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Postgraduate Training

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"A clinical evaluation of the ability of finishing files to supplement the removal of bacteria and endotoxin from primarily infected root canals (Part I- initial evaluation)"

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"Antibacterial Efficacy and Discoloration Effect of a Novel Intra-Canal Antibiotic Dressing for Regenerative Endodontics"

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Austenite and Martensite: Phases Simplified. 04/2019
AAE Annual Session, Montreal, Canada

Clinical Applications of 3D Digital Visualization in Endodontics 04/2018
AAE Annual Session, Denver, CO

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Community Service

Bethesda Project 09/2015 – 06/2017
- Prepare food for the homeless and participate in the community

Moveable Feast 08/2013 – 05/2015
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Homeless Resource Day 08/2012 – 05/2015
- Performed oral cancer screenings for homeless people

Professional Organizations/Leadership Experience

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Professional membership

American Association of Endodontics 08/2014 – Present
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Professional membership

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Abstract

Title of Thesis: A clinical evaluation of the ability of finishing files to supplement the removal of bacteria and endotoxin from primarily infected root canals (Part I- Initial evaluation)

Eunice Kim, Master of Science 2020

Thesis directed by: Frederico Martinho, DDS, MSc, PhD, Endodontic Division, School of Dentistry

The aim of this study was to evaluate the ability of XP-endo Finisher (XPF) to supplement the removal of bacteria and endotoxin from primary endodontic infections. This randomized controlled trial included eight subjects. Instrumentation was performed using Vortex Blue (VB) or XP-endo Shaper (XPS), followed by supplemental instrumentation with XPF. Bacterial and endotoxin samples were taken using paper points. Samples were collected before and after instrumentation and after XPF. Bacteria was present in all canals. After XPF, bacterial mean was reduced from 255 ± 311.82 CFU/mL to 2.5 ± 7.07 CFU/mL ($p= .056$). Endotoxin was detected in all canals by the LAL method (KQCL test). After XPF, endotoxin mean was reduced from .85 ± .26 EU/mL to .03 ± .01 EU/mL ($p= .00004$). The findings of this study showed that the supplemental use of XPF after instrumentation significantly reduced endotoxin but not bacteria present in primary endodontic infections.

A clinical evaluation of the ability of finishing files to supplement the removal of bacteria and endotoxin from primarily infected root canals (Part I- Initial evaluation)

by
Eunice Kim

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, Baltimore in partial fulfillment
of the requirements for the degree of
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2020

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List of Abbreviations

AAEF	American Association of Endodontists Foundation
AES	apical enlargement size
A-phase	austenitic phase
BHI	brain heart infusion
CEC	contracted access cavity
CFU	colony-forming units
CHX	chlorhexidine
EK	Eunice Kim
ER	Elaine Romberg
FM	Frederico Martinho
H_2O_2	hydrogen peroxide
IL-1ß	interleukin 1 beta
IRB	Institutional Review Board
KQCL	Kinetic chromogenic LAL assay
LAL	Limulus Amebocyte Lysate
LPS	lipopolysaccharide
MAPK	mitogen-activated protein kinase
M-phase	martensitic phase
NaOCl	sodium hypochlorite
NF-κB	nuclear factor kappa B
NiTi	nickel titanium alloy
OM	Omid Dianat

PIPS	erbium:yttrium aluminum garnet laser
RB	Reciproc
TEC	traditional access cavity
TLR4	toll-like receptor 4
TNF-α	tumor necrosis factor alpha
UMSOD	University of Maryland School of Dentistry
VB	Vortex Blue
WL	working length
XPF	XP-endo Finisher
XPS	XP-endo Shaper

Introduction

Background

Bacteria and their by-products are the main cause of pulpal and periapical disease (1). The persistence of bacteria in root canals after endodontic treatment is associated with root canal failure and is indicated by a lack of healing or the development of apical disease (2). The purpose of root canal treatment is to eliminate bacteria and their byproducts, such as endotoxin, from the root canal system; however, no endodontic file currently on the market is capable of completely removing all microbes (3, 4).

The root canal system has a complex anatomy with numerous possible variations, including canals that are oval or C-shaped, isthmuses between canals, or canals that bifurcate. The apical third of the root can also have additional portals of exits including lateral, secondary and accessory canals (5). A majority of the current file systems available have a circular shape, sculpting a round cross-sectional shape, while most canals have an oval or irregular shape, leaving behind untouched recesses in the extremities of the canal wall (4). This results in inadequate root canal cleaning, shaping, and disinfection of the root canal system (6).

Over the years, many studies have investigated different disinfection protocols, such as file systems, irrigation techniques, and root canal medications, with or without supplemental steps, such as ultrasonic activation and photodynamic therapy (7-14). No method of protocol has been proven to provide complete sterility in root canal systems.

With the limited ability of current file systems and disinfection protocols to clean and remove bacteria and endotoxins from root canal infections, a new file called the XP-endo Finisher (XPF; FKG Dentaire SA, La Chaux-de-Fonds, Switzerland) was recently

launched into the market. The XP-endo Finisher files have an adaptive core technology that can adapt to the root canal morphology based on the shape-memory principles of the NiTi alloy and facilitate bacteria and debris removal, as well as the overall disinfection of the canal (15). However, clinical studies have not yet confirmed these abilities.

Bacteria and endotoxin in root canal infections

Primary root canal infection is a polymicrobial infection comprised of gram-positive and gram-negative microorganisms with gram-negative anaerobic bacteria dominating the community (16-25). The most commonly encountered bacteria in root canal infections are Streptococcus spp., Fusobacterium spp., Prevotella spp., Porphyromonas spp., and *Parvimonas micra* (10, 18, 22-23, 25).

Lipopolysaccharide (LPS), also known as endotoxin, is the major surface molecule and virulence factor of gram-negative bacteria (26). It consists of three distinct structural regions: O-antigen, core, and lipid A. In particular, lipid A structures isolated from different bacterial species can vary in the number and length of fatty acid side chains, the presence of terminal phosphate moieties and other structural modifications. The recognition of LPS structures by the host innate immune system promotes the synthesis and secretion of two classes of immune response effectors, cytokines and chemokines that coordinate innate and adaptive immune system response.

Endotoxin is recognized by the innate immune system via the toll-like receptor 4 (TLR4) pathway. Diversity in TLR4-directed recognition and signaling pathways has been identified across the species, with polymorphisms in the genes encoding these pathways resulting in clinical variability in response to disease. Martinho *et al* (21) investigated the

bacterial community involved in primary endodontic infection and evaluated its ability to activate macrophage toll-like receptor through p38 mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NF-κB) signaling pathways. The authors concluded that the bacteria within the canal system were potent activators of TLR4 through MAP3K-MKK3/6-p38 and NF-kB downstream signaling.

LPS present in root canal infections, when in contact with periapical tissues via the apical foramen is a potent stimulus for different cells, leading to a periapical inflammatory response and ultimately bone destruction (27-30). Dahlen (27) administered small concentrations of *Fusobacterium nucleatum* and *Bacteroides oralis* inside the root canals of rats and verified that locally applied antigens from oral microorganisms can stimulate an immune response both in the lymph nodes and the spleen, resulting in circulating antibodies. Martinho *et al* (30) compared the inflammatory potential of LPS from *Fusobacterium nucleatum* and *Porphyromonas gingivalis* strains that were clinically isolated from root canal infections to stimulate macrophage production of interleukin 1 beta (IL-1ß) and tumor necrosis factor alpha (TNF-α). The authors concluded that *F. nucleatum* and *P. gingivalis* isolated from root canals led to increased macrophage production of IL-1ß and TNF-α, critical in the immunopathogenesis of apical periodontitis.

Over the years, clinical studies have attempted to investigate and quantify LPS in infected root canals and correlate it to the presence of clinical signs and symptoms (7, 9, 31-34). Martinho *et al* (35) conducted a systematic review and meta-analysis examining the impact of endotoxin in root canal infections. They concluded that there is strong evidence suggesting endotoxin are related to the presence of clinical signs, symptoms, and radiographic features in patients with endodontic infections. The level of endotoxin is

intimately related to the severity of endodontic disease in a patient (33, 36-37). Higher levels of endotoxin have been detected in acute apical abscesses and in cases with the presence of clinical signs and symptoms, such as tenderness to percussion, pain on palpation, swelling, and presence of sinus tract (7, 9, 21, 33, 36-38). Additionally, higher levels of endotoxin in root canal infections are related to greater bone destruction (7, 32-34, 39).

Disinfection of bacteria and endotoxin from root canals

When considering the importance of endotoxin in root canal infections and their relationship with the presence and development of clinical signs and symptoms, an optimal disinfection protocol for root canal therapy should not only reduce the number of bacteria but also the level of endotoxin present in the root canal system. Over the years, different clinical studies have tested the effectiveness of several file systems and instrumentation techniques in reducing bacterial load and levels of endotoxin present in root canal infections. Martinho and Gomes (7) evaluated the effectiveness of 2.5% sodium hypochlorite (NaOCl) using hand-files with step-back technique to reduce bacterial load and endotoxin levels from root canal infections. The authors found a mean bacterial load reduction of 99.78% but only 59.99% in the reduction of endotoxins. Such findings indicated that the chemomechanical preparation with 2.5% NaOCl in step-back technique using hand-files was more effective in reducing bacteria than endotoxin present in root canal infections.

Gomes *et al* (8) then compared the effectiveness of 2.5% NaOCl and 2% chlorhexidine (CHX) gel using hand-files with step-back technique in reducing endotoxins

levels from primarily infected root canals. The authors determined that 2.5% NaOCl (57.98%) was more effective in reducing endotoxins than 2% CHX-gel (47.12%). However, no irrigant was effective in completely eliminating endotoxins from primarily infected root canals. Martinho *et al* (9) investigated the performance of chemomechanical preparation with 2.5% NaOCl + 17% EDTA and Mtwo® rotary NiTi file system in removing endotoxin from primary root canal infection. Analysis revealed that the endotoxin content was significantly reduced by 98.06% after instrumentation, leading to the conclusion that 2.5% NaOCl + 17% EDTA and Mtwo® rotary NiTi system were effective in reducing endotoxin load from primary root canal infection.

Martinho *et al* (10) examined the effectiveness of two single-file reciprocating NiTi systems, Waveone and Reciproc, and two multi-files rotary NiTi systems, ProTaper and Mtwo, in bacterial and endotoxin removal from primarily infected root canals. Data obtained in this study indicated no difference in the median percentage values of endotoxin reduction achieved with single-file reciprocating NiTi systems [Waveone (95.15%) and Reciproc (96.21%)] and with the multi-files rotary NiTi systems [ProTaper (97.98%) and Mtwo (96.34%)]. All four file systems were effective in reducing bacteria with no statistical difference among them. The authors concluded that single-file reciprocating NiTi systems and multi-file rotary NiTi systems tested showed similar effectiveness in reducing endotoxins and bacteria, but no system was able to completely eliminate their presence.

In a similar study, Marinho *et al* (11) evaluated the effectiveness of Reciproc files to remove bacteria and endotoxins from root canals in comparison to multi-file rotary NiTi systems (Mtwo, ProTaper, and Race). All systems yielded highly significant reductions in bacterial loads and endotoxin levels, respectively – Reciproc (99.34% and 91.69%), Mtwo

(99.86% and 83.11%), ProTaper (99.93% and 78.56%) and Race (99.99% and 82.52%). No difference was found among the instrumentation systems tested. The authors determined that the single-file reciprocating NiTi system was just as effective as the multi-file rotary NiTi systems for the removal of bacteria and endotoxins from root canals.

Marinho *et al* (40) evaluated the influence of the apical enlargement size (AES) using 2.5% NaOCl and different sequences of Mtwo® rotary NiTi files in reducing endotoxins from infected root canals. The results demonstrated that a higher percentage of endotoxin reduction was achieved with increased apical enlargement. This suggests that increasing apical enlargement size could predict the reduction of endotoxin levels present in infected root canals.

Machado *et al* (41) investigated the effectiveness of Hyflex CM and ProTaper Next files to remove bacteria and endotoxin from root canal systems. Subjects were randomly divided to receive one of the two file types and samples were taken before and after instrumentation. Both systems were shown to be effective in reducing bacteria and endotoxins in primary endodontic infections with no statistical difference between the systems.

Nickel-Titanium instruments in Endodontics

History and introduction of Nickel-Titanium instruments in Endodontics

Nickel titanium alloy (NiTi), also known as nitinol was developed by W. F. Buehler in 1963 and introduced into dentistry soon afterwards (42). Nickel titanium files, or NiTi files, were first introduced into endodontics by Walia *et al* (43) in 1988 and have dominated endodontics since their debut. NiTi rotary instruments were introduced in the mid-1990s

and resulted in more efficient cutting, less transportation, and better centering during root canal preparation (44). NiTi is superior to stainless steel when comparing shape memory and flexibility and is also more predictable, with less errors during procedures. Shape memory is defined as the ability of the alloy to revert back to its original shape and this occurs when the transition temperature is reached (45). One of the valuable characteristics of NiTi alloy that makes it suitable for endodontics is shape memory.

NiTi exists in three forms: austenite, stress induced martensite and martensite (42). The austenite form is a body-centered cubic lattice, also known as the parent phase. It is a stable phase that typically occurs in high temperature (100°C). Martensite is the daughter phase that exists as a hexagonal lattice. NiTi alloy is more elastic and ductile in the martensitic phase than the austenitic phase. Martensite occurs in cooler temperatures while austenite exists in higher temperatures. The transition temperature range for NiTi alloy depends on composition and manufacturing. Stress-induced martensite is a deformed orientation that occurs during external stress. Phase changes occur either during temperature change or during stress (42).

Properties of the XP-endo Finisher

In order to achieve optimal cleansing of the root canal system, different supplemental disinfection strategies have been proposed in the literature. Recently, for maximum three-dimensional debridement and enhanced irrigation, a new type of anatomical finishing file named XP-endo Finisher (FKG Dentaire, La Chaux-de-Fonds, Switzerland), has been proposed as an adjunct and final step for root canal disinfection after instrumentation with any NiTi file system of a minimum diameter ISO 25. The XP-

endo Finisher is a non-tapered rotary NiTi instrument made of a unique alloy called MaxWire (Martensite-Austenite Electropolish Flex, FKG Dentaire). This file changes its shape according to temperature (Fig 1). At room temperature, in the martensitic phase (M-phase), the file is straight. However, when subjected to body temperature, it enters an austenitic phase (A-phase) and assumes a spoon shape when placed inside the canal in rotation mode. According to the manufacturer, the A-phase shape allows the file to access and clean areas that other instruments might not be able to reach, without damaging dentin or altering the original canal shape (15). There are two sizes of XP-endo sizes of finisher files available on the market. While the XP-endo Finisher consists of a size 25 tip, the XP-endo Finisher R displays a larger core diameter (tip size 30), resulting in a stiffer file to possibly increase efficiency when removing root canal filling material during retreatment procedures.

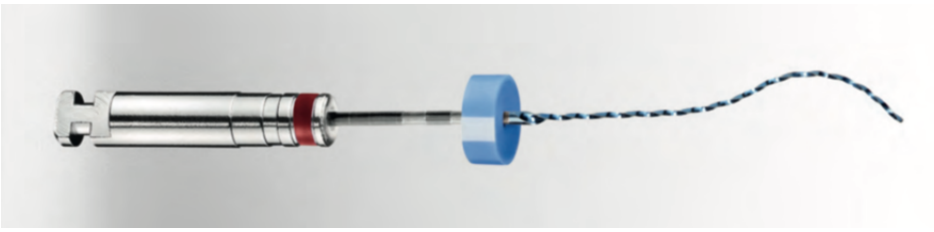

Figure 1. XP-endo Finisher (15)

Previous studies have demonstrated promising results regarding the ability of finisher files to remove smear layer, dental debris, calcium hydroxide, antibiotic pastes, gutta percha, and root filling remnants (46-60). Evidence of bacterial disinfection by the finisher file has been demonstrated in a few *in vitro* studies (56, 61-63) without clinical confirmation. Alves *et al* (56) evaluated the disinfecting ability of chemomechanical

preparation with the adjunctive step of XP-endo Finisher and passive ultrasonic irrigation *in vitro*. Their results indicated that although both supplemental approaches led to a decrease in bacteria presence, only the XP-endo Finisher showed a significant reduction in bacterial count after chemomechanical preparation. Additionally, neither of them was effective in predictably disinfecting the isthmus and recess areas.

Azim *et al* (61) determined the *in vitro* the efficiency of standard needle irrigation, sonically agitating with EndoActivator, XP-endo Finisher, and erbium:yttrium aluminum garnet laser (PIPS) in eliminating bacteria from the root canal system. The results indicated that all four irrigation protocols significantly eliminated bacteria in the canal, ranging in a 89.6%-98.2% reduction. However, the XP-endo Finisher had the highest reduction in bacterial load compared to the other three techniques and was able to eliminate bacteria up to 50 μm into the dentinal tubules. Bao *et al* (62) also evaluated the *in vitro* efficacy of the XP-endo Finisher compared with two different protocols, focusing on biofilm removal from apical root canals. The authors showed that the XP-endo Finisher showed the most efficient biofilm removal efficacy inside and outside the artificial groove followed by passive ultrasonic irrigation and conventional needle irrigation. They concluded that the XP-endo Finisher, as an irrigation agitation technique may help remove biofilm from hard-to-reach areas in the root canal system.

Bedier *et al* (64) evaluated the antibacterial effect of different instrumentation and irrigation protocols using the XP-endo Shaper (XPS) combined with conventional irrigation (XPS/C) or the XP-endo Finisher (XPF) (XPS/XPF) and iRaCe with conventional irrigation (iRaCe/C) or combined with an XPF (iRaCe/XPF). The authors observed an average bacterial reduction of 33.28% for XPS/C, 45.17% for XPS/XPF,

11.81% for iRace/C, and 62.91% for iRaCe/XPF. This study also found that XPF increased bacterial reduction up to a depth of 50 μm in the dentinal tubules when supplemented with the iRaCe system, but XPS combined with XPF did not show a significant difference in bacterial reduction compared to only XPS. Carvalho *et al* (65) compared the XP-endo Finisher (XPF) in addition to Reciproc (RB) or XP-endo Shaper (XPS) in reducing bacterial load in oval shaped canals with 2.5% NaOCl or 0.9% saline solution. The results showed that both RB and XPS effectively reduced bacterial levels while the XPF increased the reduction of bacterial loads in both file systems. 2.5% NaOCl significantly enhanced disinfection compared to saline.

In contrast, several studies have not shown a significant reduction in bacteria or endotoxin following supplemental instrumentation with the XP-endo Finisher. Sasanakul *et al* (63) compared disinfection of large root canals with ultrasonic irrigation, Navitip FX, XP-endo Finisher, and circumferential filing using 1.5% NaOCl in order to determine the most favorable irrigation protocol for regenerative endodontics. They concluded that the adjunctive step using Navitip FX most effectively reduced the number of bacterial without dentin removal. Tufenkçi and Yilmaz (66) investigated the difference *in vitro* in the reduction of *Enterococcus faecalis* using the XP-endo Finisher in teeth that have a traditional access cavity (TEC) compared to a contracted access cavity (CEC) design. The authors found that the CEC-Reciproc-XPF (82.8%) group had the lowest level of bacterial reduction but the access cavity designs lead to similar reductions of *E. faecalis* levels and the supplemental use of XPF did not show any significant differences. It is worthwhile to point out that no current clinical study has evaluated the ability of the XP-endo Finisher to remove bacteria and endotoxin from primarily infected root canals.

Purpose

The aim of this study was to evaluate the ability of the XP-endo Finisher to supplement the removal of bacteria and endotoxin from primary infected root canals after instrumentation.

Hypotheses

1) Null hypothesis: There is no significant difference in bacteria before or after the use of the finisher file.

   General research hypothesis: There is a difference in the bacterial levels when the root canal instrumentation is supplemented with a finisher file as compared to when no finisher file is used.

2) Null hypothesis: There is no significant difference in endotoxin before or after the use of the finisher file.

   General research hypothesis: There is a difference in the endotoxin levels when the root canal instrumentation is supplemented with a finisher file as compared to when no finisher file is used.

Material and Methods

The study design was developed by Dr. Frederico Martinho (FM) and funded by the American Association of Endodontists Foundation (AAEF). The study was completed by endodontic resident Eunice Kim (EK) at the University of Maryland School of Dentistry (UMSOD). Institutional review board (IRB) approval was obtained from the University of Maryland, Baltimore (HP-00082693). All research was conducted at the UMSOD.

Patients were treated in the post-graduate clinic by third year endodontic residents (EK) and Omid Dianat (OD). They were calibrated by an endodontic faculty member (FM). The calibration for the instrumentation technique was completed on extracted teeth. Maxillary and mandibular molar teeth were accessed and instrumented using the experimental protocol.

Patient selection

Eight patients, one molar per patient, presented to the postgraduate endodontic clinic at UMSOD with primary endodontic infection (diagnosed with pulp necrosis) comprised the study population. Radiographic examination confirmed the presence of a periapical radiolucency. A detailed dental and medical history were obtained from each patient. The endodontic faculty member (FM) evaluated the subjects to verify that the inclusion and exclusion criteria were met. Patients, who met the inclusion criteria and wished to participate in the study, were consented by a member of the study team. Endodontic residents, who were a part of the research team, did not consent their own patients to be in the study.

Inclusion criteria included the following:

1. Patient with tooth diagnosed with pulp necrosis and a periapical radiolucency.
2. Treatment provided must be initial root canal treatment.
3. Treatment must be on a maxillary or mandibular molar with intact pulp chamber walls and presence of one palatal or distal canal, respectively.
4. Teeth considered to have a favorable prognosis at the time of treatment.
5. Teeth planned to get permanent restorations at the time of treatment.

6. Patient must be at least 18 year of age or older.
7. If patient is diagnosed with diabetes mellitus, it must be controlled.

Exclusion criteria were as follows:

1. Presence of periodontal pockets greater than 4mm.
2. Teeth that cannot be isolated with a rubber dam.
3. Presence of spontaneous pain.
4. Patients who received antibiotic treatment in the past 3 months.
5. Canals that are bleeding and/or have purulent or serous exudate.
6. Presence of calcified canals.
7. Patients who are medically compromised, e.g. uncontrolled diabetes.

Randomization and blinding were allocated by an endodontist not involved in the study using the method described in the Foundations of Behavioral Research (67). Group assignments were randomly generated by Elaine Romberg (ER) and another individual not involved in the study. The envelopes were numbered in order from one to N and locked in ER's office. When patient number one was identified for the study, the corresponding envelope was given to the provider. The envelope was only revealed to the provider during treatment.

Sample collection

Eight molars were selected and randomly divided into groups (Appendix Table 1). Samples were taken at four separate times: crown swabs to check sterility, before instrumentation, after instrumentation, and after supplement instrumentation.

Clinical procedures

A two-stage access cavity preparation was performed under manual irrigation with sterile saline and by using a sterile high-speed carbide bur (Fig 2A). The first stage involved the removal of contaminants, including carious lesions and restorations. Then the access cavity was disinfected according to the protocol described by Martinho *et al* (7) and a swab sample was taken of the crown.

The method of disinfection for the operative field was previously described (7). Briefly, teeth were isolated with a rubber dam. The crown and surrounding structures were disinfected with 30% hydrogen peroxide (H_2O_2) (volume/volume [V/V]) for 30 seconds, followed by 5.25% NaOCl for 30 seconds, and then inactivated with 5% sodium thiosulfate (Fig 2B). Disinfection of the external surfaces of the crown was checked by taking a swab sample from the crown surface and streaking it onto blood agar plates, which was incubated at 37°C in anaerobic atmosphere.

In the second stage, the pulp chamber was accessed, and the mesial and distal canals in maxillary molars and mesial canals in mandibular molars were sealed with Top dam light curing resin gingival dam (FGM, California) before sampling the palatal canal from upper molars and distal canals from lower molars (Figs 2C and 2D).

Sampling procedure

Sampling was only taken from the palatal (maxillary molars) or distal (mandibular molars) canals. For endotoxin samples, a single, sterile paper point (Dentsply-Maillefer, Balaigues, Switzerland) was introduced to the full length of the canal, which was determined radiographically and retained in position for 60 seconds (Figs 2E and 2F).

Immediately after, the sample was placed in pyrogen-free glass and suspended in 1 mL of *Limulus Amebocyte Lysate* (LAL) water (Lonza, Walkersville, MD, USA) and frozen at −80°C for further quantification of endotoxin by using a kinetic chromogenic LAL kit (KQCL-Kit) (Lonza, Walkersville, MD, USA). For bacterial samples, this sampling procedure was done with three paper points and placed in a sterile tube containing 1 mL of liquid dental transport medium (Anaerobe Systems, Morgan Hill, CA). For all following instrumentation protocols, the canal and the pulp chamber were irrigated with 2.5% NaOCl. After determining working length (WL) with an electronic apex locator (Root ZX II, J. Morita, USA) and establishing patency by taking a #10 K-file past the canal terminus, root canal instrumentation was completed using the files according to manufacturer instructions.

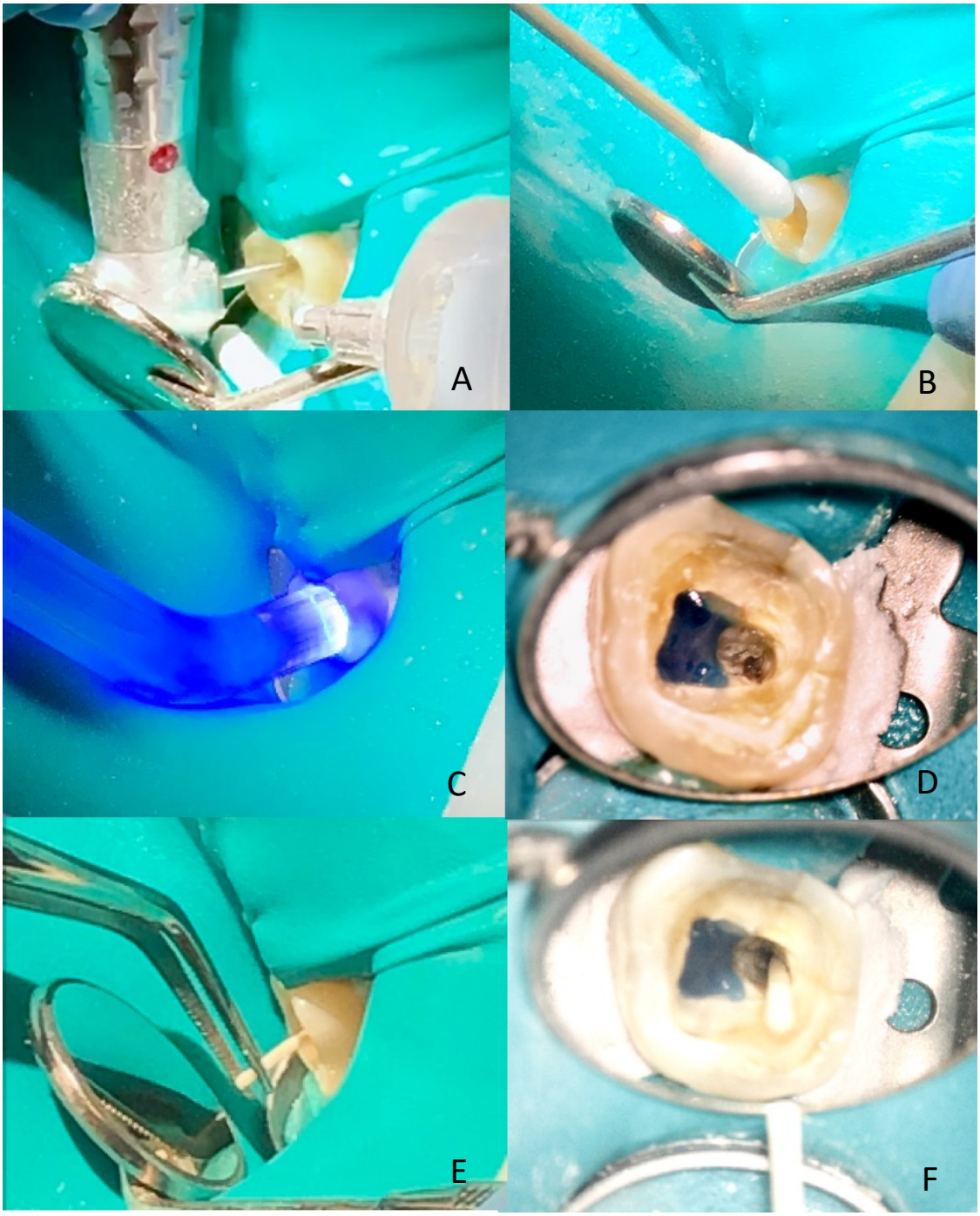

Figure 2. Crown access, disinfection, and root canal sampling. A. Two-step access with syringe irrigation; **B.** Swab decontamination; **C.** Light curing Top dam; **D.** Canals sealed with Top Dam resin; **E.** Root canal sampling with paper point from the buccal view; **F.** Paper point placed in position in the canal.

Instrumentation procedure

Root canals were instrumented using Vortex Blue and XP-endo Shaper file according to the protocol described below:

Vortex Blue rotary groups: the canal initially was irrigated using 2.5% NaOCl, hand instrumented to #15 K-file, and crown-down technique to size 40/.04 was taken to resistance or WL (500 rpm, 1.5Ncm). If resistance was encountered before WL was achieved, the operator moved on to smaller sized instruments until WL was achieved. Between instruments, a #10 K-file was used to maintain patency and root canals were irrigated with 2.5% NaOCl. Then, NaOCl was inactivated with 5 mL sterile 0.5% sodium thiosulfate for one-minute and then removed with 5 mL sterile saline. Bacterial and endotoxin samples were taken after instrumentation.

XP-endo Shaper groups: the canal initially was irrigated, and a glide path was created using a #15 K-file followed by Scout files 17/.04 (FKG Dentaire) (400 rpm, 1.5Ncm) used to the WL. According to the manufacturer's instructions, the tip of the XP-endo Shaper was inserted into the canal, retracted slightly and engaged in rotation mode. With gentle strokes, the file was progressively taken down to WL. If WL is not reached in three to five strokes, stop instrumentation, irrigate the canal, recapitulate and proceed again to the WL. Once WL was reached, irrigation and instrumentation using gentle movements in and out to WL for another ten strokes was performed. After, NaOCl was inactivated with 5 mL sterile 0.5% sodium thiosulfate during a one-minute period, which was then removed with 5 mL sterile saline. Bacterial and endotoxin samples were taken after instrumentation.

Supplemental instrumentation- XP-endo Finisher

Following instrumentation, XP-endo Finisher was used to perform a supplementary cleaning approach. All finisher files were placed in a contra-angle hand piece (VDW), cooled for a few seconds (Endo-Ice; Coltene, Cuyahoga Falls, OH), and removed, in rotation mode, from the plastic tube by applying a lateral movement. Each canal was filled with 2.5% NaOCl and the XP-endo Finisher file, in rotation, was inserted. Finisher file was activated (800 rpm and 1 Ncm) for one-minute using slow, gentle lengthwise movements 1mm short of the WL. The Finisher file was brushed against the canal walls during the procedure. Finally, each root canal was irrigated with 5 mL of 2.5% NaOCl and inactivated with 5 mL sterile 0.5% sodium thiosulfate for one-minute and then removed with 5 mL sterile saline. After supplemental instrumentation was completed, bacterial and endotoxin samples were collected. All clinical samples were collected in the Endodontics Division and immediately transported to the laboratory, which were then processed for bacterial and endotoxin analyses.

Laboratory Procedures

Determination of bacterial colony-forming unit counts (CFU/mL)

Bacteria was cultured for analysis as previously reported (7). Briefly, transport media containing root canal samples was thoroughly vortexed for 60 seconds and serially diluted in tubes up to 10^{-4} containing anaerobe broth (FAB; Lab M, Bury, UK) (Figs 3A-C). Fifty μL of diluted solution were plated onto 5% defibrinated sheep blood anaerobe agar (FAA; Lab M) using sterile plastic spreaders (Figs 3D-E). Plates were incubated at

37°C in an anaerobic chamber for 14 days (Fig 3F). After incubation, colony-forming units (CFU) were counted (Figs 3G-H).

For the CFU count, the following calculation was performed: Step 1. The amount plated (e.g. 0.05 mL) was multiplied by the dilution factor (e.g. 0.01) to yield the overall CFU count; Step 2. Divided the CFU from the dilution (e.g. 69) by the result from step 1 (0.0005) to yield 138,000 CFU. This means that the original 1 mL of sample that was diluted contains 138,000 CFU, which can be expressed as 138,000 CFU/mL or 1.38×10^5 CFU/ mL.

Culture plates were made using Brain heart infusion (BHI; Sigma-Aldrich, St. Louis, MO) media supplemented with Vit K/Hemin/5% sheep blood. BHI media was made by adding 24.5g of BHI powder to 500ml of water. The agar mixture was autoclaved at 121°C for 15 minutes. The Vitamin K (1mg/mL) was prepared with 0.1g Vit K in 100mL of 100% alcohol and kept in darkness. Hemin solution (5mg/mL) was prepared by mixing 0.5g Hemin in 10mL of 1N sodium hydroxide and added to distilled water to volumize to 100mL. After autoclaving the BHI media, Vit K, Hemin, and 5% blood sheep were added, and plates poured.

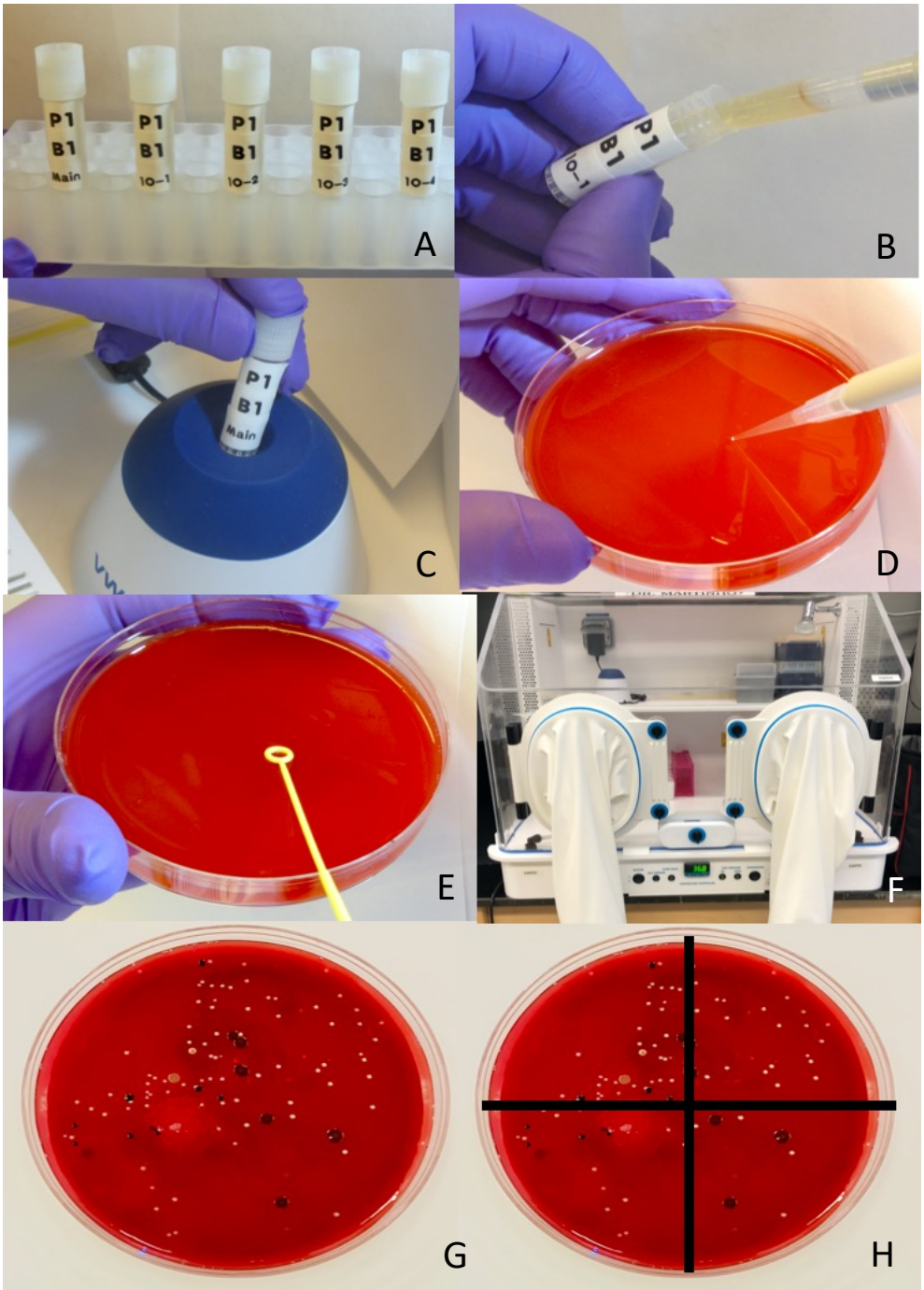

Figure 3. Laboratory procedures. A, B, C. Serial dilution of bacteria samples. **D, E.** Inoculation of bacterial samples in agar plates. **F.** Incubation in anaerobic chamber. **G.** Bacteria on agar plates after 14 days of incubation in anaerobic chamber. **H.** Bacterial CFU count on agar plate.

Quantification of endotoxin (EU/mL) – KQCL Test

The kinetic chromogenic LAL assay (Lonza, Walkersville, MD, USA) was used for the quantification of endotoxin. The KQCL-assay calculated the concentration of endotoxin in unknowns by comparison to the performance of a series of endotoxin standards.

Sample preparation

Root canal samples collected after access, after instrumentation and after supplemental instrumentation with the finisher files were removed from -80°C freezer and reconstituted with 1 mL of LAL reagent water (Lonza, Walkersville, MD, USA). Endotoxin present in the paper points were extracted under mechanical agitation for sixty seconds using a vortex mixer. After, the endotoxin samples were incubated in a water bath at 37°C prior to reagent preparation.

Reagent preparation/ preparation of standard curve

All reagents were removed from the freezer and equilibrated to room temperature prior to use.

In order to calculate endotoxin concentrations in each sample, the KQCL test must be referenced to a valid standard curve. For the standard curve, a series of endotoxin dilution from the endotoxin supplied in the kit was performed as followed (Table 1):

1. A solution containing 5.0 EU/ml endotoxin was prepared by adding 0.1 ml of the 50.0 EU/ml endotoxin stock into 0.9 ml of LAL reagent water. This solution was then vigorously vortexed for at least 1 minute before proceeding.

2. For the preparation of 0.5 EU/mL solution, 0.1 ml of the 5.0 EU/ml endotoxin solution was transferred into 0.9 ml of LAL reagent water. This solution was then vigorously vortexed for at least 1 minute before proceeding.
3. For the preparation of 0.05 EU/mL solution, 0.1 ml of the 0.5 EU/ml endotoxin solution was transferred into 0.9 ml of LAL reagent water in a suitable container and labeled 0.05 EU/ml. This solution was then vigorously vortexed for at least 1 minute before proceeding.
4. For the preparation of 0.005 EU/mL solution, 0.1 ml of the 0.05 EU/ml endotoxin solution was transferred into 0.9 ml of LAL reagent water. This solution was then vigorously vortexed for at least 1 minute before proceeding.

Table 1. Series of endotoxin dilution for the standard curve for KQCL test.

Endotoxin Concentration (EU/mL)	Volume of LAL Reagent Water	Volume of Endotoxin Solution Added to LAL Water
50	-	-
5.0	0.9 mL	0.1 ml of 50.0 EU/mL solution
0.5	0.9 mL	0.1 ml of 5.0 EU/mL solution
0.05	0.9 mL	0.1 ml of 0.5 EU/mL solution
0.005	0.9 mL	0.1 ml of 0.05 EU/mL solution

Test procedure

For the test procedure, a 96-well template was created in the WinKQCL™ Software (Fig 4). The assay type was selected as Kinetic-QCL with the following default template parameters (Delta t = 150 seconds; Measurement filter = 405 nm; Delta mOD = 200; Number of reads = 40). Initially, 100 µL of the LAL reagent water blank, endotoxin standards (0.005, 0.05, 0.5, 5.0, 50 EU/mL), samples, and samples + spike were added to appropriate wells of the 96-well microplate according to the following template (Fig 4). After, the plate was pre-incubated for 10 min at 37°C. Near the end of the pre-incubation period, the Kinetic-QCL reagent vials were reconstituted with 2.6 mL LAL reagent water and mixed gently. Next, 100 µL of the Kinetic-QCL reagent was added to all of the wells of the microplate in the first column (A1-H1), continuing in sequence until the last column. The reagent was added quickly as recommended in the kit instructions and the WinKCL software initiated the test. Replicate samples were run in order to establish good technique and low coefficient of variation. In order to verify possible product inhibition from each testing samples with the LAL reaction, samples were spiked with 10 µL of the 5.0 EU/ml solution into each well as indicated in the figure below according to the manufacturer instructions.

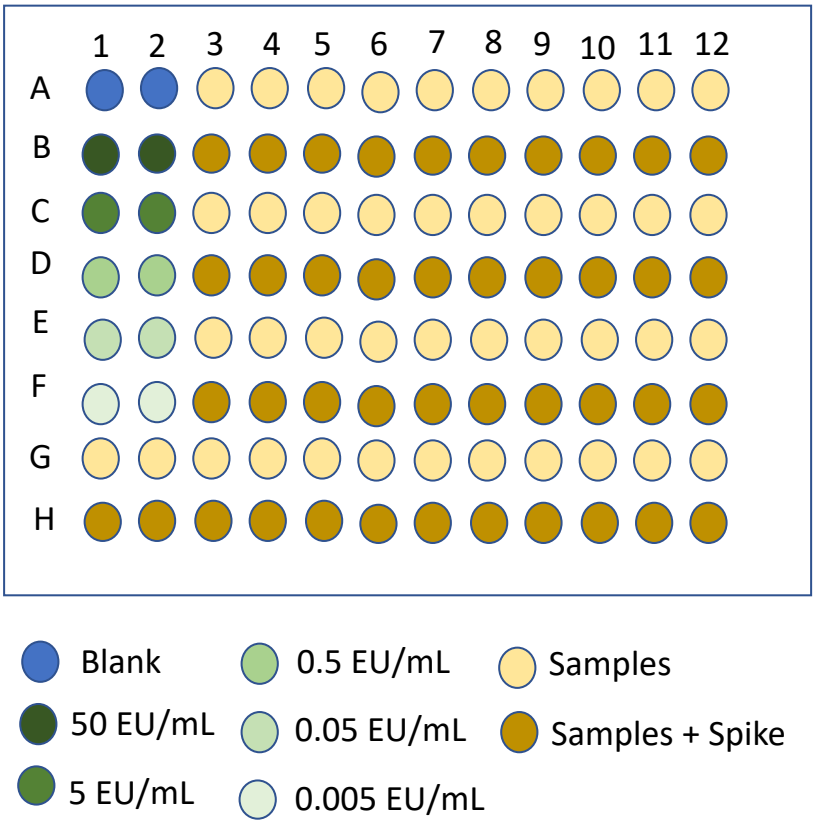

Figure 4. Endotoxin Assay. 96 well plate template for the endotoxin assay containing blank, standard curve concentrations (50, 5, 0.5, 0.05, 0.0005 EU/mL), samples, and samples + spike.

Calculation of endotoxin concentration

From the beginning of the kinetic test and continuously throughout the assay, the microplate reader/WinKQCL™ Software monitored the absorbance at 405 nm of each well of the microplate. Using the initial absorbance reading of each well as its own blank, the reader determined the time required for the absorbance to increase 0.200 absorbance units. The WinKQCL™ Software automatically performed a log/log linear correlation with r= .999 (Fig 5).

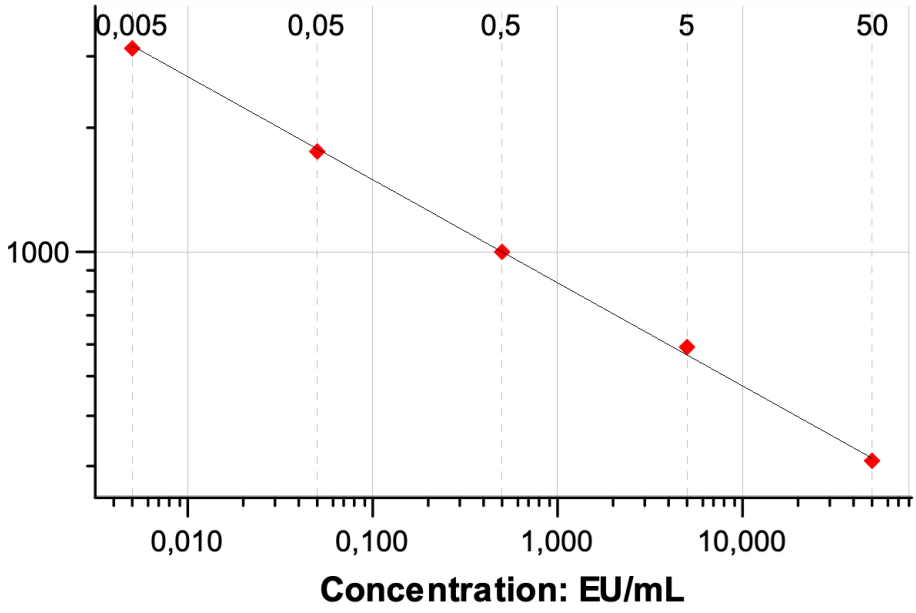

Figure 5. Calibration of endotoxin values, r= .999.

Power analysis

Post hoc power analysis (Javastats.com) was used to determine whether a sufficient number of subjects were utilized in this study.

Statistical analysis

The data collected (CFU/mL and EU/mL) were analyzed using Statistical Package for Social Sciences (SPSS software, IBM 2020; SPSS Institute Inc., Cary, NC, USA). Paired t-tests were performed to compare the number of bacteria and endotoxin before and after supplemental instrumentation with the finisher file. A $p \leq .025$ was considered significant (two-tailed test).

Results

This study investigated the ability of the XP-endo Finisher file, after instrumentation, to supplement the removal of bacteria and endotoxins. Initially, to test for sterility, swab samples of the crown surface were taken before accessing into the pulp chamber. All samples had no bacterial growth, validating the disinfection protocol for the study (Appendix Table 2). Bacteria and endotoxins were both initially present in the root canals, before instrumentation, of all teeth (Appendix Table 2).

Bacterial analyses

The level of bacteria before and after supplemental instrumentation with the XP-endo Finisher is presented in Table 2. Without finisher file usage, bacterial levels ranged from 0-800 CFU/mL. After supplemental instrumentation with the XP-endo Finisher, one root canal (Subject 2, 12.5%) still contained recoverable bacteria. After supplemental instrumentation with the XP-endo Finisher, the mean value of bacteria present in root canal infections was reduced from 255 ± 311.82 CFU/mL to 2.5 ± 7.07 CFU/mL ($t= 2.286$, $p= .056$). There was no significant difference in bacterial levels with and without the finisher file. However, the use of finisher files increased the number of root canals yielding negative bacterial culture from two to seven out of eight.

Post hoc power for this analysis was .48. The difference between the actual results before use of the finisher file and after the use of the finisher file was 232.5. The minimal detectable difference needed to find significance was 258.97. It was suggested that a second required sample size was n=8.

Table 2. Comparison of bacterial levels (CFU/mL) before use of finisher file vs. after use of finisher file.

Subject Number	Before use of finisher file (CFU/mL)	After use of finisher file (CFU/mL)	Matched pairs t	p	Post hoc power analysis
1	400	0	2.286	.056*	.48
2	200	20			
3	600	0			
4	20	0			
5	0	0			
6	800	0			
7	0	0			
8	20	0			
	255 ± 311.82	2.5 ± 7.07			
	Mean CFU/mL ± SD				

*Significant p value, p≤.025

Endotoxin Analyses

The level of endotoxin before and after supplemental instrumentation with the XP-endo Finisher file is presented in Table 3. After instrumentation but before finisher file use, endotoxin levels ranged from .55 to 1.32 EU/mL. All eight root canals still contained recoverable endotoxin after supplement instrumentation with the finisher files. There was a mean endotoxin level of .85 ± .26 for no finisher file and .03 ± .01 with a finisher file. There was a significant difference in endotoxin levels after instrumentation when the finisher file was included. The finisher files removed significantly more endotoxin in the canals than conventional rotary instruments alone (t= 9.063, p= .00004). Post hoc power for this analysis was .98 (Table 3). It was suggested that a power of n=3 would be needed to replicate the result in a second analysis.

Table 3. Comparison of endotoxin levels (EU/mL) before use of finisher file vs. after use of finisher file.

Subject number	Before use of finisher file (EU/mL)	After use of finisher file (EU/mL)	Matched pairs t	p	Post hoc power analysis
1	1.06	0.05	9.063	.00004	.98
2	0.83	0.03			
3	0.56	0.03			
4	0.71	0.02			
5	0.93	0.03			
6	1.32	0.02			
7	0.80	0.04			
8	0.55	0.01			
	.85 ± .26	.03 ± .01			
	Mean EU/mL ± SD				

*Significant p value, p≤.025

Discussion

Currently, chemical, and mechanical debridement help reduce bacterial load that persists within the root canal system, but incomplete disinfection continues to be a challenge in endodontic treatment. Innovative rotary files continue to be developed to improve mechanical debridement and contribute to significant decreases in bacterial load, creating optimal environment for healing. The XP-endo Finisher was introduced into endodontics as a supplemental instrument to be utilized following conventional instrumentation. The present study evaluated the ability of the XP-endo Finisher to supplement the removal of bacteria and endotoxin from primarily infected root canals after instrumentation. Data obtained in this study showed that there was no significant difference in bacterial reduction with and without the finisher file. However, the XP-endo Finisher

supplemented the removal of endotoxin after root canal instrumentation in primary endodontic cases.

In this study, a two-stage access cavity preparation was performed. The first stage involved the removal of contaminants, including carious lesions and restorations. Then the access cavity was disinfected according to the protocol described by Martinho *et al* (7). A swab sample was taken of the crown and streaked onto blood agar to verify disinfection. All samples had no bacterial growth, validating the disinfection protocol for the study. In the second stage of the access, the pulp chamber was accessed and the mesial and distal canals in maxillary molars and mesial canals in mandibular molars were sealed. The samples were taken from the palatal canal of the maxillary molars and distal canal of the mandibular molars. These canals were selected due to their larger size (85-86), which assisted the placement of the paper point before instrumentation.

In this research, bacteria and endotoxin were collected with a paper point technique, which is the most widely used technique for microbiological root canal sampling (7, 10-13). Paper points are manufactured into a standard shape that fits the shape of the canal, facilitating the sampling procedure. In contrast, a disadvantage of the paper point technique is that it can only sample the lumen of the canal and cannot access microorganisms in areas such as isthmuses, dentinal tubules, and fins (83). This may produce false-negative results and underestimate the presence of bacteria inside the canals.

For the root canal sampling, a sterile paper point was introduced into the full-length of the canal and retained in position for 60 seconds. The endotoxin sample was performed first, followed by the bacterial sample. The root canals were sampled at three different

times: before instrumentation, after instrumentation, and after supplemental instrumentation with the XP-endo Finisher.

Bacteria were detected in all root canal samples taken before instrumentation. The level of bacteria in primary endodontic infection with apical periodontitis ranged from 42,000 to 122,000 CFU/mL, which is comparable to previous studies (7, 68-69). The chemomechanical protocols using Vortex Blue or XP-endo Shaper files and irrigation with 2.5% NaOCl were effective in reducing the bacterial load to a mean CFU/mL value of 255 ± 311.82, ranging from 0-800 CFU/mL. Two out of eight canals showed no microbial growth after chemomechanical preparation. Conventional root canal instrumentation and irrigation with 2.5% NaOCl was the most effective step in reducing bacterial load from infected root canals, which has been demonstrated in previous studies (7-11, 40-41, 64-65, 71).

After supplemental instrumentation with the XP-endo Finisher, the mean value of bacteria was reduced from 255 ± 311.82 CFU/mL to 2.5 ± 7.07 CFU/mL. There was no significant difference in bacterial levels before and after supplemental instrumentation with the finisher file; thus, the null hypothesis was accepted. Despite no difference in the levels of bacterial reduction, the use of finisher files increased the number of root canals yielding negative bacterial culture from two to seven out of eight canals. The sample that yielded a positive culture was taken from a maxillary second molar. Maxillary second molars have complex anatomy, including isthmuses, ramifications, accessory, and lateral canals. According to Tomaszewaska *et al* (72), 21.9% of maxillary second molars showed the presence of lateral canals in the palatal canal, which may not be accessible even with finisher files. The limited ability of XP-endo Finisher in disinfecting isthmuses and recess

areas has been demonstrated by Alves *et al* (56). Considering the limited ability of the XP-endo Finisher in completely eliminating bacteria from root canal system, the null hypothesis presented in this study was accepted.

Although there is no established threshold for how much bacteria can be left in the canal while having a healed outcome, Sjögren *et al* (84) reported that the success rate of endodontic treatment is 26% higher if the root canal is bacteria free at the time of root canal obturation. However, Peters *et al* (79) and Molander *et al* (80) could not find a significant difference in healing outcomes based upon the presence or absence of bacteria at obturation. Despite no established threshold, there may be clinical importance with the findings even though no significant difference was found. We did not find significant difference due to the large standard deviation before and after supplemental instrumentation with the finisher file. It should be noted that the XP-endo Finisher did eliminate a considerable number of bacteria.

Controversy exists on the ability of XP-endo Finisher to increase root canal cleaning and disinfection (56, 61-62, 64, 66). Alves *et al* (56) evaluated the disinfecting ability of chemomechanical preparation with the adjunctive step of XP-endo Finisher and passive ultrasonic irrigation *in vitro*. Their results indicated that only the XP-endo Finisher showed a significant reduction in bacterial count after chemomechanical preparation. Azim *et al* (61) reported that the XP-endo Finisher had the highest reduction in bacterial load compared to the other three techniques and was able to eliminate bacteria up to 50 μm into the dentinal tubules. Bedier *et al* (64) also found that the XP-endo Finisher increased bacterial reduction up to a depth of 50 μm in the dentinal tubules when supplemented with the iRaCe system. However, the authors reported that the supplemental use of XP-endo

Finisher did not show a significant difference in bacterial reduction when supplemented with the XP-endo Shaper. Bao *et al* (62) showed that the XP-endo Finisher showed the most efficient biofilm removal efficacy inside and outside the artificial groove when compared to passive ultrasonic irrigation and conventional needle irrigation. More recently, Tufenkçi and Yilmaz (66) showed that the supplemental use of the XP-endo Finisher did not show significant difference in the reduction of *Enterococcus faecalis* in teeth that had a traditional access cavity (TEC) versus a contracted access cavity (CEC) design. This study also found no significant reduction in bacterial count after supplemental instrumentation with the Finisher.

It is important to highlight that this study is the first randomized and blinded controlled trial to evaluate the ability of the XP-endo Finisher in supplementing bacteria and endotoxin removal from primarily infected root canals. Randomization and blinding were performed to minimize risk of bias. The researcher who processed the samples was double-blinded and the subjects were single-blinded in this study. All operators were aware of the instruments used only at the time of treatment.

Different endotoxin assays applying the LAL principle have been used for the detection of endotoxin in root canal infections. The kinetic chromogenic LAL assay (KQCL test) for this study was selected according to Martinho *et al* (73). This study compared different LAL assays for the detection of endotoxin in root canal infections, determining that the KQCL test was one of the superior methods for endotoxin analysis, allowing for precision and better reproducibility. The KQCL test used in the present study was a kinetic chromogenic LAL assay that used synthetic peptide-pNA chromogenic substrate, which was cleaved by a clotting enzyme, imparting a yellow color to the solution.

The strength of the yellow color was determined at an optical density (OD) = 405 nm (73). In this method, the OD was read at multiple time points because the reaction proceeded with no termination step (z ≈ 60 minutes), allowing the concentration of endotoxin to be quantified over a broader range of sensitivity (0.005-50 EU/mL) when compared to other LAL methods (73).

Endotoxin was detected in all root canal samples in this study. The mean levels of endotoxin recovered from primary root canal infection ranged between 16.42 and 47.20 EU/mL, which were similar to findings from previous studies on primary endodontic infection (7, 68). The variation of endotoxin concentration is partly due to the nature of endodontic infections, indicating whether there is a higher amount of gram-negative species. Studies have demonstrated that increased endotoxin levels can be detected in patients with clinical symptomatology (21, 32-35, 37-38, 76) and greater areas of bone destruction in periapical tissues (29, 76).

After supplemental instrumentation with the XP-endo Finisher, the mean value of endotoxin was significantly reduced from .85 ± .26 EU/mL to .03 ± .01 EU/mL ($p= 0.00004$). Therefore, the general research hypothesis for endotoxin reduction presented in this study was accepted. To the best of our knowledge, there are no *in vitro* or *in vivo* studies examining the effect of the XP-endo Finisher on endotoxin levels and this study is the first *in vivo* study examining this. Although minimum amounts of endotoxin were still detected in all root canal samples after conventional instrumentation and after supplemental instrumentation with the XP-endo Finisher, there is no established threshold for how much endotoxin can be left in the canal while having a healed outcome. Despite this fact, successful primary endodontic therapy is achievable in 88% of root canal treatments (78).

Although there is no established threshold for the concentration of endotoxin that can have healing, a study by Hausmann found that a low concentration of 30 EU/ml was able to stimulate bone resorption in tissue culture (87). It is important to mention that despite the limited number of samples analyzed in this study, the post hoc power analysis of .98 showed that the ability of the XP-endo Finisher in reducing endotoxins were reliable.

Future studies

Future randomized clinical trials should be aimed at evaluating the supplemental use of irrigants with the XP-endo Finisher in facilitating the removal of bacteria and endotoxin from the root canal system. Due to non-significant results of bacterial reduction in this study, future studies should again examine whether the finisher file is more effective at reducing bacteria than without the finisher file.

Conclusion

In conclusion, the findings of this study showed that the supplemental use of the XP-endo Finisher after root canal instrumentation was effective in significantly reducing endotoxin but not bacteria present in primary endodontic infections.

Appendix

Appendix Table 1. Distribution of the groups to be tested according to the instrumentation protocol

Groups	Description
G1	XP-endo Shaper file + XP-endo Finisher (25/.00)
G2	XP-endo Shaper file+ XP-endo Finisher R (30/.00)
G3	Vortex blue + XP-endo Finisher (25/.00)
G4	Vortex blue + XP-endo Finisher R (30/.00)

Appendix Table 2. Results: sterility test and before instrumentation

Subject number	Sterility test results (CFU/mL)	Analysis before instrumentation	
		Bacterial levels (CFU/mL)	Endotoxin levels (EU/mL)
1	0	96,000	44.02
2	0	74,000	18.61
3	0	106,000	27.50
4	0	72,000	16.42
5	0	42,000	25.92
6	0	122,000	47.20
7	0	58,000	24.98
8	0	66,000	25.01

References

1) Kakehashi S, Stanley HR, Fitzgerald RJ. The Effects of Surgical Exposures of Dental Pulps in Germ-Free and Conventional Laboratory Rats. Oral Surg Oral Med Oral Pathol 1965;20:340-9.

2) Nair PN. On the causes of persistent apical periodontitis: a review. Int Endod J 2006;39:249-81.

3) Peters OA, Laib A, Gohring TN, Barbakow F. Changes in root canal geometry after preparation assessed by high-resolution computed tomography. J Endod 2001;27:1–6.

4) Gulabivala K, Patel B, Evans G, Ng YL. Effects of mechanical and chemical procedures on root canal surfaces. Endod Topics 2005;10:103–22.

5) DeDeus QD. Frequency, location and direction of the lateral, secondary, and accessory canals. J Endod 1975;1:361-6.

6) Lacerda MFLS, Marceliano-Alves MF, Perez AR, Provenzano JC, Neves MAS, Pires FR, Goncalves LS, Rocas IN, Siquira FJ Jr. Cleaning and shaping oval canals with 3 instrumentation systems: a correlative micro-computed tomographic and histologic study. J Endod 2017;43:1878-1884.

7) Martinho FC, Gomes BP. Quantification of endotoxins and cultivable bacteria in root canal infection before and after chemomechanical preparation with 2.5% sodium hypochlorite. J Endod 2008;34:268-72.

8) Gomes BP, Martinho FC, Vianna ME. Comparison of 2.5% sodium hypochlorite and 2% chlorhexidine gel on oral bacterial lipopolysaccharide reduction from primarily infected root canals. J Endod 2009;35:1350-3.

9) Martinho FC, Chiesa WM, Marinho AC, Zaia AA, Ferraz CC, Almeida JF, Souza-Filho FJ, Gomes BP. Clinical investigation of the efficacy of chemomechemical preparation with rotary nickel-titanium files for removal of endotoxin from primarily infected root canals. J Endod 2010;36:1766-9.

10) Martinho FC, Gomes AP, Fernandes AM, Ferreira NS, Endo MS, Freitas LF, Camões IC. Clinical comparison of the effectiveness of single-file reciprocating systems and rotary systems for removal of endotoxins and cultivable bacteria from primarily infected root canals. J Endod 2014;40:625-9.

11) Marinho AC, Martinho FC, Gonçalves LM, Rabang HR, Gomes BP. Does the Reciproc file remove root canal bacteria and endotoxins as effectively as multifile rotary systems? Int Endod J 2015;48:542-8.

12) Herrera DR, Martinho FC, de-Jesus-Soares A, Zaia AA, Ferraz CCR, Almeida JFA, Gomes BPFA. Clinical efficacy of EDTA ultrasonic activation in the reduction of endotoxins and cultivable bacteria. Int Endod J 2017;50:933-40.

13) Rabello DGD, Corazza BJM, Ferreira LL, Santamaria MP, Gomes APM, Martinho FC. Does supplemental photodynamic therapy optimize the disinfection of bacteria and endotoxins in one-visit and two-visit root canal therapy? A randomized clinical trial. Photodiagnosis Photodyn Ther. 2017;19:205-211.

14) Martinho FC, Gomes CC, Nascimento GG, Gomes APM, Leite FRM. Clinical comparison of the effectiveness of 7- and 14-day intracanal medications in root canal disinfection and inflammatory cytokines. Clin Oral Investig 2018;22:523-530.

15) FKG Dentaire SA The XP-endo Finisher file brochure. Available at: http://www.fkg.ch/ products/endodontics/final-preparation/xp-endo-finisher. Accessed December 1, 2016.

16) Bystrom A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. Scand J Dent Res 1981;89:321-8.

17) Sundqvist G. Ecology of the root canal flora. J Endod 1992;18:427-30.

18) Sundqvist G. Taxonomy, etiology, and pathogenicity of the root canal flora. Oral Surg Oral Med Oral Pathol 1994;78:522-30.

19) Siqueria JF, Flavio RF, Alves FR, Rocas IN, Pyrosequencing analysis of the apical root canal microbiota. J Endod 2011;1499-1503.

20) Murad CF, Sassone LM, Faveri M, Hirata R Jr, Figueiredo L, Feres M. Microbial diversity in persistent root canal infections investigated by checkerboard DNA-DNA hybridization. J Endod 2014;40:899-906.

21) Martinho FC, Leite FR, Chiesa WM, Nascimento GG, Feres M, Gomes BP. Signaling pathways activation by primary endodontic infectious contents and production of inflammatory mediators. J Endod 2014;40:484-9.

22) Ferreira NS, Martinho FC, Cardoso FG, Nascimento GG, Carvalho CA, Valera MC. Microbiological profile resistant to different intracanal medications in primary endodontic infections. J Endod 2015;41:824-30.

23) Gomes BP, Berber VB, Kokaras AS, Chen T, Paster BJ. Microbiomes of Endodontic-Periodontal Lesions before and after Chemomechanical Preparation. J Endod 2015;41:1975-84.

24) Keskin C, Demiryürek EÖ, Onuk EE. Pyrosequencing Analysis of Cryogenically Ground Samples from Primary and Secondary/Persistent Endodontic Infections. J Endod 2017;43:1309-16.

25) Bouillaguet S, Manoil D, Girard M, Louis J, Gaïa N, Leo S, Schrenzel J, Lazarevic V. Root Microbiota in Primary and Secondary Apical Periodontitis. Front Microbiol 2018;9:2374.

26) Sperandeo P, Martorana AM, Polissi A. Lipopolysaccharide biosynthesis and transport to the outer membrane of gram-negative bacteria. Subcell Biochem 2019;92:9-37.

27) Dahlén G. Immune response in rats against lipopolysaccharides of Fusobacterium nucleatum and Bacteroides oralis administered in the root canal. Scand J Dent Res 1980;88:122-9.

28) Horiba N, Maekawa Y, Abe Y, Ito M, Matsumoto T, Nakamura H, Ozeki M. Cytotoxicity against various cell lines of lipopolysaccharides purified from Bacteroides, Fusobacterium, and Veillonella isolated from infected root canals. J Endod 1989;15:530-4.

29) Hong CY, Lin SK, Kok SH, Cheng SJ, Lee MS, Wang TM, Chen CS, Lin LD, Wang JS. The role of lipopolysaccharide in infectious bone resorption of periapical lesion. J Oral Pathol Med 2004;33:162-9.

30) Martinho FC, Leite FR, Nóbrega LM, Endo MS, Nascimento GG, Darveau RP, Gomes BP. Comparison of Fusobacterium nucleatum and Porphyromonas gingivalis Lipopolysaccharides Clinically Isolated from Root Canal Infection in the Induction of Pro-Inflammatory Cytokines Secretion. Braz Dent J 2016;27:202-7.

31) Schein B, Schilder H. Endotoxin content in endodontically involved teeth. J Endod 1975;1:19-21.

32) Horiba N, Maekawa Y, Abe Y, Ito M, Matsumoto T, Nakamura H. Correlations between endotoxin and clinical symptoms or radiolucent areas in infected root canals. Oral Surg Oral Med Oral Pathol 1991;71:492-5.

33) Jacinto RC, Gomes BP, Shah HN, Ferraz CC, Zaia AA, Souza-Filho FJ. Quantification of endotoxins in necrotic root canals from symptomatic and asymptomatic teeth. J Med Microbiol 2005;54:777-83.

34) Cardoso FG, Ferreira NS, Martinho FC, Nascimento GG, Manhães LR Jr, Rocco MA, Carvalho CA, Valera MC. Correlation between Volume of Apical Periodontitis Determined by Cone-beam Computed Tomography Analysis and

Endotoxin Levels Found in Primary Root Canal Infection. J Endod 2015;41:1015-9.

35) Martinho FC, de Rabello DGD, Ferreira LL, Nascimento GG. Participation of endotoxin in root canal infections: A systematic review and meta-analysis. Eur J Dent 2017;11:398-406.

36) Martinho FC, Chiesa WM, Leite FR, Cirelli JA, Gomes BP. Correlation between clinical/radiographic features and inflammatory cytokine networks produced by macrophages stimulated with endodontic content. J Endod 2012;38:740-5.

37) Sousa EL, Martinho FC, Nascimento GG, Leite FR, Gomes BP. Quantification of endotoxins in infected root canals and acute apical abscess exudates: monitoring the effectiveness of root canal procedures in the reduction of endotoxins. J Endod 2014;40:177-81.

38) Gomes BP, Endo MS, Martinho FC. Comparison of endotoxin levels found in primary and secondary endodontic infections. J Endod 2012;38:1082-6.

39) Martinho FC, Chiesa WM, Leite FR, Cirelli JA, Gomes BP. Antigenic activity of bacterial endodontic contents from primary root canal infection with periapical lesions against macrophage in the release of interleukin-1beta and tumor necrosis factor alpha. J Endod 2010;36:1467-74.

40) Marinho AC, Martinho FC, Zaia AA, Ferraz CC, Gomes BP. Influence of the apical enlargement size on the endotoxin level reduction of dental root canals. J Appl Oral Sci 2012;20:661-6.

41) Machado CAD, Souza ACA, Loureiro C, Martinho FC, Cinta LTA, Dezan Jr E, Jacinto RC. Comparison of two rotary systems in bacteria/lps removal from endodontic infections: randomized clinical trial. Braz Oral Res. 2019;33:e039.

42) Thompson SA. An overview of nickel-titanium alloys used in dentistry. Int Endod J 2000;33:297–310.

43) Walia HM, Brantley WA, Gerstein H. An initial investigation of the bending and torsional properties of Nitinol root canal files. J Endod 1988;14:346-51.

44) Glosson CR, Haller RH, Dove SB, del Rio CE. A comparison of root canal preparations using Ni-Ti hand, Ni-Ti engine-driven, and K-Flex endodontic instruments. J Endod 1995;21:146-51.

45) Wang FE, Pickart SJ, Alperin HA. Mechanism of the TiNi martensitic transformation and the crystal structures of TiNi-II and TiNi-III phases. J Appl Phys 1972;43:97 –112.

46) Zand V, Mokhtari H, Reyhani MF, et al. Smear layer removal evaluation of different protocol of Bio Race file and XP- endo Finisher file in corporation with EDTA 17% and NaOCl. J Clin Exp Dent 2017;9:1310-1314.

47) Leoni GB, Versiani MA, Silva-Sousa YT, et al. Ex vivo evaluation of four final irrigation protocols on the removal of hard-tissue debris from the mesial root canal system of mandibular first molars. Int Endod J 2017;50:398-406.

48) Turkaydin D, Demir E, Basturk FB, Sazak Övecoglu H. Efficacy of XP-Endo Finisher in the Removal of Triple Antibiotic Paste from Immature Root Canals. J Endod 2017;43:1528-1531.

49) Wigler R, Dvir R, Weisman A, Matalon S, et al. Efficacy of XP-endo finisher files in the removal of calcium hydroxide paste from artificial standardized grooves in the apical third of oval root canals. Int Endod J 2017;50:700-705.

50) Uygun AD, Gündoğdu EC, Arslan H, Ersoy İ. Efficacy of XP-endo finisher and TRUShape 3D conforming file compared to conventional and ultrasonic irrigation in removing calcium hydroxide. Aust Endod J 2017;43:89-93.

51) Gokturk H, Ozkocak I, Buyukgebiz F, Demir O. Effectiveness of various irrigation protocols for the removal of calcium hydroxide from artificial standardized grooves. J Appl Oral Sci 2017;25:290-298.

52) Gokturk H, Bayram E, Bayram HM, Aslan T, et al. Effect of double antibiotic and calcium hydroxide pastes on dislodgement resistance of an epoxy resin-based and two calcium silicate-based root canal sealers. Clin Oral Investig 2017;21:1277-1282.

53) Hamdan R, Michetti J, Pinchon D, Diemer F, et al. The XP-Endo Finisher for the removal of calcium hydroxide paste from root canals and from the apical third. J Clin Exp Dent 2017;9:855-60.

54) Keskin C, Sariyilmaz E, Sariyilmaz Ö. Efficacy of XP-endo Finisher File in Removing Calcium Hydroxide from Simulated Internal Resorption Cavity. J Endod 2017;43:126-130.

55) Wigler R, Dvir R, Weisman A, et al. Efficacy of XP-endo finisher files in the removal of calcium hydroxide paste from artificial standardized grooves in the apical third of oval root canals. Int Endod J 2017;50:700-705

56) Alves FR, Andrade-Junior CV, Marceliano-Alves MF, et al. Adjunctive Steps for Disinfection of the Mandibular Molar Root Canal System: A Correlative Bacteriologic, Micro-Computed Tomography, and Cryopulverization Approach. J Endod 2016;42:1667-1672.

57) Silva EJNL, Belladonna FG, Zuolo AS, et al. Effectiveness of XP-endo Finisher and XP-endo Finisher R in removing root filling remnants: a micro-CT study. Int Endod J 2018;51:86-91.

58) Aksel H, Küçükkaya Eren S, Askerbeyli Örs S, Serper A, Ocak M, Çelik HH. Micro-CT evaluation of the removal of root fillings using the ProTaper Universal Retreatment system supplemented by the XP-Endo Finisher file. Int Endod J 2019;52:1070-1076.

59) Elnaghy AM, Mandorah A, Elsaka SE. Effectiveness of XP-endo Finisher, EndoActivator, and File agitation on debris and smear layer removal in curved root canals: a comparative study. Odontol 2017;105:178-83.

60) Zhao Y, Fan W, Xu T, Tay FR, Gutmann J, Fan B. Evaluation of several instrumentation techniques and irrigation methods on the percentage of untouched canal wall and accumulated dentine debris in C-shaped canals. Int Endod J 2019;52:1354-1365.

61) Azim AA, Aksel H, Zhuang T, Mashtare T, Babu JP, Huang GT. Efficacy of 4 Irrigation Protocols in Killing Bacteria Colonized in Dentinal Tubules Examined by a Novel Confocal Laser Scanning Microscope Analysis. J Endod 2016;42:928-34.

62) Bao P, Shen Y, Lin J, Haapasalo M. In Vitro Efficacy of XP-endo Finisher with 2 Different Protocols on Biofilm Removal from Apical Root Canals. J Endod 2017;43:321-325.

63) Sasanakul P, Ampornaramveth RS, Chivatxaranukul P. Influence of Adjuncts to Irrigation in the Disinfection of Large Root Canals. J Endod 2019;45:332-7.

64) Bedier MM, Hashem AAR, Hassan YM. Improved dentin disinfection by combining different-geometry rotary nickel-titanium files in preparing root canals. Restor Dent Endod 2018;43:e46.

65) Carvalho MC, Zuolo ML, Arruda-Vasconcelos R, Marinho ACS, Louzada LM, Francisco PA, Pecorari VGA, Gomes BPFA. Effectiveness of XP-endo Finisher in the reduction of bacterial load in oval-shaped root canals. Braz Oral Res 2019;33:e021.

66) Tüfenkçi P, Yilmaz K. The effects of different endodontic access cavity design and Using XP-endo Finisher on the reduction of Enterococcus faecalis in the root canal system. J Endod 2020;46:419-24.

67) Kerlinger, F.N. Foundations of behavioral research. 1986; third edition. Holt, Rinehart and Winston.

68) Vianna ME, Hortz H-P, Conrads G, Zaia AA, Souza-Filho FJ, Gomes BPFA. Effect of root canal procedures on endotoxins and endodontic pathogens. Oral Microbiol Immunol 2007;22:411–8.

69) Siqueira JF Jr, Rôças IN, Paiva SS, Guimarães-Pinto T, Magalhães KM, Lima KC. Bacteriologic investigation of the effects of sodium hypochlorite and chlorhexidine during the endodontic treatment of teeth with apical periodontitis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;104:122–30.

70) Vianna ME, Horz HP, Gomes BP, Conrads G. In vivo evaluation of microbial reduction after chemo-mechanical preparation of human root canals containing necrotic pulp tissue. Int Endod J 2006;39:484–92.

71) Alves FR, Pavia PL, Marceliano-Alves MF, Cabreira LJ, Lima KC, Siqueira Jr JF, Rocas IN, Provenzano JC. Bacteria and hard tissue debris extrusion and intracanal bacterial reduction promoted by XP-endo Shaper and Reciproc instruments. J Endod 2018;44:1173-8.

72) Tomaszewaska IM, Jarzebska A, Skinningsrud B, Pekala PA, Wronski S, Iwanaga J. An original micro-CT study and meta-analysis of the internal and external anatomy of maxillary molars- implications for endodontic treatment. Clin Anat 2018;31:838-53.

73) Martinho FC, Chiesa WM, Zaia AA, Ferraz CC, Almeida JF, Souza-Filho FJ, Gomes BP. Comparison of endotoxin levels in previous studies on primary endodontic infections. J Endod 2011;37:163-7.

74) Hurley JC. Endotoxemia: methods of detection and clinical correlates. Clin Micro- biol Rev 1995;8:268–92.

75) Cohen J, McConnell JS. Observations on the measurement and evaluation of endo- toxemia by a quantitative limulus lysate microassay. J Infect Dis 1984;150:916–24.

76) Schein B, Schilder H. Endotoxin content in endodontically involved teeth. J Endod 1975;1:19–21.

77) Xavier AC, Martinho FC, Chung A, Oliveira LD, Jorge AO, Valera MC, Carvalho CA. One-visit versus two-visit root canal treatment: effectiveness in the removal of endotoxins and cultivable bacteria. J Endod 2013;39:959-64.

78) de Chevigny C, Dao TT, Basrani BR, Marquis V, Farzaneh M, Abitbol S, Friedman S. Treatment outcome in endodontics: The Toronto study—Phase 4: Initial treatment. J Endod 2008;34:258–263.

79) Peters LB, Wesselink PR, Moorer WR. The fate and the role of bacteria left in root dentinal tubules. Int Endod J 1995;28:95–9.

80) Molander A, Warfvinge J, Reit C, Kvist T. Clinical and radiographic evaluation of one- and two-visit endodontic treatment of asymptomatic necrotic teeth with apical periodontitis: A randomized clinical trial. J Endod 2007;33:1145–8.

81) Teles AM, Manso MC, Pina C, Cabeda J. A review of microbiological root canal sampling: updating an emerging picture. Arch Oral Res 2013;9:41-59.

82) Tavares WLF, Neves de Brito LC, Teles RP, Massara MLA, Ribeiro Sobrinho AP, Haffajee AD. Microbiota of deciduous endodontic infections analyzed by MDA and Checkerboard DNA-DNA hybridization. Int Endod J 2011;44:225-35.

83) Sathorn C, Parashos P, Messer HH. How useful is root canal culturing in predicting treatment outcome? J Endod 2007;33:220-5.

84) Sjogren U, Figdor D, Persson S, Sundqvist G. Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. In Endod J 1997;30:297-306.

85) Acosta-Vigouroux SA, Bossans AT. Anatomy of the pulp chamber floor of the permanent maxillary first molar. J Endod 1978;4:214-9.

86) Skidmore AE, Bjorndal AM. Root canal morphology of the human mandibular first molar. Oral Surg Oral Med Oral Pathol 1971;32:778-84.

87) Hausmann E, Raisz LG, Miller WA. Endotoxin: Stimulation of bone resorption in tissue culture. Science 1970;168:862-4.