greater area covered by debris and invasion of dentinal tubules entrance; Score 4 - Root surface completely covered by debris and invisible dentinal tubule entrance (Fig 1).

Statistical analysis

Microbiological data were tested for adherence to the normal curve using Shapiro-Wilk test, and for homogeneity of variances using Lèvene test. No normal distribution and homogeneity of variances were found for the percentage data of initial collection/after final cleaning ($p < 0.05$). Microorganism reduction data from initial/final collection were then analyzed using Krukal-Wallis test.

Analysis of root surfaces images was performed using Kruskal-Wallis test. The level of significance was 5%.

Results

Mean optical density (μm) of the culture medium for final microbiological collection showed statistically significant bacterial reduction when compared to the initial one ($p > 0.05$) (Table I). The experimental groups did not show statistically significant differences ($p = 0.196$).

Scanning electron microscopy

Analysis of root surfaces images after using SAF, XPF, and PUI systems revealed no statistically significant difference ($p = 0.414$) among groups, even when surfaces were evaluated by thirds (cervical - $p = 0.254$; middle - $p = 0.120$; apical - $p = 0.982$). When compared within the same group, the thirds did not present statistically significant differences.

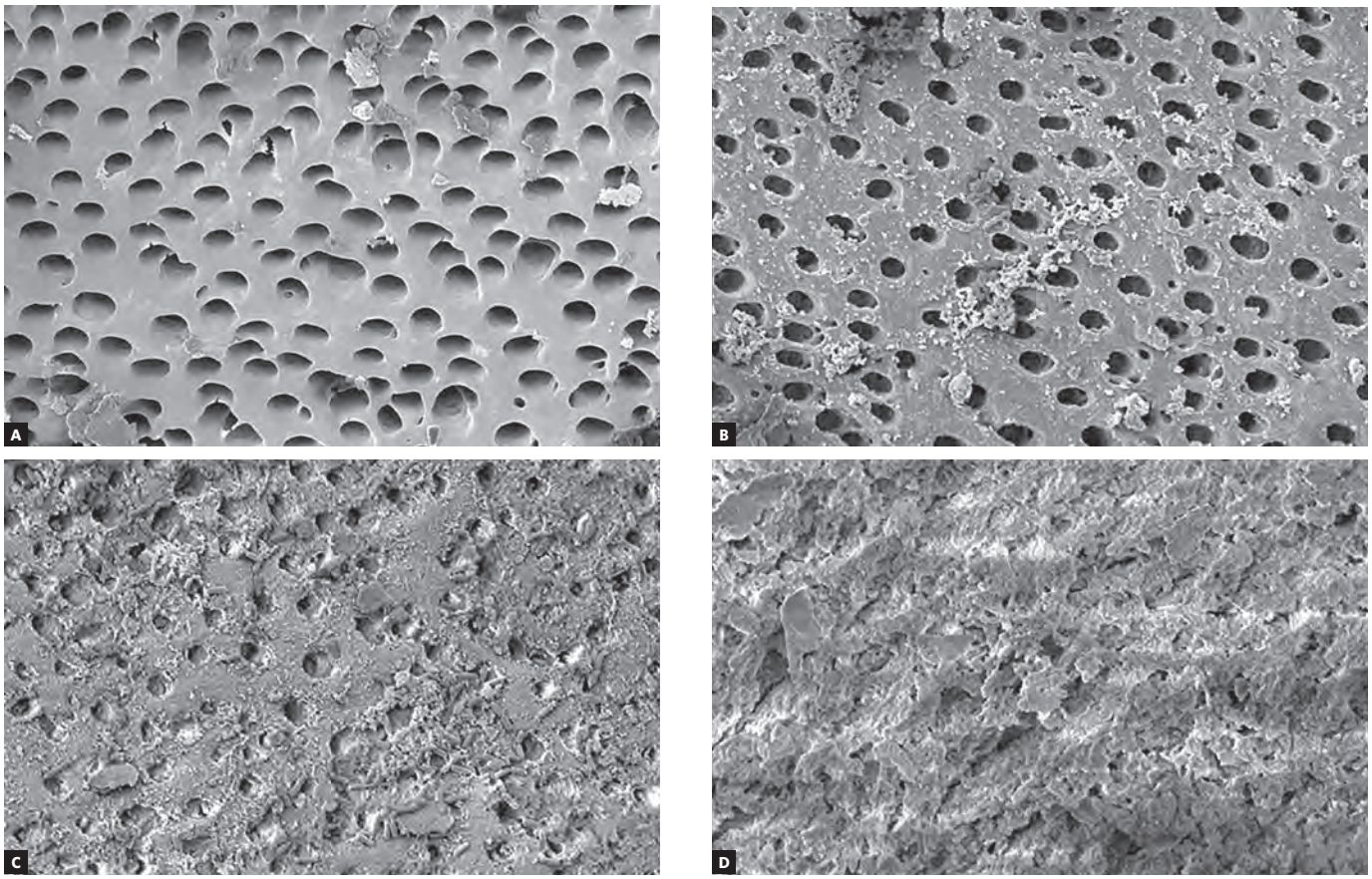

Figure 1. Classification of SEM images for the presence of debris on root dentinal surface. 1. Score 1 - Root surface with presence of debris and visible dentinal tubules entrance; 2. Score 2 - Root surface with presence of debris and invasion of dentinal tubules entrance; 3. Score 3 - Root surface with presence of greater area covered by debris and invasion of dentinal tubules entrance; 4. Score 4 - Root surface completely covered by debris and invisible dentinal tubule entrance.

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Table 1. Mean optical density (μm) of the culture medium for initial and final microbiological collection.

Experimental groups	Microbiological collections			
	Initial	Mean/SD optical density of medium	Final	Mean/SD optical density of medium
SAF	+++	0.350 ± 0.066	+++	0.047 ± 0.080
XPF	+++	0.218 ± 0.058	+++	0.004 ± 0.006
PUI	+++	0.218 ± 0.058	+++	0.007 ± 0.011

(+++) Presence of bacteria (- - -) absence of bacteria ($p > 0.05$). * statistically significant difference. SD: Standard deviation.

Discussion

Root canal preparation was performed with BioRace system in continuous rotary movement up to instrument #60 followed by complementary protocols for final cleaning. In all the experimental groups, bacterial reduction could be observed compared to initial microbiological collections and after final cleaning.

Siqueira et al[20] revealed a significant reduction in the number of colony forming units (CFUs) when using SAF with its own irrigation device and Biorace (#40.04) with a positive pressure irrigation technique with 2.5% NaOCl. SAF proved to be more effective, with 20% of samples indicating positive cultures, whereas BioRace presented 55%. SAF performance with its own irrigation device with 2.5% NaOCl in a study by Alves et al[21] showed a relation with the time of use. After 6 minutes of action, a reduction of 54.5% in bacterial population (CFUs) was observed, followed by 45.5% after 4 minutes and 18% after 2 minutes. Using the same methods, they observed a bacterial reduction of 38.84% when using SAF with 6% NaOCl with irrigation apparatus for 4 minutes.[22]

In the present study, SEM analysis showed that the complementary protocols used (SAF, XPF, and PUI) were not fully effective in complete removal of debris on the root surface. When comparing cervical, middle, and apical thirds, no difference could be observed within the same group and among different groups. These complementary strategies were analyzed through different methodos.[23-30]

Studies have suggested that the area close to the apical foramen is not completely cleaned when the PUI system is used,[31,32] with removal of debris being larger in the cervical third.[33] Results similar to those obtained by Paranjpe et al,[22] with the use of SAF, in which no significant debris removal was observed at 1 mm and 3 mm in apical direction, were attributed to inadequate apical irrigation.

Different studies have demonstrated the effectiveness of SAF in the removal of debris in all areas of the root canal, in 95%, 90%, and 85% of cervical, middle, and apical thirds, respectively,[35] and 100% using 1% NaOCl and EDTA.[15] With an innovative design, the SAF system was created with a recommendation for preparing the root canal with a single instrument capable of cleaning a larger area and promoting minimum wear of the narrowest part of the dentinal structure. Its structure comprises a hollow, compressible, thin-walled body composed of a delicate NiTi trellis covered by an abrasive layer. Its action promotes dentine wear by vibration movement (3000 to 5000 vibrations per minute) at low amplitude (0.4 mm) promoted by a specific apparatus. When inserted into the root canal, this instrument adapts to its shape, both longitudinally and transversally, which favors a three-dimensional action.[15-17]

It is assumed that the mechanical action of instruments is indispensable for biofilm rupture.[1,2] A previous study has demonstrated that debris removal from the root canal increases with progressive widening by instrumentation,[34] removing more contaminated dentine and providing better access for efficient irrigation and disinfection of the root canal system. In the present study, the use of SAF was a final cleaning strategy. Root canal preparation was performed up to instrument BR7 60, and conicity of 0.02, which allowed better mechanical removal of infected dentine

and increased the diameter of the canal. As a result, it had a flow of the irrigating solution along canal walls and increased its chemical action.

A study performed with μTC showed superior results in the removal of debris after XPF and PUI systems (89.7% and 94.1%, respectively) when compared to conventional irrigation system and SAF (45.7% and 41.3%, respectively).[23] However, investigations performed by μTC also showed superiority of SAF (80.7%) over PUI system (60.3%) when the debris removal area was evaluated.[24]

Trope and Debelian[18] reported that XPF is an alternative for final cleaning of root canals in a three-dimensional shape without unnecessary dentine removal. XPF is an instrument capable of reacting at different temperature levels. When heated, the instrument is linear (phase M), but when exposed to the internal temperature of the root canal, it changes its shape to phase A, which allows the instrument to expand its range to 6 mm in diameter, cleaning a 100-fold larger area than a file of the same caliber when in rotational motion by having access to regions previously untouched by conventional instruments. In addition, the file causes turbulence of the irrigating solution that potentiates its antimicrobial properties.

In the present study, throughout root canal preparation, a positive pressure irrigation technique was used in all experimental groups.It was during final cleaning of the complementary protocol with PUI that the irrigating substance was activated. It is noteworthy that the SAF protocol was altered to reflect the same clinical conditions under which XPF and the ultrasonic irrigation technique were used.

SAF of 1.5 mm results in an apical size equivalent to instrument #40[15,24] and showed better results in teeth with oval root canals.[20] Further studies should be performed with the aim of studying teeth with more complex anatomy and/or with the largest caliber instrument (2.0 mm).

In the present study, a reduction of bacterial contamination was observed using SAF, XPF, and passive ultrasonic irrigation protocols.

Conclusion

Complementary sanitization protocols reduced bacterial contamination.

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